Responses of selected chickpea cultivars to imidazolinone herbicide

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Abstract

Limitations to broadleaf weed management options in chickpea present obstacles for stable production. Even with low weed incidence, chickpea yield can be severely affected, creating need for an integrated weed management system. Due to zero-tillage commonly practiced in Saskatchewan, there is heavy reliance on herbicides. The chickpea breeding program at the Crop Development Centre, University of Saskatchewan, has developed chickpea cultivars with resistance to imidazolinone (IMI) class of herbicides. The objectives of this study were: (i) to examine the reaction of four chickpea cultivars – CDC Luna, CDC Corinne, CDC Alma, and CDC Cory - to imazamox, imazethapyr, and a combination of imazamox and imazethapyr under field conditions; and (ii) to examine cultivar responses to IMI applications at different growth stages: 2-4 node, 5-8 node, and 9-12 node stage. Field experiments were conducted over five site years in Saskatchewan, Canada in 2012 and 2013. For each experiment, visual injury ratings, plant height, node, and internode length were recorded at 7, 14, 21, and 28 days after each herbicide application (DAA). Days to flowering (DTF), days to maturity (DTM), number of primary branches, pods per plant, harvest index, and seed yield were additional measurements for elucidating physiological responses.

Conventional cultivars, CDC Luna and CDC Corinne, had moderate to severe visual injury scores compared to resistant cultivars, CDC Alma and CDC Cory, with minimal to no visual injury after IMI treatment. Height stopped increasing and node development slowed for conventional cultivars treated with IMI herbicides. This

susceptibility to IMI herbicides was also recognized with a delay in the DTF and DTM. Despite significant negative response, CDC Luna and CDC Corinne were able to recover throughout the field season, resulting in no yield loss from IMI treatments. Resistant cultivars CDC Alma and CDC Cory demonstrated no negative response from IMI herbicide application compared with the untreated controls. Growth, in terms of height and node development, DTF, DTM, and yield were not significantly different between IMI treated and control treatments. Resistant cultivars tolerated IMI herbicide at all growth stages tested. These results demonstrate potential for use of IMI herbicides in chickpea, expanding the currently limited options for broadleaf weed control.

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List of Abbreviations

AHAS	Acetohydroxyacid Synthase
ALS	Acetolactate Synthase
BCAA	Branched Chain Amino Acids
DAA	Days after Application
DAS	Days after Sowing
DTF	Days to Flowering
DTM	Days to Maturity
HR	Herbicide Resistant
IMI	Imidazolinone
PPO	Protoporphyrinogen oxidase
SCMR	SPAD Chlorophyll Meter Reading

1.0 Introduction

1.1 Background

Chickpea (*Cicer arietinum L.*) is an important food legume grown worldwide. Canada is among the top 10 chickpea producing countries with 90% of Canadian production occurring in Saskatchewan (Saskatchewan Pulse Growers, 2009). Production of chickpea on the Canadian Prairies is hindered by many factors including ascochyta blight disease, a short growing season, and limited options for weed management. While improved disease resistant and early maturing cultivars have been developed (Gan et al., 2009), weed control still remains problematic.

Chickpea is a weak competitor. Yields of chickpea are severely affected by even low weed incidence. Without weed control, yield loss upwards of 80% has been reported (Al-Thahabi et al., 1994). An integrated weed management system is desirable for stable chickpea production. Due to zero tillage practices in Saskatchewan, there is significant dependence on herbicides for weed control, of which only few options exist for use in chickpea. There is a necessity to expand chemical weed control options for improved integrated weed management in chickpea.

Current chemicals for broadleaf weed control in chickpea consist of a glyphosate pre-plant burnoff, sulfentrazone applied pre-emergence, and metribuzin applied up until the 3 node stage (Saskatchewan Ministry of Agriculture, 2013). Metribuzin is the only herbicide that can be applied post-emergence, but injury may

still result (McVicar et al., 2007). Broadleaf weed control later in the season is relatively non-existent at the present time. Herbicides that can be applied throughout the growing season are required for recurrent weed control and stable chickpea production.

Tar'an et al. (2010) identified four chickpea accessions with good plant appearance and minor chlorosis at 21 days after application of 35% imazamox and 35% imazethapyr at a rate of 30 g a.i/ha. With further crossing and selection, imidazolinone resistant chickpea cultivars such as CDC Alma and CDC Cory adapted to Western Canadian environments have been developed. However, detailed information on their reaction to imidazolinone application especially under field conditions is lacking. The use of these cultivars may broaden herbicide options for an integrated weed management system in chickpea.

1.2 Objectives and Hypotheses

The main objectives of this research were: (1) to examine and compare two conventional and two IMI resistant chickpea cultivars for resistance to imazethapyr, imazamox, and combination imazamox + imazethapyr herbicide at two application rates; and (2) to evaluate two conventional and two IMI resistant chickpea cultivars for resistance to combination imazamox + imazethapyr herbicide applications at early, mid, and late growth stages.

The research was designed to test the following hypotheses: (1) No injury is observed on IMI resistant cultivars CDC Alma and CDC Cory from the applications of

IMI herbicides; and (2) No injury is observed on IMI resistant cultivars CDC Alma and CDC Cory chickpea cultivars from IMI herbicide applications at 2-4 node, 5-8 node, and 9-12 node growth stages.

2.0 Literature Review

2.1 Chickpea Production

Chickpea (*Cicer arietinum* L.) is a cool, long season specialty crop belonging to the family *Fabaceae.* Originating from Turkey, chickpea is primarily grown in semi-arid regions around the world (Redden and Berger, 2007). Southwest Saskatchewan and Southeast Alberta dominate Canadian chickpea production. In 2012, total chickpea production in Saskatchewan was estimated at 141,300 tonnes with average yield of 1,996 kg/ha (Saskatchewan Ministry of Agriculture, 2013). Average yield in 2012 exceeded the 10-year provincial average by 36%.

Canada is one of the global chickpea producers alongside India, Pakistan, Bangladesh, Turkey, Mexico, and Australia (Reddy et al., 2007). The United Sates is Canada's largest market for chickpea exports. In 2009, Canada held 45% share of the United States' chickpea import market (Agriculture and Agri-Food Canada, 2010). India and Pakistan are also significant importers of Canadian chickpeas with a combined total value of 16.6 million dollars of Canadian chickpea exports in 2009. Agriculture and Agri-Food Canada (2011) predicts the continuation of increased Canadian production due to high demands for Canadian exports by the United States and Middle Eastern and South Asian markets.

2.2 Characteristics and growth habit

There are two types of chickpeas, kabuli and desi. Large, cream-coloured, round to ram-head shaped seeds with a thick seed coat and white flowers characterize the kabuli chickpea. Desi chickpea are characterized by smaller, angular seeds with a thick, pigmented seed coat and pink or purple flowers (Maiti and Wesche-Ebeling, 2001). Desi type chickpeas may have purplish stems due to varying levels of anthocyanin, whereas kabuli type has green stems and foliage (Pundir et al., 1985). Semi-erect and semi-spreading are the most prevalent growth structures of chickpea, however some cultivars grow erectly with the advantage of easier mechanical cultivation. On the Canadian prairies, both fern and unifoliate leaf types have been grown, however, fern-type leaf structure is most common as they are expected to capture solar radiation more efficiently (Muehlbauer and Singh, 1987) and tend to be less susceptible to ascochyta blight. Due to their deep tap root and ability to respond to water stress, chickpea can access moisture from greater depths than other pulses (Benjamin and Nielsen, 2006).

For optimum growth in Canada, warm temperatures of 20-30˚C days and 18-20˚C nights are required. For germination, soil temperature of 15˚C is ideal, however kabuli type can be planted into 10˚C soil and desi type can withstand 5˚C soil temperature (Agriculture and Agri-Food Canada, 2008). Chickpea is a cool, long-season legume crop with indeterminate growth, which can present problems in Saskatchewan's short growing season. Seed set and maturity are typically forced by moisture and/or nitrogen stress (Miller et al., 2002), which is not indicative of the end of Saskatchewan's growing season. Early frost events on immature crops will

result in decreased crop quality due to high amounts of green seeds. Earlier maturing chickpea cultivars have been developed allowing for production in the Northern Great Plains, however moderate risk is still involved (Gan et al., 2009).

2.3 Limitations to Production

Chickpea production on the Canadian Prairies is challenged due to multiple debilitating stressors. Crops are weakened from adverse environmental conditions, disease pressure, insect pests, and weed incidence.

2.3.1 Abiotic Stress

Chickpea production is largely affected by climatic conditions. Although drought is considered the number one constraint in Middle Eastern and Asian growing regions (Johansen et al., 1994), production in the Northern Great Plains is limited by a short growing season, characterized by decreasing temperatures and excess moisture late in the season (Anbessa et al., 2007a). In Saskatchewan's brown and dark brown soil zones, usually less than 120 days are frost-free (Bueckert and Clarke, 2013). Early frost can increase the amount of immature green seed in harvested chickpea, causing reductions in grade and value (Saskatchewan Pulse Growers, 2000). Adapting chickpea for production on the Canadian prairies involves addressing the strong indeterminancy that causes delayed maturity.

Gan et al. (2009) studied how crop management affected maturity dates of four chickpea cultivars. Their results indicated that cultivar choice could advance

maturity by 2-7 days. Additionally, moderate rates of nitrogen fertilizer and choosing cereal stubble over summer fallow seedbeds effectively reduced maturity dates by 15 days. Anbessa et al. (2007b) also studied the effect of short internode length, double podding and early flowering on maturity. While short internode length negatively affected maturity, earlier flowering and double podding were positively associated with early maturity. These traits reduced days to maturity by up to 7 days. To minimize risk, earlier maturing cultivars should be used alongside effective crop management schemes.

2.3.2 Disease

Of the numerous diseases affecting chickpea such as Botrytis grey mould, Fusarium wilt, stem and root rot, Ascochyta blight is the most destructive with substantial economic impacts. Aerial plant parts are attacked by the fungus *Ascochyta rabiei* resulting in necrotic lesions, stem breakage, seed abortion, and plant death (Shtienberg et al., 2006). Upwards of 90% yield losses can result from no disease management (Sabbavarapu et al., 2013). Planting resistant cultivars is essential for disease control. Cultural practices such as planting disease-free seed and crop rotation can be used in conjunction with chemical treatments for optimal disease management. Foliar applications of boscalid (Lance®, BASF Canada), pyraclostrobin (Headline EC®, BASF Canada) and chlorothalonil (Bravo 500®, Syngenta Canada) aid in control of *Ascochyta* spp., and *Botrytis cinerea* pathogens (McVicar et al., 2007; Saskatchewan Ministry of Agriculture, 2013; Singh et al., 2007). Chemicals fludioxonil + metalaxyl-M and S-isomer + thiabendazole (Apron

Maxx RTA®, Syngenta Canada) and Metalaxyl (Allegiance FL®, Bayer CropScience) can be used as seed treatments for control of *Pythium*.

2.3.3 Insects

In Canada, few insect pests cause significant damage in chickpea. Cutworms, wireworms, and alfalfa looper are considered minor pests, but rarely affect more than a few hectares within a field (Saskatchewan Pulse Growers, 2010). Chickpea are unattractive plant hosts due to hairy leaves, stems and pods that secrete malic acid. Seeds treatments of thiamethoxam (Cruiser 5FS®, Syngenta Canada) can control wireworms and aerial or ground applications of lambda-cyhalothrin (Matador®, Syngenta Canada or Silencer®, MANA Canada) can be used for cutworm control if necessary (Saskatchewan Ministry of Agriculture, 2013).

2.3.4 Weeds

With the high competitive ability of weeds, exploitation of moisture, light and nutrient resources, a limiting environment for the crop is generated. Pulse crops are of particular concern due to weak competitive ability from slow seedling growth, low stature, and canopy closure developing late in the season (Saskatchewan Pulse Growers, 2000; Solh and Pala, 1990). Problematic weeds found in Saskatchewan include: kochia (*Kochia scoparia*), wild mustard (*Sinapsis arvensis*), Canadian thistle (*Cirsium arvense*), Russian thistle (*Salsola iberica*), perennial sowthistle (*Sonchus arvensis*), green foxtail (*Setaria viridis*), wild buckwheat (*Polygonum convolvulus*),

wild oats (*Avena fatua*), field pennycress (*Thlaspi arvense*), and common lamb's-quarters (*Chenopodium album*), to name a few (Dale and Thomas, 1987; Government of Saskatchewan, 2013). Early research has shown substantial losses of chickpea yield caused by weed presence in Mediterranean climates as well as Western Canadian regions (Kukula et al., 1983; Knott and Halila, 1986; Drew, 1982; Curran et al., 1987). These and other studies demonstrate the need for an integrated weed management system to reduce weed pressure on vulnerable pulse crops.

Al-Thahabi et al. (1994) demonstrated how severely chickpea yield was affected by weed presence. Chickpeas grown without weed removal suffered seed yield losses of up to 81% compared to weed free controls. Another study in Australia demonstrated a similar outcome with low weed abundance, <10 plants/m^2, resulting in an approximate 50% reduction in chickpea yields (Whish et al., 2002).

Further, weed interference at critical crop stages can influence yields substantially. In general, a competitive advantage will be given to weed species that emerge before, or simultaneously with, crop emergence (O'Donovan et al., 1985; Bosnic and Swanton, 1997). Crops, however, have the potential to recover from initial high weed densities upon weed removal before a critical stage (Dawson, 1986; Knezevic et al., 2002). A study in Jordan indicated the critical weed free period for chickpea is 35-49 days (Al-Thahabi et al., 1994), whereas a Tunisian study estimated the critical period as 28 to 70 days after emergence depending on the site and weed severity (Knott and Halila, 1986). An Iranian study also

established the critical weed-free period at 24-49 days after emergence (Mohammadi et al., 2005). This translates to the four or five-leaf stage up until early to late flowering. The critical weed-free period will differ with environment though, and should be used only as an estimate for efficient weed control timing.

An integrated management system is required to control weed incidence, preventing severe chickpea yield losses (Whish et al., 2002). Due to zero tillage practices in Saskatchewan, there is significant dependence on herbicides for weed control, of which only few options exist for use in chickpea. There is a necessity to expand chemical weed control options for improved integrated weed management in chickpea.

2.4 Weed management

2.4.1 Mechanical and Cultural Control

For optimal weed control, an integrated weed management system should be implemented that uses multiple control strategies. Most cost-effective is the prevention of weed development and dispersal (Yenish, 2007). These strategies include planting weed-free seed and cleaning farm equipment before entry and upon removal from fields. Mechanical weed control options are very limited in chickpea crops. In less industrialized nations hand pulling, hoeing, or human powered equipment is common, however labour costs are expensive (Solh and Pala, 1990). Although tillage can be used aggressively prior to planting, due to narrow crop spacing, continued mechanical control throughout the critical weed-free period

may cause extensive crop damage. Due to zero-tillage practiced in Saskatchewan, mechanical weed control is not feasible as part of an integrated management system.

Cultural weed control methods in chickpea are also limited. Management strategies to increase crop health and competitiveness are futile on the generally weak competitive ability of chickpea. For example, the cultural practice of increasing plant density to minimize weed incidence impairs chickpea health and quality. To the detriment of weed control, decreasing plant density helps reduce disease and ensure the largest seed size possible, an important quality component (Gan et al., 2002). Managing sowing dates may provide some cultural benefit. While major differences can be observed in spring versus fall sowing in Mediterranean climates (Yau, 2005), in semiarid regions, such as the Canadian Prairies, sowing dates are only separated by a few weeks. Earlier sowing dates can improve yield and quality (Miller et al., 2006), while early stand establishment is important for a competitive edge on weeds (Knezevic et al., 2002). Crop rotation may be considered the most important cultural control method in chickpea. While minimal options are available for use in chickpea, using rotational crops with comprehensive weed control strategies maintains low weed populations, providing advantage for following year chickpea crop (Yenish, 2007).

2.4.2 Chemical Control

With zero-tillage practices and the cost of manual labour prohibitive, herbicides are heavily relied on for weed management. Zero-till farming, practiced

in Saskatchewan, creates a dependence on chemical methods for sufficient weed control.

Grassy weeds in chickpea are currently controlled with chemicals clethodim (Centurion®, Bayer CropScience), sethoxydim (Poast Ultra®, BASF Canada), and quizalofop (Assure II®, DuPont Canada). These herbicides provide good to excellent control of barnyard grass, wild oat, green and yellow foxtail, and volunteer barley and wheat (Saskatchewan Ministry of Agriculture, 2013). All of these chemicals belong to group 1 herbicides that control weeds through the inhibition of acetyl CoA carboxylase (ACCase). To maintain efficacy and avoid herbicide resistance development in weeds, it is important to use herbicides with different modes of action. Potential use of IMIs in chickpea would provide a different mechanism for control over grassy weeds.

While select chemicals are currently available for grassy weed control, broadleaf weed control poses a major problem in chickpea. Only two herbicides are registered for broadleaf weed control in chickpea, sulfentrazone (Authority 480®, Nufarm Canada) and metribuzin (Sencor®, Bayer CropScience), of which metribuzin is the only chemical that can be applied post-emergence (Saskatchewan Ministry of Agriculture, 2013). Metribuzin can only be applied up until the 3 node stage (6cm height), after which significant crop injury may result (McVicar et al., 2007). Kay and McMillian (1990) applied metribuzin at 0.105 kg a.i/ha on 5-15 cm tall chickpeas and found significant damage. Even when applied pre-emergent, chickpeas were moderately susceptible at rates of 0.28 kg a.i/ha and higher. Tar'an et al. (2013)

demonstrated similar results of high crop injury with post-emergent applications of metribuzin.

Sulfentrazone is a group 14 herbicide that inhibits protoporphyrinogen oxidase (PPO) preventing chlorophyll and heme biosynthesis. Depending on soil characteristics sulfentrazone has a half-life of 110 – 280 days (Grey et al, 1997) which provides soil residual activity. Sulfentrazone must be applied pre-emergence, providing control over wild buckwheat, kochia, lamb's-quarters, and redroot pigweed (Saskatchewan Ministry of Agriculture, 2013) with no evidence of crop injury (Lyon and Wilson, 2005).

Alternative herbicides have been widely tested for potential use in chickpea around the globe. While there has been limited success for post-emergence herbicides, multiple studies have suggested minimal crop injury for numerous additional herbicides applied pre-emergent: trifluralin, pendemethalin, simazine, metolachlor (Bhan and Kukula, 1987; Solh and Pala, 1990), sulfentrazone, and isoxaflutole (Lyon and Wilson, 2005; Datta et al., 2009a).

Khan et al. (2006) demonstrated that pre-emergent applications of methabenzthiazuron, terbutryn, and linuron resulted in minimal chickpea injury. At a rate of 1.25 g a.i/kg applied pre-sowing, terbutryn actually improved grain yield by 19.4%. Similar to that result, in Turkey, Kantar et al. (1999) demonstrated that methabenzthiazuron, terbutryn, and linuron applications slightly increased chickpea yields from the handed weeded control of 823 kg/ha to 873 kg/ha, 900 kg/ha, and 943 kg/ha, respectively. Felton et al. (2004) analyzed isoxaflutole as pre-emergence broadleaf weed control for use on chickpea in Australia. Although one

variety, 91025-3021, was more severely affected, all seven genotypes assessed showed minimal crop injury when isoxaflutole was applied at 2 or 7 days after sowing (DAS). Datta et al. (2009b) experienced similar results testing susceptible and resistant cultivars with isoxaflutole applied 1 DAS. Susceptible cultivars showed phytotoxicity and inhibition of shoot growth whereas resistance cultivars were largely unaffected.

A few pre-emergent broadleaf herbicides have potential for use in chickpea, however sustained weed control is absent (Lyon and Wilson, 2005). Post-emergent herbicides are required for prolonged, seasonal weed control. Due to regional regulations, few options exist for herbicide use in Saskatchewan chickpea production. As previously described, metribuzin is the only chemical registered for use post-emergence on chickpea (McVicar et al., 2007). Establishing chickpea cultivars that are resistant to additional herbicides with different modes of actions is ideal for an enhanced weed management system.

2.4.3 Herbicide Resistance

Heavy reliance on chemical weed control over the last 50 years has shifted weed population dynamics and has resulted in a growing number of herbicide resistant (HR) weed species. From the first identification of HR weeds in the 1960s in the United States (Holt, 1992) to now, with over 880 cases of resistance reported across 65 countries (Heap, 2014), managing HR has become a global concern.

The rate of resistance evolution is dependent on the frequency of mutation in the initial population, inheritance, dominance, and selection by herbicides (Jasieniuk

et al., 1996). High mortality of susceptible weeds under herbicide treatment rapidly leads to increased frequency of resistant biotypes in the population, demonstrating herbicide selection pressure as most influential in HR evolution. The rapid progression of HR weeds also may be exacerbated by the increasing development and use of HR crops. The swift adoption of HR crops, due to improved weed control and greater yields, created heavy reliance on herbicides with single modes-of-action throughout rotations (Beckie et al., 2006). Repeated applications of the same mode-of-action herbicide create an intense selection pressure for resistance in weeds (Holt and Lebaron, 1990; Owen and Zelaya, 2005). More problematic, weeds with multiple resistance and cross resistances are now becoming prevalent.

Debate exists whether low dosage of herbicide accelerates resistance. The theory is based on surviving cross-pollinated species accumulating all minor resistance traits in the population. Rigid ryegrass is an example of rapid evolution of herbicide resistance through high survival under low rates of diclofop (Manalil et al., 2011; Manalil, 2014). The recommendation is to use full herbicide rates to ensure high mortality and limit resistant gene flow. In contrast, other studies suggest low herbicide rates may slow resistance evolution by maintaining susceptible biotypes for dilution of resistance in a population (Friesen et al., 2000).

Managing resistance is important for the efficacy of current herbicides in maintaining adequate weed control. In general, it is recommended that herbicides are used at full rates, tank-mixes be used when possible, chemical rotations involving multiple modes of action, cultural and mechanical control be used in

conjunction with chemical, and a zero tolerance policy is employed to destroy all survivors (Prather et al., 2000).

Despite resistance first reported from triazine herbicides, resistance to ALS inhibitors rapidly evolved and is now the most widespread resistance in weed species (Heap, 2014). Resistance to ALS inhibitors is prevalent because of the high frequency and repetitive herbicide use combined with high soil residual activity (Tranel and Wright, 2002). Point mutations at several locations within the gene encoding ALS can cause an amino acid substitution resulting in resistance (Boutsalis et al., 1999; Park and Mallory-Smith, 2004). Regardless of the ubiquity of resistance to ALS inhibitors, they remain important herbicides in chemical rotations.

2.5 Imidazolinones

2.5.1 Imidazolinones and Pulse Crops

Group 2 herbicides, encompassing imidazolinones (IMIs), have been widely used for weed control because of the limited soil persistence, favourable toxicological properties, and broad spectrum of weed control (Hanson et al., 2007). These herbicides act through the inhibition of acetolactate synthase (ALS), which interrupts the synthesis of branched-amino acids (Shaner et al., 1984). Although more weed species are resistant to ALS-inhibiting herbicides than any other herbicide class (Tranel and Wright, 2002), they remain important for integrated weed management systems.

Thus far, IMIs cannot be used in chickpea. Lyon and Wilson (2005) evaluated chickpea injury with pre-emergence application of imazethapyr. They observed reduced plant height, chlorosis and delayed maturity when application rates were 0.053 kg a.i/ha. However, a lower rate at 0.026 kg a.i/ha applied in combination with sulfentrazone, reduced injury symptoms to a commercially acceptable level. Tar'an et al. (2013) tested pre- and post-emergent applications of imazethapyr and post-emergent applications of imazamox on multiple chickpea cultivars with negative results. Crop injury, delayed maturity, and increased risk of ascochyta blight were observed on all cultivars to varying degrees.

Despite high susceptibility of chickpea, IMIs have been effective for use on other pulse crops such as soybean, field pea, dry bean (Shaner and Hornford, 2005; Hanson and Thill, 2001) and lentil (Fedoruk and Shirtliffe, 2011). The usefulness of IMIs in other pulses has sustained interest in developing IMI resistance in chickpea. Toker et al. (2012) experimented with induced mutation to develop IMI resistant chickpea. The study was deemed successful when mutant *C. reticulatum* Ladiz had no IMI herbicide injury compared to the susceptible parents. Exploiting natural genetic variation, Tar'an at el. (2010) identified four chickpea accessions with good plant appearance and minor chlorosis at 14 and 21 days after application of 35% imazamox and 35% imazethapyr at a rate of 30 g a.i./ha. Through conventional breeding and selection, imidazolinone resistant chickpea cultivars adapted to Western Canadian environments have been developed. The use of these cultivars may broaden the herbicide options for an integrated weed management system in chickpea.

2.5.2 Mechanisms of ALS inhibition

Imidazolinones are group 2 herbicides that act by inhibiting acetolactate synthase (ALS) enzyme, also called acetohydroxyacid synthase (AHAS). Leaves and roots absorb IMI herbicides, which are then translocated to the actively growing tissues via xylem and phloem (Ballard et al., 1995). Once IMI herbicides bind to the ALS enzyme, the capability of the plant to synthesize branch chain amino acids (BCAA) isoleucine, leucine and valine is reduced, causing the plant to starve to death (Shaner et al., 1984). This property has been exploited for control of broadleaf and grass weeds worldwide (Tranel and Wright, 2002). Due to repeated applications, resistance to ALS inhibition has been naturally selected in weed populations. However, this trait is also purposefully being selected for in-crop breeding programs to develop resistant cultivars that could expand weed control options.

Although inactivation through rapid metabolism is a common mechanism of resistance to IMI herbicide in many leguminous species (Bukun et al., 2012; Ballard et al., 1995), primary resistance is gained from single mutations in the ALS coding sequence which leads to an altered form of the enzyme (Zhou et al., 2007; Boutsalis et al., 1999). Point mutations potentially occur in the coding sequence in one of the five highly conserved domains resulting in a substitution of an amino acid (Lamego et al., 2009). Substitution at one or more of the following five amino acids has been shown to confer resistance to ALS inhibiting herbicides: Ala122, Pro197, Ala205, Trp574, and Ser653 (Lamego et al., 2009).

Jander et al. (2003) sequenced the single gene, *CSR1*, which encodes the catalytic subunit of ALS in Arabidopsis mutant isolates. They determined that

imidazolinone resistance resulted from either a base pair change from Ser653 to Asn or at Ala122 to Thr. Sathasivan et al. (1991) similarly determined imidazolinone resistance in mutant Arabidopsis when a single-point mutation occurred at nucleotide 1958 of the coding sequence, resulting in the Ser-653-Asn change.

Amino acid substitutions conferring resistance to ALS inhibition does not demonstrate cross-resistance to all ALS inhibiting herbicides. While the substitution of Ser653 to Asn results in tolerance to imidazolinone, cross tolerance to sulfonylurea and triazolopyrimidine is not achieved (Roux et al., 2004). Likewise, sulfonylurea and triazolopyrimidine resistance from the substitution of Pro197 to Ser does not translate to imidazolinone tolerance (Roux et al., 2004; Park and Mallory-Smith, 2004).

Recent research by Thompson and Tar'an (2014) identified the point mutation in chickpea at nucleotide 675 resulting in an amino acid substitution of Ala205 to Val205 conferring IMI resistance. This mutation was identified in the AHAS1 gene on chromosome 5. Segregation analyses suggested inheritance of IMI resistance follows a semi-dominant, single gene model. Thompson and Tar'an (2014) also successfully developed a SNP marker targeting the point mutation which can be utilized in breeding of IMI resistant cultivars. This research provides background for newly identified IMI resistance in chickpea.

3.0 Materials and Methods

3.1 Research Component 1 – IMI Resistance

3.1.1 Trial Design

Field research was conducted over five site years with three locations in Saskatchewan; Saskatoon (52° 9' N, 106° 32' W) and Elrose (51°17' N, 107°58' W) in 2012 & 2013 and Moose Jaw (50° 11' N, 106° 0' W) in 2013. Saskatoon sites were characterized by Dark Brown Chernozemic soil with 3.5 - 4.5% organic matter and pH of 6.1 -6.7, while Elrose and Moose Jaw had Brown Chernozemic soil with 2.5 – 3.5% organic matter and pH greater than 7.5 (Rostad et al, 1987). Trials were planted on wheat stubble at all site-years except Saskatoon in 2012, which was planted under chemical fallow.

Four cultivars were examined in this study; two conventional (susceptible) – CDC Luna (kabuli) and CDC Corinne (desi); and two resistant near-isogenic lines – CDC Alma (kabuli) isogenic of CDC Luna and CDC Cory (desi) isogenic of CDC Corinne. These near-isogenic lines differ at the ALS gene, allowing easy comparison of the responses to IMI herbicides. Table 3.1 lists the pedigree of each cultivar.

Table 3.1 - Pedigree of the four cultivars used for the evaluation of their response to different rates of IMI herbicides.

Name	Type	Pedigree
CDC Luna	Kabuli	FLIP91-123C/FLIP84-79C//FLIP90127C
CDC Alma	Kabuli	CDC Luna *3//FLIP97-133C/ICCX860047-9
CDC Corinne	Desi	Single plant selection from landrace ICC12512
CDC Cory	Desi	CDC Corinne*2//ICC12512-9/ICCX860047-9

To prevent seed-borne fungal diseases, seeds were pre-treated with mefenoxam + fludioxonil (Apron Maxx®, Syngenta Canada) at a rate of 3.25 ml/1000g. A glyphosate burn-off was applied pre-emergence at 900 g a.i/ha and pre-emergence liquid UAN was applied at 0.87 L actual/100m^2 all site years. Seeds were sown at a rate of 43 plants per meter square in Elrose (25-Apr-2012, 13-May-2013), Saskatoon (18-May-2012, 15-May-2013), and Moose Jaw (9-May-2013). Plot dimensions were 2.28 m by 3.66 m for a seeded area of 8.34 m^2 per plot. Each plot was comprised of 6 crop rows. Adjacent plots were separated by 0.762 m width. Faba bean border rows separated replications and parallel trials. Fungicide was applied on multiple dates to control Ascochyta blight incidence (Table 3.2).

Table 3.2 - Fungicide application information including date, chemical, and rate for all site years.

Year	Location	Application	Date	Chemical	Rate
2012	Elrose	1st	June 22, 2012	Proline®	371 ml/ha
	Saskatoon	1st	July 6, 2012	Proline®	371 ml/ha
	Saskatoon	2nd	July 24, 2012	Bravo®	2.5 L/ha
	Saskatoon	3rd	August 18, 2012	Headline®	420 ml/ha
2013	Elrose	1st	July 9, 2013	Bravo®	2.5 L/ha
	Saskatoon	1st	July 2, 2013	Proline®	371 ml/ha
	Moose Jaw	1st	June 18, 2013	Proline®	371 ml/ha
	Moose Jaw	2nd	July 2, 2013	Bravo®	2.5 L/ha

3.1.2 Treatments

To determine the effects of multiple IMI chemicals on each cultivar, the experiment design was a split plot with four replications. Herbicide was the main plot and cultivar was the sub-plot with herbicide and cultivars arranged in a randomized complete block within main and sub-plots. The chemicals tested were imazamox, imazethapyr, and the combination imazamox (35%) + imazethapyr

(35%). Using recommendations for field pea, each chemical treatment was applied at 1x and 2x rates. Hand weeded control plots were used for each cultivar with no herbicide application. The treatment combination list follows:

1. CDC Luna – Control
2. CDC Alma – Control
3. CDC Cory – Control
4. CDC Corinne – Control
5. CDC Luna - 1X imazethapyr (50 g a.i./ha)
6. CDC Alma - 1X imazethapyr (50 g a.i./ha)
7. CDC Cory - 1X imazethapyr (50 g a.i./ha)
8. CDC Corinne - 1X imazethapyr (50 g a.i./ha)
9. CDC Luna - 2X imazethapyr (100 g a.i./ha)
10. CDC Alma - 2X imazethapyr (100 g a.i./ha)
11. CDC Cory - 2X imazethapyr (100 g a.i./ha)
12. CDC Corinne - 2X imazethapyr (100 g a.i./ha)
13. CDC Luna - 1X imazamox + imazethapyr (30 g a.i./ha)
14. CDC Alma - 1X imazamox + imazethapyr (30 g a.i./ha)
15. CDC Cory - 1X imazamox + imazethapyr (30g a.i./ha)
16. CDC Corinne - 1X imazamox + imazethapyr (30 g a.i./ha)
17. CDC Luna - 2X imazamox + imazethapyr (60 g a.i./ha)
18. CDC Alma - 2X imazamox + imazethapyr (60 g a.i./ha)
19. CDC Cory - 2X imazamox + imazethapyr (60 g a.i./ha)
20. CDC Corinne - 2X imazamox + imazethapyr (60 g a.i./ha)
21. CDC Luna - 1X imazamox (20 g a.i./ha)
22. CDC Alma - 1X imazamox (20g a.i./ha)

23. CDC Cory - 1X imazamox (20 g a.i./ha)
24. CDC Corinne - 1X imazamox (20 g a.i./ha)
25. CDC Luna - 2X imazamox (40 g a.i./ha)
26. CDC Alma - 2X imazamox (40 g a.i./ha)
27. CDC Cory - 2X imazamox (40 g a.i./ha)
28. CDC Corinne - 2X imazamox (40 g a.i./ha)

All plots were hand weeded to remove any confounding effects from weed competition. All herbicide treatments were applied at the 2-4 node growth stage. The applications corresponded to 8-Jun-2012 & 4-Jun-2013 in Saskatoon, 31-May-2012 & 4-Jun-2013 in Elrose, and 29-May-2013 in Moose Jaw.

Treatments were prepared and applied based on plot dimensions. Chemicals were measured (as per below) and added to 1.5 L of water.

a) 1x imazethapyr = 3.125 ml/ 1.5 L water + 3.75 ml Agral 90
b) 2x imazethapyr = 6.25 ml/ 1.5 L water +3.75 ml Agral 90
c) 1x imazamox + imazethapyr = 0.6429 g/ 1.5 L water + 7.5 ml Merge
d) 2x imazamox + imazethapyr = 1.286 g/ 1.5 L water + 7.5 ml Merge
e) 1x imazamox = 0.4286 g/ 1.5 L water + 7.5 ml Merge
f) 2x imazamox = 0.8571 g/ 1.5 L water + 7.5 ml Merge

Solutions were mixed in 2 L bottles by inversion. Treatments were applied at a rate of 100 L/ha with pressure of 40 PSI. Six airmix 019, flat fan, teejet 100-1 nozzles were used at a spacing of 45 cm. The chemical was applied 30 cm above the plant canopy using either a small plot tractor sprayer or hand held wand. All treated plots received the appropriate rate within a 5% error margin.

3.1.3 Data Collection

To determine IMI resistance, chickpea plots were subject to visual injury ratings and other physiological measurements. Visual injury, plant height, number of nodes, and chlorophyll content were assessed throughout the growing season at 7 day intervals after application. Six plants in each plot were tagged at 7 days after application (DAA) for repeated assessment. Days to flowering (DTF) and days to maturity (DTM) were recorded when 80% of the plot had begun flowering or reached maturity, respectively. At harvest, plant measurements included plant dry weight, pods per plant, seeds per plant, seeds per pod, green seed percentage, height, number of nodes, number of primary branches, 1000 seed weight, harvest index, and yield.

Visual injury ratings were conducted at 7 day intervals starting at 7 DAA up until 28 DAA. Untreated controls were compared with treated plots on a whole plot basis. Injury was scored based on a 0 – 100 scale. A rating of 0% signified no plant damage and 100% signified plant death across the entire plot. Injury rating >10% was classified as unacceptable damage. Scoring was based on the severity of plant stunting, chlorosis, and other changes in morphology such as increased lateral branching and leaves becoming thin or pine-like.

Height and node measurements were taken on 7 DAA intervals until 28 DAA at Elrose and Saskatoon. A meter stick was placed at ground level beside tagged plants. The primary stem was raised against the meter stick. The height measurement was taken at the apical meristem of the primary stem. Height measurements were taken for the six-tagged plants within each plot and averaged.

The number of nodes were manually counted for the six-tagged plants within each plot and averaged.

Chlorophyll content was measured using a SPAD-502DL Plus meter at weekly intervals starting at 7 DAA. Preliminary sampling confirmed variation of SPAD chlorophyll meter reading (SCMR) between the first and third leaf positions. The SCMRs did not have significant variation at the first fully expanded leaf within cultivars. With this result and the mode of action of IMI herbicides considered, the first fully expanded leaf was selected for continued sampling. Six plants, avoiding tagged plants, were randomly selected at each sampling interval. Using scissors, the first fully expanded leaf was removed and placed into a labeled plastic sample bag. Sample bags were placed on ice until the entire trial was completed. Samples were stored at 4°C until SCMR were completed within 48 hours of sampling. Leaflets were placed under the sensor of the SPAD-502DL Plus meter using tweezers, avoiding the midrib. Three readings for each leaflet were taken and the average was recorded. The six leaflet readings per plot were averaged, for one SCMR for each plot.

At all locations, DTF and DTM were recorded when 80% of the plot had reached flowering (DTF) and maturity (DTM).

At maturity, 5-Sep-2012 & 19-Sep-2013 (Elrose) and 28-Sep-2012 & 24-Sep-2013 (Saskatoon), the six-tagged plants from each plot were removed by hand. The plant samples were placed into labeled paper sleeves and boxed. Boxes were placed on driers for three days to remove moisture. Once dry, samples were individually processed. The 6 plants from each plot were visual assessed for overall uniformity. Unrepresentative plants were removed from the sample. Each of the remaining

plants within a sample were measured for height, number of nodes, number of branches from the primary stem, and number of pods per plant. After removing roots, the total dry weight of all the plants was taken on the above ground biomass including seeds using a scale, and then averaged. The plants were then processed using a mechanical thresher. Using a calibrated seed counter and a scale, the number of seeds per sample and seed weight was measured. Harvest index was calculated based on dry weight and seed weight.

Entire plots at Elrose (29-Sep-2012 and 2-Oct-2013), Saskatoon (7-Oct-2013), and Moose Jaw (1-Oct-2013) were harvested using a small plot combine. Seeds were collected in harvest bags and placed on the driers until moisture content was approximately 12%. Seed was cleaned using a size 15 round sieve to remove dirt, debris, weed seed and small, shriveled chickpea seed. Large debris was removed by hand. Seed was weighed and yield was calculated based on plot dimensions. Yield was adjusted to account for the percent of green by weight in each yield sample. Therefore, adjusted yield measurements were yield with green seed removed. Using a seed counter and scale, subsamples of greater than 200 seeds were used to calculate 1000 seed weights.

In 2012, Saskatoon plots were not harvested before season end. The majority of plots did not reach maturity before snow covered the trial. Plots were reassessed in spring 2013 and deemed unworthy for collection of yield data.

3.1.4 Statistical Analyses

Data were analyzed using SAS version 9.3 (SAS Institute, 2002-2010 Cary, NC, USA). Homogeneity of variance across site-years was tested for each measurement using Levene's test in a general linear model procedure (PROC GLM). This test determined whether site years could be combined based on the interaction of cultivar by herbicide by location by year. Analysis of variance (ANOVA) of DTF, DTM, final height, node, and internode lengths, yield, harvest index, and seed characteristics was performed using PROC MIXED in a split-plot model with herbicide as the main plot and cultivar as the sub-plot. Replication was considered a random effect and location, herbicide and cultivar were fixed effects. Location as a fixed effect was based on the sizable environmental differences between sites. Response of cultivars could be determined across environments and similar trends identified. Means were separated using Tukey's statistic at $P < 0.05$. Repeated measures ANOVA using mixed model procedure was performed for injury, height, node, internode and SCMR. First order ante dependence was selected as the covariance model based on Akaike's Information Criteria (AIC) and Bayesian Information Criteria (BIC). Correlations between Ascochyta blight incidence, visual injury, and yield components were conducted using Pearson's correlation.

3.2 Research Component 2 – Timing of IMI Applications

3.2.1 Trial Design

Field research was conducted over five site years with three locations in Saskatchewan; Elrose and Saskatoon in 2012 & 2013 and Moose Jaw in 2013. Four cultivars were examined in this study; two conventional – CDC Luna (kabuli) and CDC Corinne (desi); and two resistant – CDC Alma (kabuli) and CDC Cory (desi). For details on Component 2 trial design, refer to section 3.1.1 Component 1 – Trial Design.

3.2.2 Treatments

To determine how IMI application at different growth stages affects each cultivar, the experiment was set up in a split plot with a randomized complete block design with four replications. Herbicide timing was the main plot and cultivar was the split plot. The chemical used for all treatments was the combination imazamox (35%) + imazethapyr (35%). Using recommendations for field pea, the chemical treatment was applied at 1x (20 g a.i/ha) and 2x (60 g a.i/ha) rates for treatments at 2-4 node, 5-8 node, and 9-12 node growth stages. Hand weeded control plots were used for each cultivar with no herbicide application. The treatment list follows:

1. CDC Luna – Control
2. CDC Alma – Control
3. CDC Cory – Control
4. CDC Corinne - Control

5. CDC Luna - 1X imazamox + imazethapyr 2-4 node stage
6. CDC Alma - 1X imazamox + imazethapyr 2-4 node stage
7. CDC Cory - 1X imazamox + imazethapyr 2-4 node stage
8. CDC Corinne - 1X imazamox + imazethapyr 2-4 node stage
9. CDC Luna - 1X imazamox + imazethapyr 5-8 node stage
10. CDC Alma - 1X imazamox + imazethapyr 5-8 node stage
11. CDC Cory - 1X imazamox + imazethapyr 5-8 node stage
12. CDC Corinne - 1X imazamox + imazethapyr 5-8 node stage
13. CDC Luna - 1X imazamox + imazethapyr 9-12 node stage
14. CDC Alma - 1X imazamox + imazethapyr 9-12 node stage
15. CDC Cory - 1X imazamox + imazethapyr 9-12 node stage
16. CDC Corinne - 1X imazamox + imazethapyr 9-12 node stage
17. CDC Luna - 2X imazamox + imazethapyr 2-4 node stage
18. CDC Alma - 2X imazamox + imazethapyr 2-4 node stage
19. CDC Cory - 2X imazamox + imazethapyr 2-4 node stage
20. CDC Corinne - 2X imazamox + imazethapyr 2-4 node stage
21. CDC Luna - 2X imazamox + imazethapyr 5-8 node stage
22. CDC Alma - 2X imazamox + imazethapyr 5-8 node stage
23. CDC Cory - 2X imazamox + imazethapyr 5-8 node stage
24. CDC Corinne - 2X imazamox + imazethapyr 5-8 node stage
25. CDC Luna - 2X imazamox + imazethapyr 9-12 node stage
26. CDC Alma - 2X imazamox + imazethapyr 9-12 node stage
27. CDC Cory - 2X imazamox + imazethapyr 9-12 node stage

28. CDC Corinne - 2X imazamox + imazethapyr 9-12 node stage

All plots were hand weeded to remove any confounding effects from weed competition. Herbicide treatments occurred at various dates throughout May, June and July (Table 3.3).

Table 3.3 - Imidazolinone herbicide application dates at Saskatoon, Elrose and Moose Jaw in 2012 and 2013.

	Saskatoon		Elrose		Moose Jaw	
	2012	2013	2012	2013	2012	2013
2-4 node	07-Jun	04-Jun	17-May	04-Jun	.	29-May
5-8 node	21-Jun	12-Jun	31-May	11-Jun	.	06-Jun
9-12 node	03-Jul	21-Jun	22-Jun	25-Jun	.	18-Jun

Treatments were prepared and applied based on plot dimensions stated in Section 3.1.1. Combination imazamox + imazethapyr was measured for a 1x rate: 0.6429 g of herbicide, and a 2x rate: 1.286 g of herbicide. Chemical and 7.5 ml of Merge was added to 1.5 L of water. Solutions were mixed in 2 L bottles by inversion. Treatments were applied at a rate of 100 L/ha with pressure of 40 PSI. Nozzles used were airmix 019, flat fan, teejet 100-1. Nozzles were spaced 45 cm apart. The chemical was applied at 30 cm above the canopy using either a small plot tractor sprayer or hand held wand. All treated plots received the appropriate rate within a 5% error margin.

3.2.3 Data Collection

To determine IMI resistance across growth stages, chickpea plots were subjected to the same visual injury ratings and physiological measurements

discussed in Section 3.1.3 Component 1 – Data Collection. Visual injury, plant height, number of nodes, and chlorophyll content were assessed throughout the growing season. At 7 days after application (DAA), six plants in each plot were tagged for repeated measurements. Records were taken of days to flowering (DTF) and days to maturity (DTM). At harvest, plant measurements included plant dry weight, pods per plant, seeds per plant, seeds per pod, percent of green seed, plant height, number of nodes, degree of primary branching, 1000 seed weight, harvest index, and yield. Refer to Section 3.1.3 Research Component 1 – Data Collection for detailed methods.

3.2.4 Statistical Analyses

Data were analyzed using SAS version 9.3 (SAS Institute, 2002-2010 Cary, NC, USA). Homogeneity of variance across site-years was tested for each measurement using Levene's test in a general linear model procedure (PROC GLM). This test determined whether site years could be combined based on the interaction of cultivar by herbicide by location by year. Analysis of variance (ANOVA) of DTF, DTM, final height, node, and internode lengths, yield, harvest index, and seed characteristics was performed using PROC MIXED in a split-plot model with herbicide as the main plot and cultivar as the sub-plot. Replication was considered a random effect and location, herbicide and cultivar were fixed effects. Location as a fixed effect was based on the sizable environmental differences between sites. Response of cultivars could be determined across environments and similar trends identified. Means were separated using Tukey's statistic at $P < 0.05$. Repeated measures ANOVA using mixed model

procedure was performed for injury, height, node, internode and SCMR. First order ante dependence was selected as the covariance model based on Akaike's Information Criteria (AIC) and Bayesian Information Criteria (BIC). Correlations between Ascochyta blight incidence, visual injury, and yield components were conducted using Pearson's correlation.

4.0 Results

4.1 Growing Season Conditions

The seasonal (May to September) weather at both Saskatoon and Elrose in 2012 was much wetter than normal (Table 4.1). The total precipitation in Saskatoon was 1.5 times higher than normal, with 63.7% of total rainfall occurring in May and June. Similarly, Elrose received 1.7 times more precipitation, with 65.8% rainfall occurring in May and June. This weather resulted in extremely wet soils and limited the days suitable for herbicide spraying and data collection. The average air temperature at both locations was slightly lower than average in May, however typical temperatures were experienced throughout the remainder of the season.

In 2013, Saskatoon and Elrose experienced less than normal total precipitation (Table 4.1). Despite this, 55.7% and 59.1% of the total seasonal rainfall occurred in June, at Saskatoon and Elrose, respectively. This was 1.7 times and 2 times more than the normal rainfall in June. Excluding June, the rest of the season was 47.0% drier than normal at both locations. Moose Jaw in 2013, on the other hand, received 17.7% more rainfall than normal throughout the season. Similar to Saskatoon and Elrose, precipitation was highest in June, accounting for 33.3% of the total seasonal rainfall.

Temperatures in 2013 slightly deviated from climate normals (Table 4.1). Specifically, May and September were slightly hotter months by approximately 1.8°C and 3.9°C, respectively, at Saskatoon and Elrose. At Moose Jaw in 2013, the

average daily temperature in July was 2.3°C cooler than normals, whereas September was 3.3°C warmer than normal. Typical temperatures were experienced in all other months at all locations.

Table 4.1 - Mean temperature and average rainfall during 2012 and 2013 growing seasons in Saskatoon, Elrose, and Moose Jaw, compared with climate normals of 1971-2000.

	2012		2013		Normals for 1971 - 2000	
Month	Mean temp (°C)	Total precipitation (mm)	Mean temp (°C)	Total precipitation (mm)	Mean temp (°C)	Total precipitation (mm)
Saskatoon						
May	10.1	108	13.5	15.9	11.5	49.4
Jun	15.8	121.1	16	105.6	16	61.1
Jul	19.7	80.9	17.2	37.6	18.2	60.1
Aug	17.3	48.5	18.5	20.4	17.3	38.8
Sep	13	0.8	15.3	10.1	11.2	30.7
Total		359.3		189.6		240.1
Elrose						
May	10.1	100.2	13	15.2	11.3	44.2
Jun	16	150.6	15.5	115.9	15.9	57.1
Jul	19.7	80.9	17.4	35.2	18.2	57.3
Aug	17.3	48.5	18.9	14.7	17.8	41.1
Sep	13	0.8	15.2	14.9	11.5	27.1
Total		381		195.9		226.8
Moose Jaw						
May			12.6	28.8	12.1	48.9
Jun			16.1	98	17.1	60.2
July			17.1	55.6	19.4	57.3
Aug			18.3	58.7	18.6	39.8
Sep			15.7	52.8	12.4	35.7
Total				293.9		241.9

Source: (Government of Canada, 2014)

4.2 Research Component 1 – IMI Resistance

4.2.1 Disease Incidence

Despite repeated fungicide applications, Saskatoon 2012 and Moose Jaw 2013 sites were severely affected by ascochyta blight. All other site years had minimal to no signs of disease. Disease ratings were conducted at both infested site years based on a 1-9 scale from Singh et al. (1981). At Saskatoon 2012, the interaction of herbicide and cultivar significantly affected the severity of ascochyta blight ($p<0.0001$). Disease severity at Moose Jaw 2013, however, was only influenced by cultivar ($p<0.001$). At both site years, kabuli cultivars CDC Luna and CDC Alma had higher disease incidence than desi cultivars (Figure 4.1 & Figure 4.2). The severity of ascochyta blight was only weakly negatively correlated to injury ratings at 7DAA ($r=-0.2129$, $p= 0.0242$), but had no correlation to injury at 14, 21, and 28 DAA. Ascochyta disease scores had strong negative correlations to seed weight per plant ($r=-0.6536$, $p<0.0001$) and pods per plant ($r=-0.6797$, $p<0.0001$) and very strong negative correlations to seeds per plant ($r=-0.7047$, $p<0.0001$) and seeds per pod ($r=-0.7933$, $p<0.0001$).

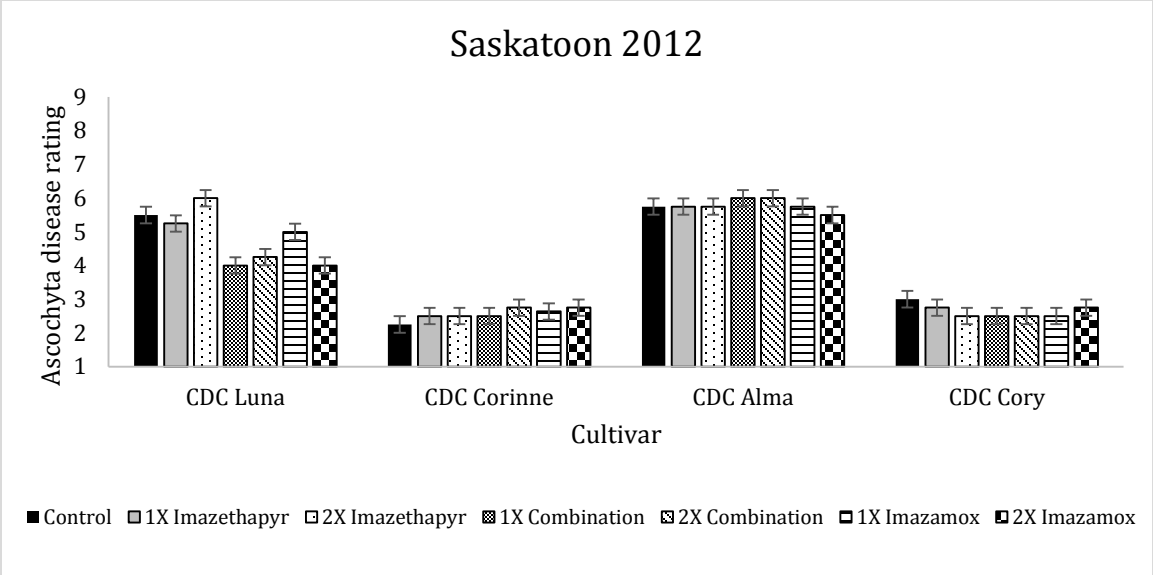


Figure 4.1 – Ascochyta blight disease scores across four chickpea cultivars at Saskatoon 2012. The scores were based on a 1-9 scale. The interaction of herbicide and cultivar was significant (p<0.0001).

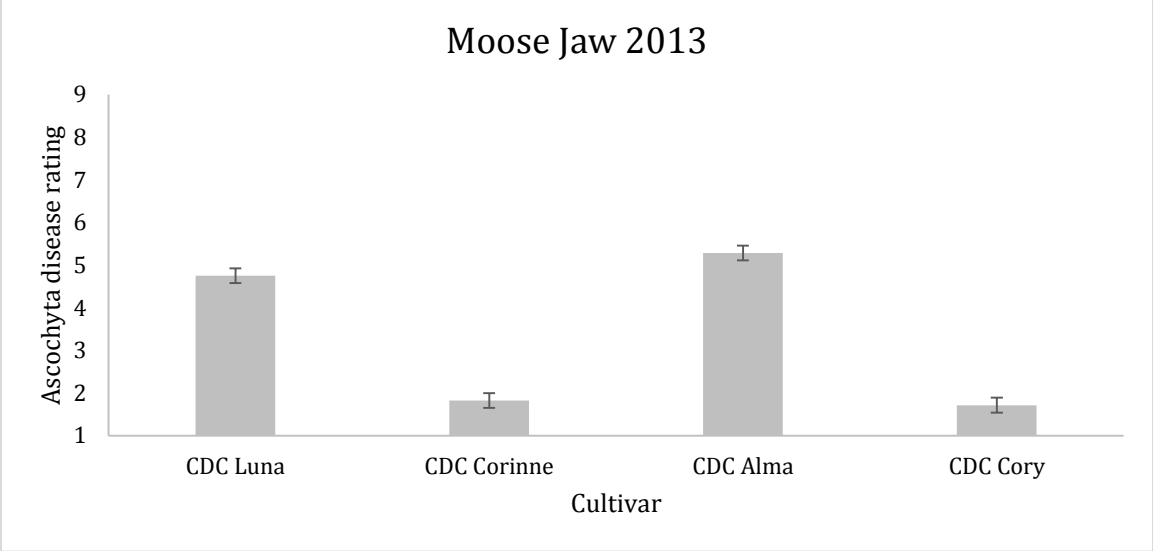


Figure 4.2 – Ascochyta blight disease scores at Moose Jaw 2013 based on a 1-9 disease scale. Cultivar was the only significant factor (p<0.0001).

4.2.2 Repeated Measures

Initial results demonstrated the differences in response of the four cultivars to IMI herbicides. Visual injury ratings demonstrated significant interactions of herbicide and cultivar ($p<0.0001$). Levene's test revealed homogeneous variance across site years, therefore, visual injury could be analyzed together (Appendix 1). CDC Luna and CDC Corinne had high injury rating from all herbicide treatments (Figure 4.3). Injury ratings at 14 and 21 DAA signified the most severe damage on these two cultivars. At 28 DAA severity of injury began to decrease. Imazethapyr was most tolerated at 1x and 2x rates with a maximum injury rating of 55% for CDC Luna and 57% for CDC Corinne. Imazamox at the 2x rate generated the most severe injury followed by the combination imazamox + imazethapyr at a 2x rate. In contrast, CDC Alma and CDC Cory had minimal to no visual injury across all treatments from 7 to 28 DAA (Figure 4.3). Cultivars CDC Alma and CDC Cory demonstrated resistance to all of the IMI herbicide applications compared with their respective controls.

Plant height measurements taken at 7 DAA intervals revealed similar growth patterns across all site years. The height of susceptible cultivars CDC Luna and CDC Corinne was negatively affected by all herbicide treatments (Figure 4.4). The height of both susceptible cultivars was arrested at 7 and 14 DAA for all IMI treatments. At 21 DAA, height began to increase again. Imazamox and the combination imazamox + imazethapyr at the 2x rate were the most debilitating treatments. Imazethapyr at both 1x and 2x rates had less severe stunting and allowed for faster recovery. In

contrast, there was no significant height alteration from the respective controls for either CDC Alma or CDC Cory for any of the IMI treatments.

Similar for all site years, all control treatments showed a steady increase in the average number of nodes over time (Figure 4.5). Herbicide treatments on CDC Luna and CDC Corinne decreased the rate in which new nodes developed. Imazamox and the combination imazamox + imazethapyr at the 2x rate stopped further node development up until 21 DAA. Imazethapyr at the 1x and 2x rate was most tolerated, however still significantly decreased the rate of node development compared to the control treatments. The rate of node development for resistant cultivars CDC Alma and CDC Cory was not affected for any of the IMI treatments.

Internode length increased slightly from 1.3 cm per node to 1.7 cm per node on average over the 28 days of measurements for the untreated controls (Figure 4.6). Average internode length decreased slightly at 14 DAA on CDC Luna and CDC Corinne and remained constant until 21DAA. CDC Alma and CDC Cory showed no change in internode length between the control and all the IMI treatments.

Leaf greenness, measured using the SPAD-502DL Plus meter, was not consistent across site years. In 2012 at Saskatoon there was no significant effect of cultivar ($p=0.126$), herbicide ($p=0.216$), nor the interaction ($p=0.412$) for level of greenness. Despite significant effects from all factors in Saskatoon 2013 (Figure 4.7), Elrose 2012 and Elrose 2013, the fluctuation in response from all cultivars displayed no obvious trend for any treatment.

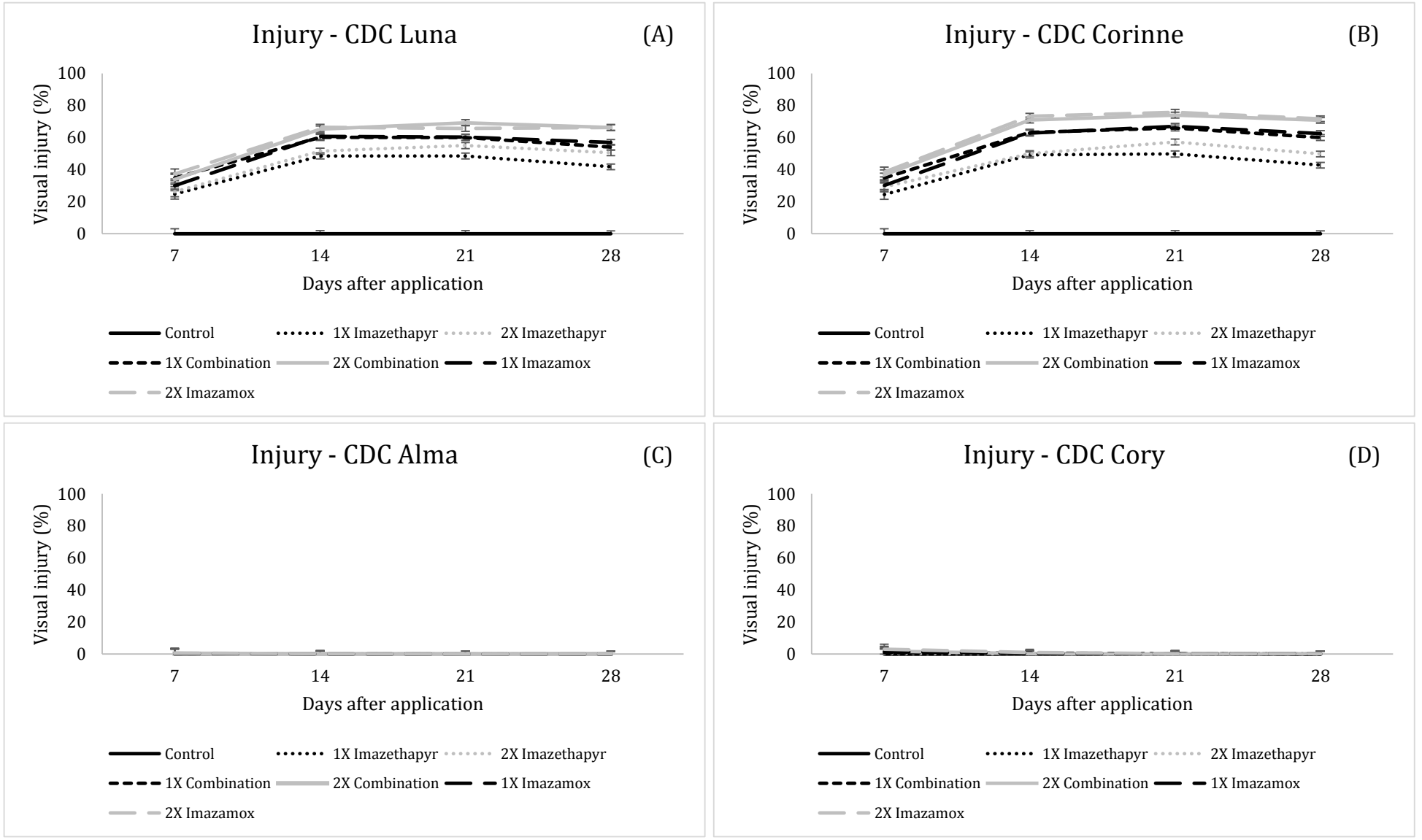


Figure 4.3 - Visual injury scores from all site-years combined for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d), over 7 day intervals after application. Visual injury was based on the whole plot using a 0-100 scale. There was a significant interaction of herbicide and cultivar (P<0.0001).

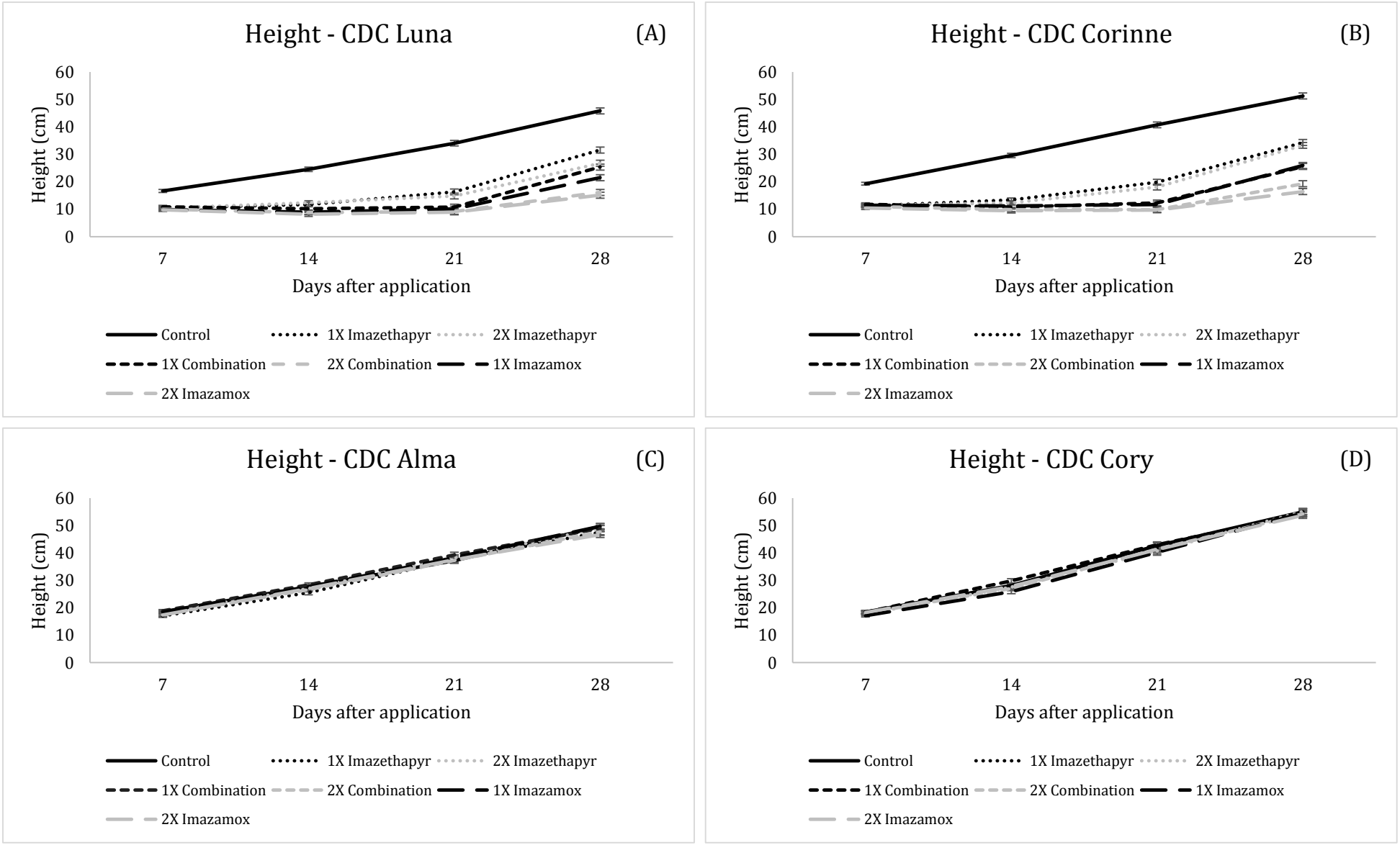


Figure 4.4 – Height measurements from a representative site year Saskatoon 2012, from CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d) of 7 day intervals after application. Height was significantly affect by the interaction of herbicide and cultivar ($p<0.0001$).

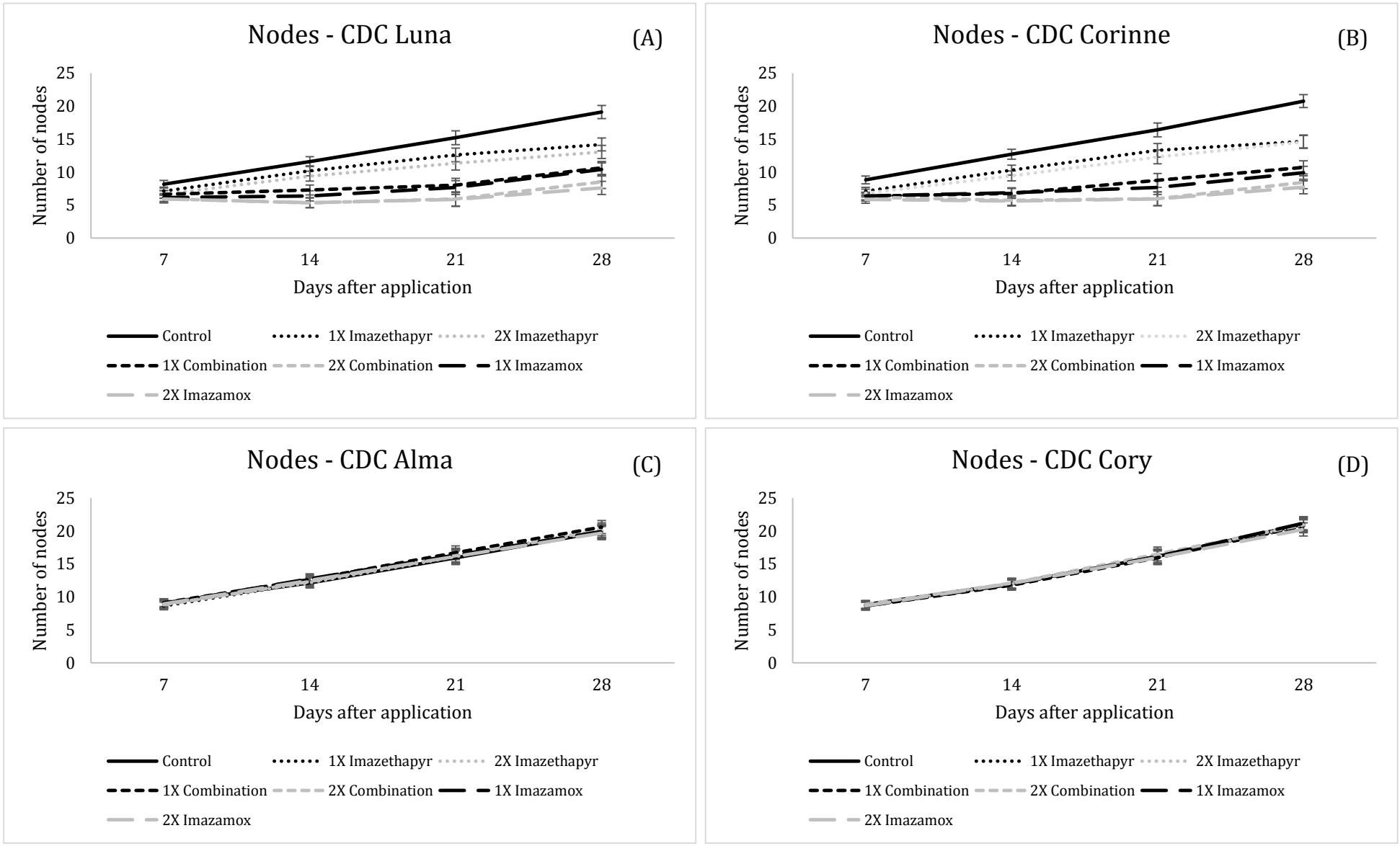


Figure 4.5 – Saskatoon 2012 representing general node developmental trends for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d) over 7 day intervals after application. The interaction of herbicide and cultivar was significant ($p<0.0001$).

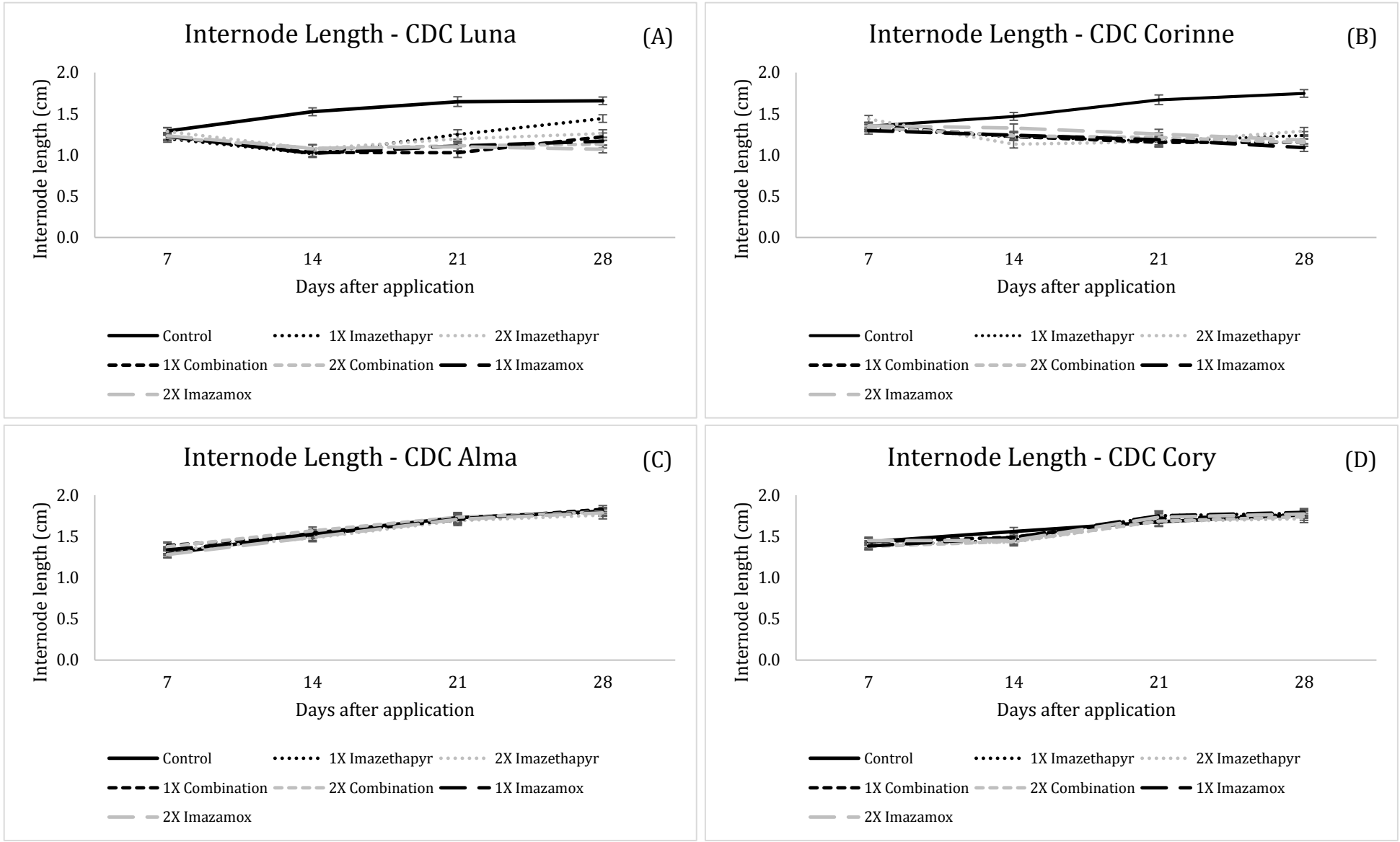


Figure 4.6 – Combined analysis of internode length at 7 day intervals after application for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d), at Elrose 2012 and 2013. Internode length was significantly affected by the interaction of herbicide and cultivar ($p<0.0001$).

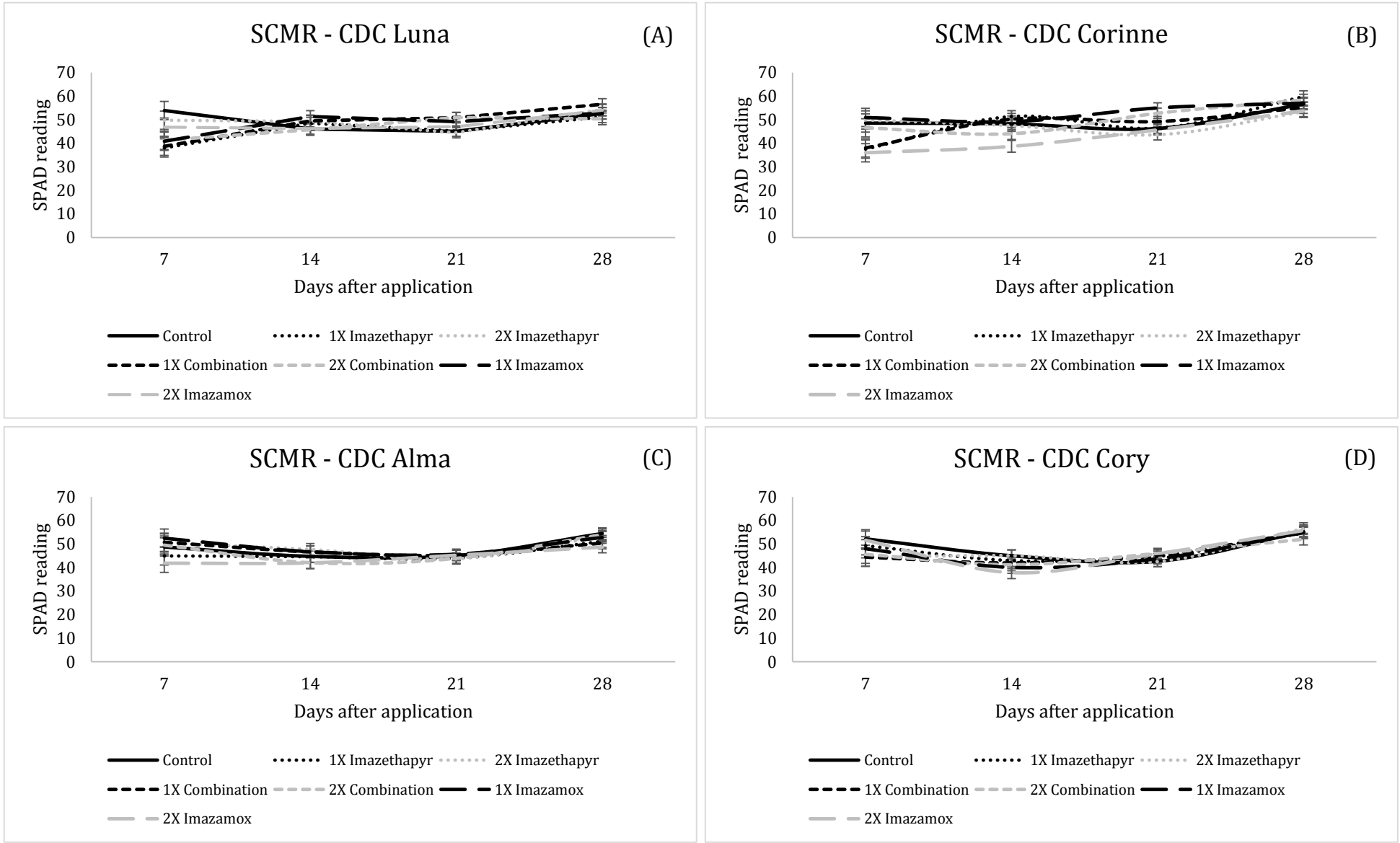


Figure 4.7 – SPAD chlorophyll meter readings from Saskatoon 2013 for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d) at 7 day intervals after application. The interaction of herbicide by cultivar by day was significant (p=0.004).

4.2.3 Harvest Measurements

Days to flowering (DTF) were significantly affected by herbicide, cultivar, and their interaction at Elrose and Saskatoon in 2012 and 2013 (Table 4.2). CDC Luna and CDC Corinne experienced a delay in DTF with all herbicide treatments compared to the controls (Table 4.3). Imazamox treatments caused the most severe delay in DTF on these two cultivars, followed by the combination of imazamox + imazethapyr. For example, at Elrose in 2012 and 2013, CDC Luna and CDC Corinne treated with 2x imazamox flowered on average 20.5 and 18.9 days later than their varietal control. In contrast, CDC Alma and CDC Cory did not differ in DTF from any herbicide treatments compared to the control. On average, CDC Alma flowered 52, 49 and 56 days after sowing (DAS) in Saskatoon 2012, Saskatoon 2013, and Elrose 2012 + 2013 combined, respectively. CDC Cory flowered 55, 49 and 64 DAS in Saskatoon 2012, Saskatoon 2013, and Elrose 2012 + 2013 combined, respectively. Moose Jaw in 2013 only showed significant differences between cultivars for DTF (Table 4.2). Susceptible cultivars, CDC Luna and CDC Corinne, flowered later than resistant cultivars, CDC Alma and CDC Cory across all herbicide treatments.

Saskatoon plots failed to reach maturity in 2012; therefore days to maturity (DTM) for this site year were excluded from further analysis. Elrose 2012, 2013 and Moose Jaw 2013 demonstrated a significant effect due to the interaction between herbicide and cultivar (Table 4.2). Although there were minor fluctuations in the DTM for all cultivars, statistically CDC Luna, CDC Alma, and CDC Cory did not change significantly after the herbicide treatments (Table 4.3). Imazamox applied to CDC Corinne, however, caused a prominent delay in maturity. In Saskatoon 2013, DTM

were only affected by cultivars. Desi cultivars, CDC Cory and CDC Corinne, tended to mature sooner than kabuli cultivars.

Table 4.2 - P values from mixed model analyses investigating the effects of herbicide and cultivar on days to flowering (DTF) and days to maturity (DTM) at all site years.

	Saskatoon 2012		Saskatoon 2013		Elrose 2012		Elrose 2013		Moose Jaw 2013	
	DTF	DTM	DTF	DTM	DTF	DTM	DTF	DTM	DTF	DTM
Herbicide	<0.0001	n/a	<0.0001	0.7833	0.0005	0.3577	0.0005	0.1983	0.2512	0.6070
Cultivar	<0.0001	n/a	<0.0001	<0.0001	<0.0001	0.0396	<0.0001	<0.0001	<0.0001	<0.0001
H x C	<0.0001	n/a	<0.0001	0.7425	0.0166	0.0368	0.0166	0.0034	0.211	0.0258

n/a – information not available due to adverse conditions preventing maturity

Final plant height was measured at maturity, and an ANOVA was performed for Saskatoon and Elrose locations. Variance was heterogeneous for all site years combined (Appendix 1), however further analysis demonstrated homogeneous variance between 2012 and 2013 in Saskatoon, additionally between 2012 and 2013 in Elrose, therefore years were combined for analysis. There was no interaction effect of herbicide, cultivar, location, and year, nor herbicide and cultivar, nor did the herbicide alone affect the final height in Elrose (Table 4.4). Height differences only existed among cultivars, demonstrating desi cultivars as taller than kabuli cultivars under the conditions at Elrose. In Saskatoon, height at maturity was affected by herbicide and cultivar, but not their interaction. CDC Luna and CDC Corinne had final heights shorter than CDC Alma and CDC Cory. Both imazamox and the combination imazamox + imazethapyr decreased final plant height in Saskatoon.

Final node measurements were also measured and homogenous variance between 2012 and 2013 in Saskatoon, as well as 2012 and 2013 in Elrose allowed for combined analysis. In Saskatoon, herbicide and cultivar were statistically significant at $p \leq 0.01$, however the interaction only caused difference in the number

Table 4.3 –Effects of IMI herbicide on the days to flowering (DTF) and days to maturity (DTM) of four chickpea cultivars at all site years.

	CDC Luna		CDC Alma		CDC Cory		CDC Corinne	
	DTF	DTM	DTF	DTM	DTF	DTM	DTF	DTM
Saskatoon 2012								
Control	51.8	.	51.8	.	55.2	.	55.0	.
1X imazethapyr	68.0	.	52.0	.	55.8	.	72.8	.
2X imazethapyr	72.5	.	52.2	.	55.2	.	73.2	.
1X combination	77.5	.	51.8	.	55.0	.	77.5	.
2X combination	80.2	.	51.8	.	55.5	.	82.2	.
1X imazamox	76.5	.	52.0	.	55.8	.	76.1	.
2X imazamox	86.8	.	55.0	.	55.0	.	87.8	.
DTF LSD (0.05)	8.3							
Saskatoon 2013								
Control	49.0	118.3	49.0	119.8	49.3	115.3	49.3	113.8
1X imazethapyr	60.3	118.0	49.5	117.8	50.5	113.8	60.0	115.3
2X imazethapyr	64.0	120.8	49.5	122.0	49.3	110.5	61.8	114.8
1X combination	63.8	120.3	49.5	122.5	48.3	116.3	68.5	119.3
2X combination	71.5	117.8	48.5	119.0	48.3	110.5	74.0	120.8
1X imazamox	63.8	122.0	49.3	120.0	49.0	112.3	71.3	116.0
2X imazamox	71.8	119.5	48.8	117.8	49.8	113.5	72.5	117.0
DTF LSD (0.05)	4.2							
DTM LSD (0.05)	17.6							
Elrose 2012								
Control	66.2	126.0	64.0	127.8	70.5	125.8	73.8	125.2
1X imazethapyr	77.5	126.0	67.2	126.5	70.5	126.2	77.0	125.0
2X imazethapyr	79.2	126.8	63.2	127.2	71.8	125.5	78.0	126.0
1X combination	80.5	127.8	64.0	125.5	73.0	127.2	85.2	128.2
2X combination	88.0	126.2	62.2	126.5	71.5	126.2	89.8	128.0
1X imazamox	82.2	126.5	64.0	126.8	72.5	125.8	88.0	128.0
2X imazamox	90.8	127.8	62.5	127.2	71.0	124.2	95.0	130.0
DTF LSD (0.05)	9.2							
DTM LSD (0.05)	5.0							
Elrose 2013								
Control	50.3	116.0	51.8	112.5	55.3	111.0	55.5	106.5
1X imazethapyr	60.5	114.8	50.5	114.8	55.5	109.0	61.8	111.3
2X imazethapyr	67.0	121.5	49.3	116.8	56.0	112.0	69.5	118.8
1X combination	61.5	119.0	48.8	117.0	54.5	108.5	70.8	114.8
2X combination	70.5	120.5	48.8	116.8	55.3	107.3	77.0	119.3
1X imazamox	64.3	119.0	49.3	115.3	56.0	111.0	72.5	119.3
2X imazamox	66.8	119.5	49.5	115.0	55.3	109.3	72.0	119.3
DTF LSD (0.05)	7.4							
DTM LSD (0.05)	12.0							
Moose Jaw 2013								
Control	53.5	127.8	46.5	129.0	54.0	119.8	55.5	117.5
1X imazethapyr	55.0	127.8	45.5	128.8	51.5	119.5	59.0	119.0
2X imazethapyr	57.3	128.0	48.0	128.5	54.3	119.8	66.3	123.8
1X combination	55.3	128.0	44.5	129.3	51.5	116.5	64.8	123.0
2X combination	57.5	129.0	46.3	127.5	51.0	119.3	65.0	123.0
1X imazamox	55.3	127.3	48.0	128.0	52.5	120.8	61.5	120.3
2X imazamox	58.3	127.3	44.3	128.0	53.8	117.0	64.3	123.3
DTF LSD (0.05)	12.7							
DTM LSD (0.05)	6.6							

of nodes at $p \leq 0.10$ significance level. Node number remained unaltered for CDC Alma and CDC Cory for all herbicide treatments. CDC Luna and CDC Corinne experienced decreased node number for all herbicide treatments compared to the respective controls. Number of nodes from Elrose 2012 and 2013 was significantly affected by herbicide, cultivar, and the interaction of herbicide and cultivar. Similar to Saskatoon, the number of nodes were fewer on CDC Luna and CDC Corinne for all herbicide treatments. Although the number of nodes were fairly constant for CDC Alma and CDC Cory, imazamox at the 1x rate caused a slight increase in nodes on CDC Cory.

Both 2012 and 2013 for Saskatoon, and 2012 and 2013 for Elrose could be analyzed in combination for final internode measurements. For Saskatoon, internode length was only affected by cultivar. The ascending cultivar order for internode length was CDC Luna, CDC Alma, CDC Cory, and then CDC Corinne. Different from Saskatoon, CDC Luna had the second longest internode length next to CDC Corinne at Elrose. The interaction of herbicide and cultivar significantly affected internode length at Elrose as well. For both CDC Luna and CDC Corinne, internode length was extended with treatments of imazamox and the combination imazamox + imazethapyr at both the 1x and 2x rates.

The number of primary branches at maturity followed a similar trend for both locations and years and can therefore be analyzed together. The only significant factor influencing branching was cultivar (Table 4.4). CDC Luna and CDC Corinne had slightly more primary branches than CDC Alma and CDC Cory.

At both locations with combined years, dry weight at maturity varied depending on cultivar (Table 4.4). Using Elrose 2012 and 2013 as an example, dry weight was slightly higher for CDC Alma (21.3 g) and CDC Cory (21.7 g) compared to CDC Luna (17.4 g) and CDC Corinne (18.5 g).

Table 4.4 – P values from mixed model analyses of the effects of herbicide and cultivar on plant height, number of branches, dry weight, seed per plant, and seed per pod from 6 sampled plants per plot at Elrose and Saskatoon in 2012 and 2013.

	Height	Branching	Dry weight	Seed weight	Pods/plant	Seed/plant	Seed/pod
Saskatoon 2012							
Herbicide	0.0266	0.2690	0.0757	0.0012	0.0490	0.0002	0.0031
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
H x C	0.2326	0.4550	0.1099	<0.0001	0.0771	<0.0001	<0.0001
Saskatoon 2013							
Herbicide	0.0266	0.2690	0.0757	0.0726	0.0571	0.0323	0.9453
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001
H x C	0.2326	0.4550	0.1099	0.1139	0.1535	0.0460	0.6788
Elrose 2012							
Herbicide	0.7004	0.2690	0.3921	0.6817	0.5606	0.5609	0.8036
Cultivar	<0.0001	<0.0001	<0.0001	0.0566	0.0068	<0.0001	<0.0001
H x C	0.9998	0.4550	0.2328	0.7292	0.5791	0.7698	0.0645
Elrose 2013							
Herbicide	0.7004	0.2690	0.3921	0.0425	0.0320	0.2359	0.9531
Cultivar	<0.0001	<0.0001	<0.0001	0.5693	<0.0001	0.8571	0.0020
H x C	0.9998	0.4550	0.2328	0.9072	0.1158	0.7853	0.7175

The results of seed characteristics varied between year and location. Saskatoon plots in 2012 were significantly affected by herbicide, cultivar and the interaction for seeds per plant and the seed to pod ratio (Table 4.4). The number of pods per plant was the only seed characteristic that was not affected by the interaction. The two kabuli cultivars, CDC Luna and CDC Alma, had lower values than desi cultivars for all seed characteristics across all treatments. Compared to the control, CDC Corinne experienced a decrease for all parameters across all herbicide

treatments but saw the most dramatic reductions with the 2x rate of imazamox. Compared to the control, seeds per plant decreased by 78.1 seeds (Table 4.5) and the ratio of seeds per pod reduced from 1.16 to 0.29 seed/pod (Table 4.6).

Table 4.5 – The number of seed per plant for all four chickpea cultivars treated with IMI herbicide at Saskatoon in 2012.

	Number of seeds per plant			
	CDC Luna	CDC Corinne	CDC Alma	CDC Cory
Control	2.5	89.9	1.7	56.7
1X imazethapyr	0.6	55.5	1.1	62.6
2X imazethapyr	2.5	37.0	3.1	56.7
1X combination	0.5	25.9	4.4	31.8
2X combination	0.6	16.8	0.7	71.4
1X imazamox	0.6	31.1	1.8	56.7
2X imazamox	0.5	11.8	1.7	41.4
LSD (0.05)	16.0			

Table 4.6 – The ratio of seeds per pod of all four chickpea cultivars treated with IMI herbicide at Saskatoon in 2012.

	Ratio of seeds per pod			
	CDC Luna	CDC Corinne	CDC Alma	CDC Cory
Control	0.09	1.16	0.05	0.90
1X imazethapyr	0.03	0.88	0.04	0.82
2X imazethapyr	0.02	0.85	0.07	0.97
1X combination	0.03	0.69	0.11	0.64
2X combination	0.03	0.45	0.03	0.97
1X imazamox	0.04	0.58	0.07	0.93
2X imazamox	0.01	0.29	0.06	0.76
LSD (0.05)	0.18			

The interaction between herbicide and cultivar was not significant for Saskatoon 2013, Elrose 2012 nor Elrose 2013 for any seed characteristic except seeds per plant in Saskatoon 2013 (Table 4.4). In most cases, cultivar was the only factor significantly influencing seed characteristics.

4.2.4 Yield Results

Due to snowfall before harvest at Saskatoon plots in 2012, the plants were not harvested, resulting in no yield data from Saskatoon 2012. For the rest of the site years, except for Elrose 2013, yield was not affected by herbicide applications but only cultivar differences were observed (Table 4.7). CDC Cory was the highest yielding cultivar in Saskatoon 2013 (4368 kg/ha), Elrose 2012 (2501 kg/ha), and Moose Jaw 2013 (3384 kg/ha). Susceptible cultivars CDC Luna and CDC Corinne had the lowest yields in Elrose 2012 and Saskatoon 2013 (Figure 4.8). Herbicide treatment affected cultivar yields in Elrose 2013. A yield reduction was observed for susceptible cultivars, CDC Luna and CDC Corinne for 2x treatments of imazethapyr, imazamox, and the combination imazethapyr + imazamox as compared to the control. On the other hand, CDC Alma and CDC Cory experienced an increase in yield for all treatments except for the combination imazethapyr + imazamox at 2x rate. CDC Cory yield increased from 3026 kg/ha in the control treatment to as high as 3979 kg/ha with the 2x imazamox treatment (Figure 4.8).

Table 4.7 - P values from mixed model analyses of yield, adjusted yield with green seed removed, 1000 seed weight and harvest index (H.I.) for all site years.

	Yield	Adjusted yield	1000 seed weight	H.I.
Saskatoon 2012				
Herbicide	n/a	n/a	0.6902	0.0009
Cultivar	n/a	n/a	<0.0001	<0.0001
Herbicide x Cultivar	n/a	n/a	0.7154	<0.0001
Saskatoon 2013				
Herbicide	0.0009	0.0399	0.6902	0.7793
Cultivar	<0.0001	<0.0001	<0.0001	0.0128
Herbicide x Cultivar	0.4794	0.6127	0.7154	0.5360
Elrose 2012				
Herbicide	0.5080	0.4270	0.0351	0.2472
Cultivar	<0.0001	<0.0001	<0.0001	0.0003
Herbicide x Cultivar	0.7563	0.6340	0.2304	0.8280
Elrose 2013				
Herbicide	0.1831	0.2869	0.6902	0.8305
Cultivar	<0.0001	0.0002	<0.0001	0.0459
Herbicide x Cultivar	0.0006	0.4055	0.7154	0.4194
Moose Jaw 2013				
Herbicide	0.7104	0.7318	0.8069	n/a
Cultivar	<0.0001	<0.0001	<.0001	n/a
Herbicide x Cultivar	0.3438	0.3373	0.1263	n/a

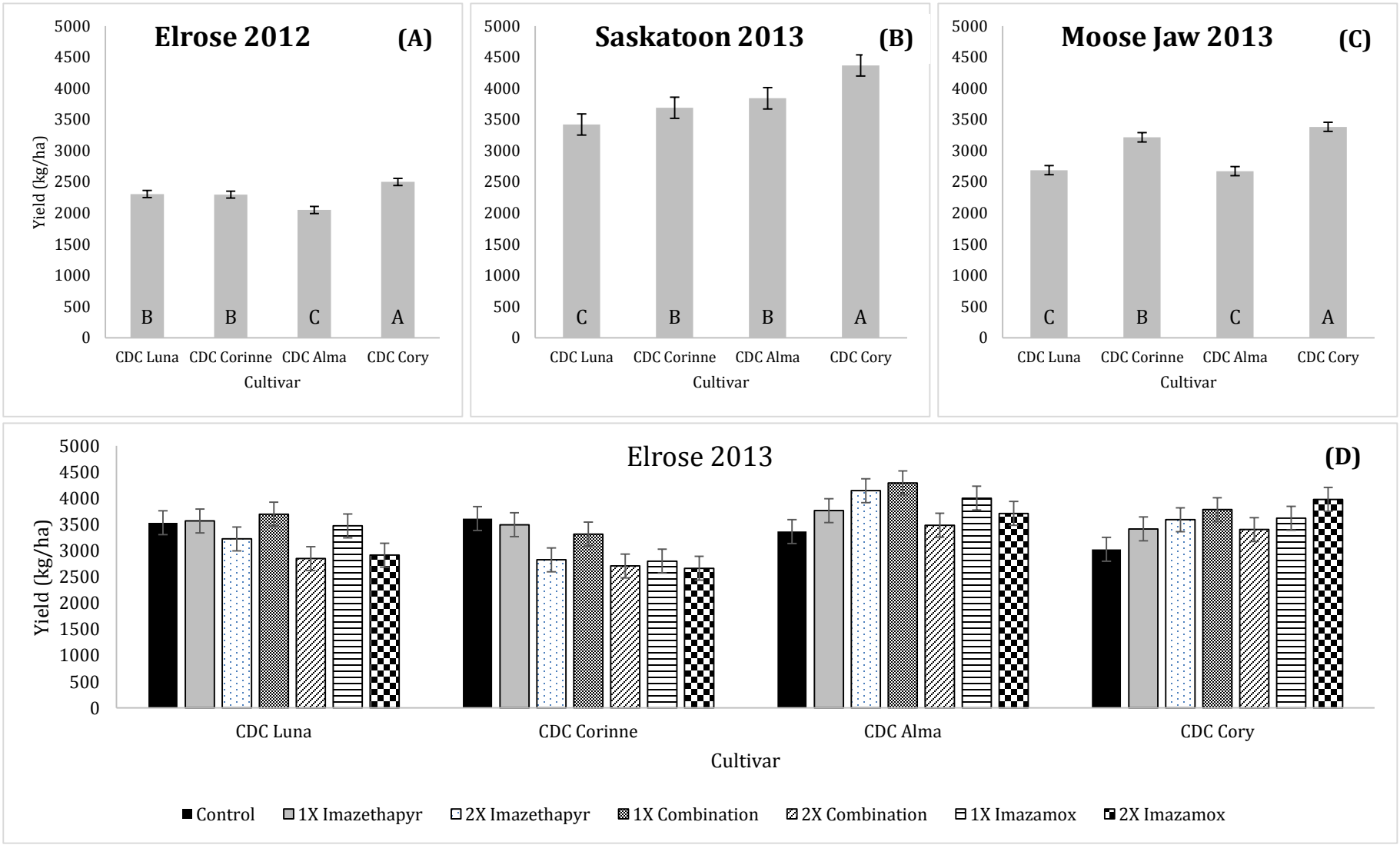


Figure 4.8 – Average yield of four chickpea cultivars across IMI herbicide treatments at Elrose 2012 (a), Saskatoon 2013 (b), Moose Jaw 2013 (c), and Elrose 2013 (d). Cultivar was the only significant factor effecting yields in Elrose 2012 (P<0.0001), Saskatoon 2013 (P<0.0001) and Moose Jaw 2013 (P<0.0001). The interaction of cultivar and herbicide was significant in Elrose 2013 (P=0.0006).

In all site years 1000 seed weights were only influenced by cultivar (Table 4.7). CDC Alma had the highest seed weight at 340 g/1000 seeds followed by CDC Luna (329 g/1000 seeds), CDC Cory (234 g/1000 seeds), and CDC Corinne (211 g/1000 seeds) across Saskatoon, Elrose and Moose Jaw in 2013. Similar trend for seed weight also occurred across locations in 2012 (Table 4.8).

Table 4.8 – 1000 seed weight (g) at all 2013 site years (Saskatoon, Elrose and Moose Jaw) combined, compared to 2012 site year (Elrose only). Saskatoon 2012 was not harvested, therefore 1000 seed weight data is unavailable.

	1000 seed weight (g)	
	2013	2012
CDC Luna	329	322
CDC Corinne	211	232
CDC Alma	341	331
CDC Cory	234	262
2013 LSD (0.05)	14.0	
2012 LSD (0.05)	20.9	

Finally, locational effects were revealed for harvest index measurements. In Saskatoon 2012 and 2013 harvest index was highest for CDC Cory and lowest for CDC Luna. In contrast, Elrose 2013 displayed the reverse trend of CDC Luna with the highest harvest index and CDC Cory with the lowest (Table 4.9).

Table 4.9 – Comparison of harvest index between Saskatoon 2012 and 2013 and Elrose 2012 and 2013.

	Harvest Index			
	Saskatoon		Elrose	
	2012	2013	2012	2013
CDC Luna	0.01	0.38	0.42	0.55
CDC Corinne	0.14	0.41	0.43	0.53
CDC Alma	0.01	0.42	0.39	0.47
CDC Cory	0.24	0.45	0.42	0.44
2012 LSD (0.05)	0.18			
2013 LSD (0.05)	0.14			

4.3 Research Component 2 – Timing of IMI Application

4.3.1 Repeated Measures

Repeated measures analysis of variance demonstrated significant effects of herbicide and cultivar for visual injury ratings throughout the season. A Levene's test demonstrated homogeneous variance for visual injury at each interval between site years, however there was an interaction of location, year, cultivar, and herbicide (Appendix 2). In Saskatoon 2012 the 2x 2-4 node stage application caused the most prolonged injury on CDC Luna and CDC Corinne (Figure 4.9). In comparison, both 1x and 2x rates applied at the 5-8 node stage in Elrose 2012 had the highest level and most prolonged injury (Figure 4.10). Despite minor site year differences, all timings of IMI applications on CDC Luna and CDC Corinne produced unacceptable injury signified by a score above 10%. CDC Alma and CDC Cory, on the other hand, remained relatively unaffected through all growth stages of IMI application. Both cultivars demonstrated strong IMI resistance.

Repeated height measurements gave an indication of how growth was affected by the timing of herbicide application. Control treatments for all cultivars presented a steady increase in height over time (Figure 4.11). Comparatively, all timings of herbicide application on CDC Luna and CDC Corinne arrested vertical growth until 21 DAA. Height started to increase again at 28 DAA for all treatments at all locations. Height for resistant cultivars, CDC Alma and CDC Cory, was unaffected by all treatments, seen through continual height increases parallel to controls.

Node measurements over time followed a similar pattern as to height measurements. All cultivars under the control treatment had a steady increase in the number of nodes over time (Figure 4.12). Node development for resistant cultivars CDC Alma and CDC Cory did not deviate from the development pattern of the control for any of the IMI herbicide timings. CDC Luna and CDC Corinne, however, experienced a decreased rate of node development until 14 DAA for all timing treatments. Nodes steadily increased again at 21 DAA.

Although internode length results were slightly irregular, they corresponded to height and node relationships. For control treatments, internode length increased marginally over time (Figure 4.13). After herbicide treatment, internode length remained constant, or somewhat decreased for CDC Luna and CDC Corinne. Internode length of CDC Alma and CDC Cory for any IMI treatment did not digress from the control.

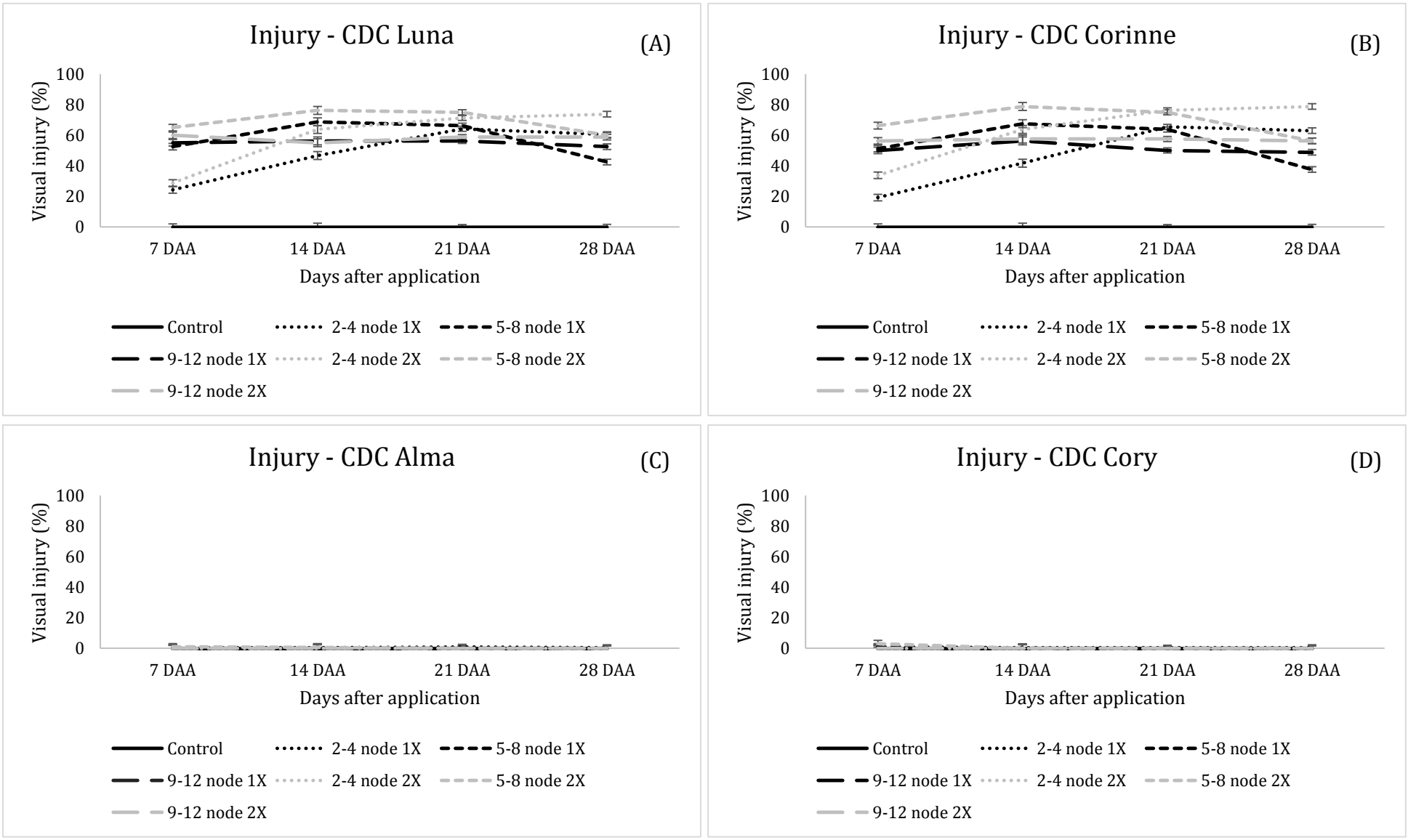


Figure 4.9 – Visual injury scores from Saskatoon 2012 for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d) at each growth stage of IMI application over 7 day intervals after application. Visual injury was based on the whole plot using a 0-100 scale. There was a significant interaction effect of herbicide and cultivar (P<0.0001).

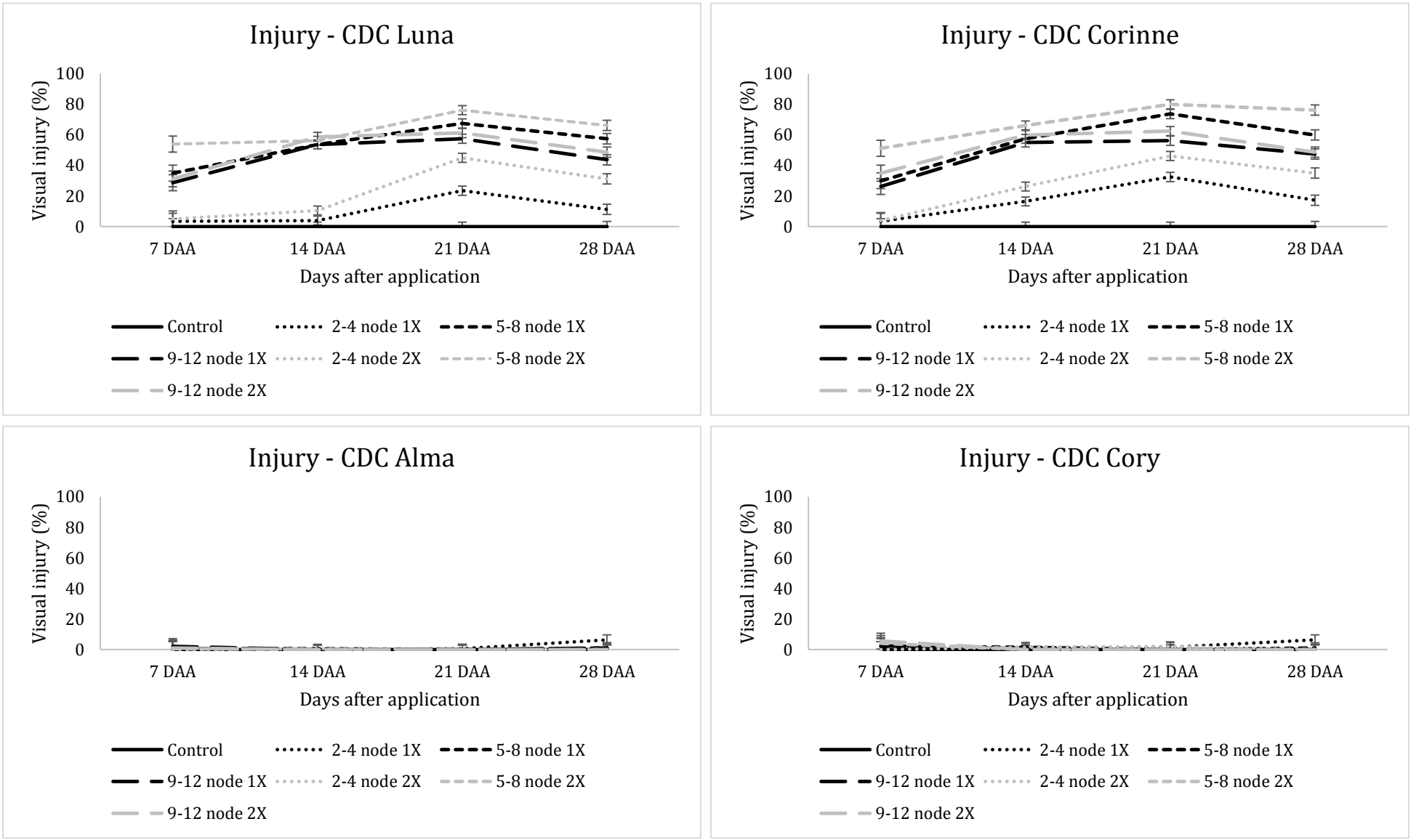


Figure 4.10 - Visual injury scores from Elrose 2012 for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d) at each growth stage of IMI application over 7 day intervals after application. Visual injury was based on the whole plot using a 0-100 scale. There was a significant interaction effect of herbicide and cultivar (P<0.0001).

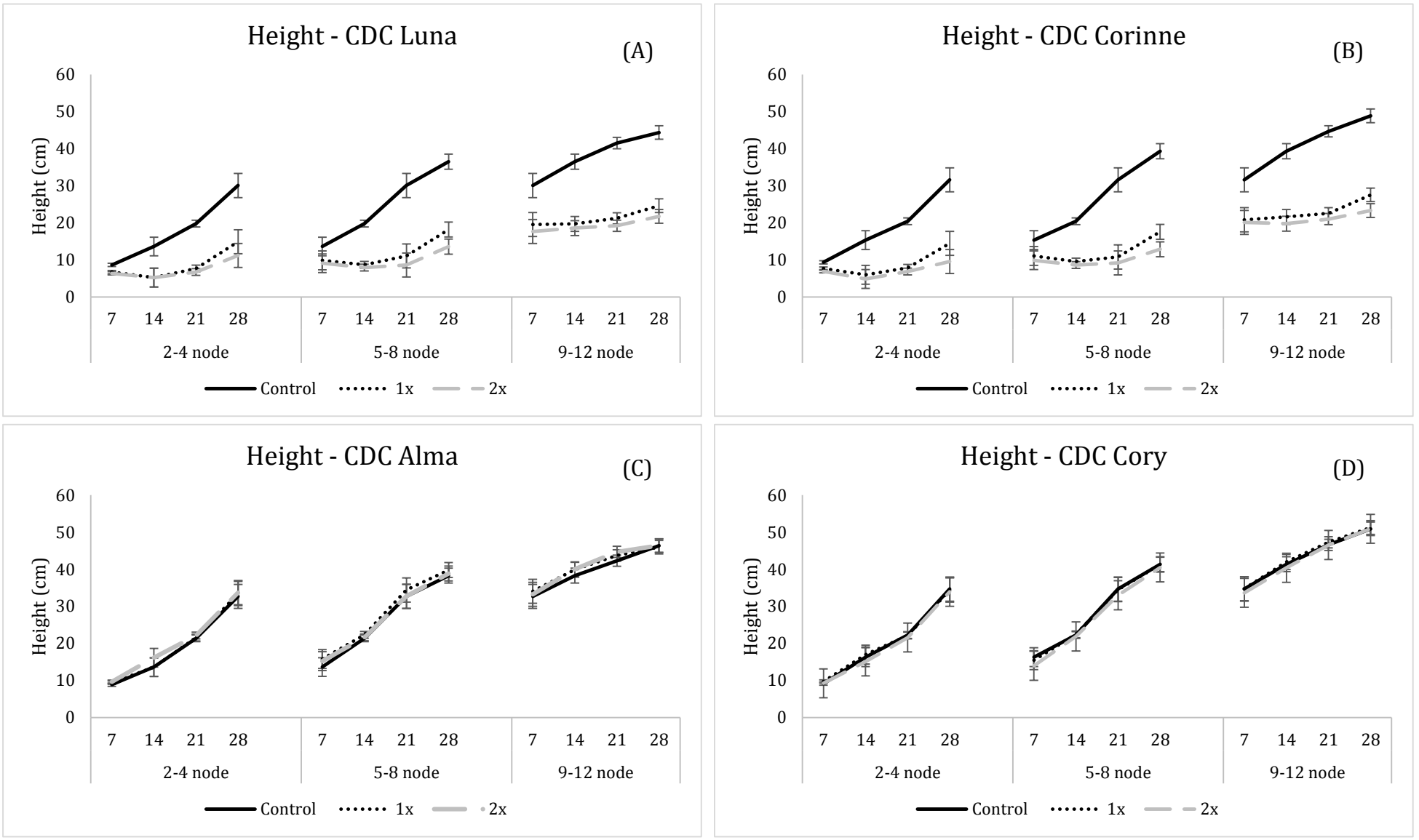
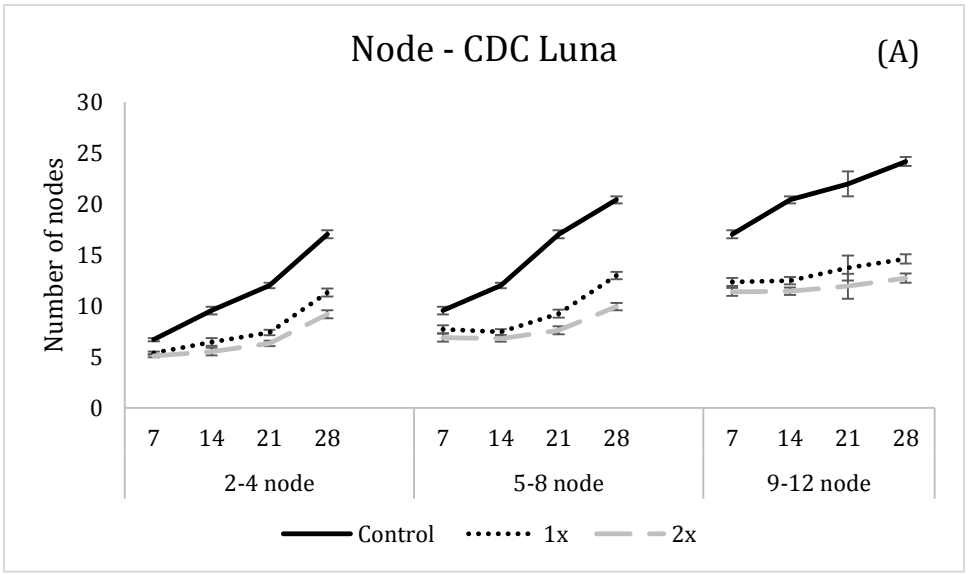
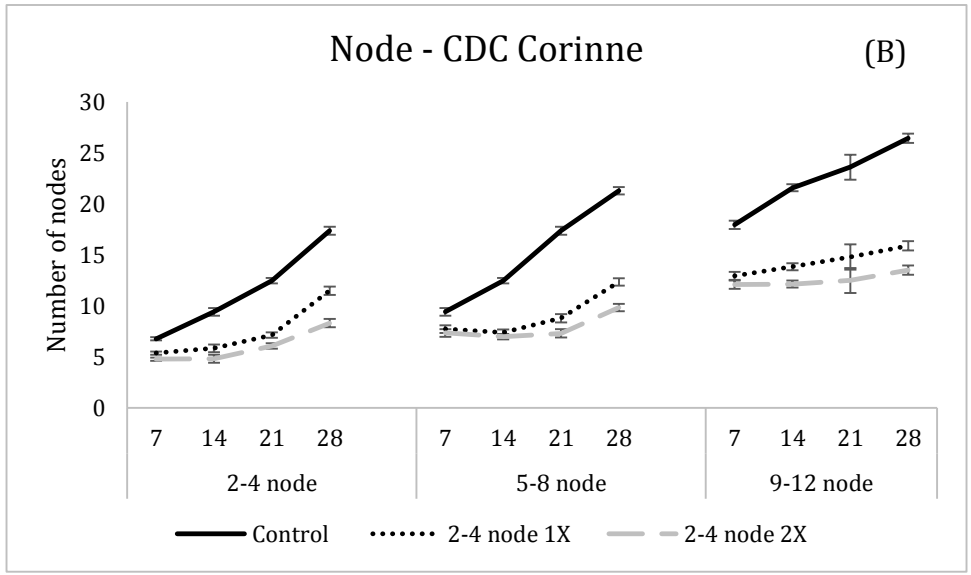
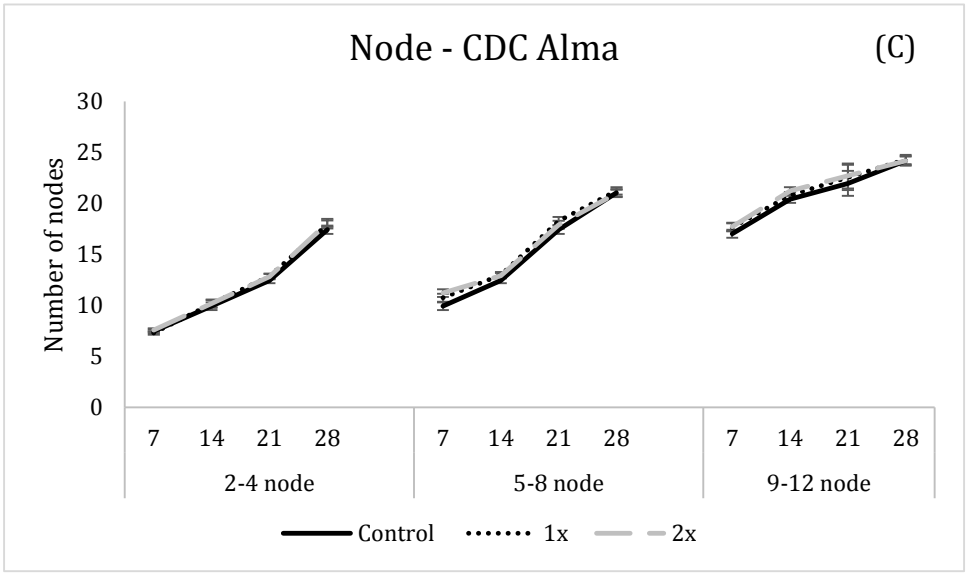


Figure 4.11 Figure – Repeated height measurements over 7 day intervals after herbicide application at different growth stages for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d) across Saskatoon and Elrose in 2013. The interaction of herbicide and cultivar over time intervals was significant (p=0.0399).







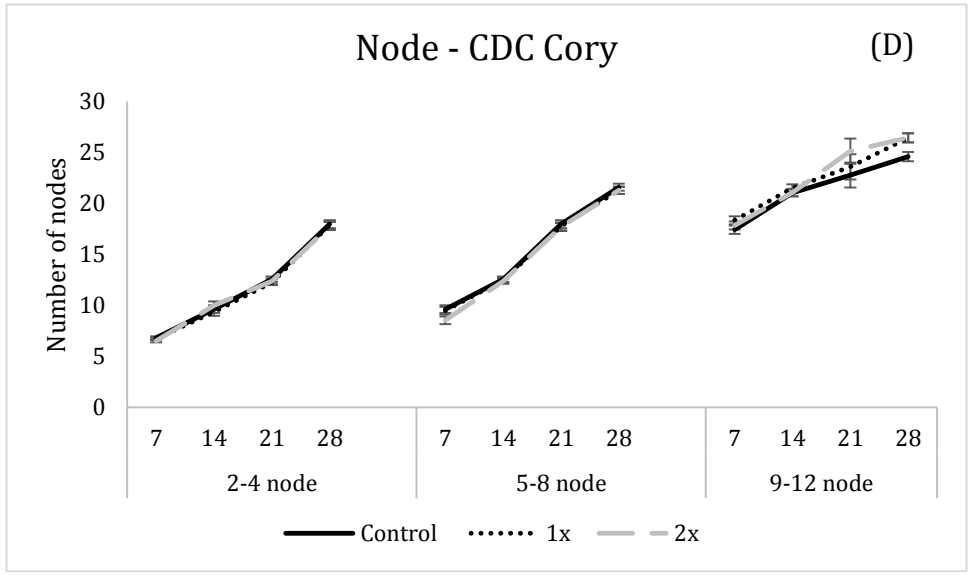
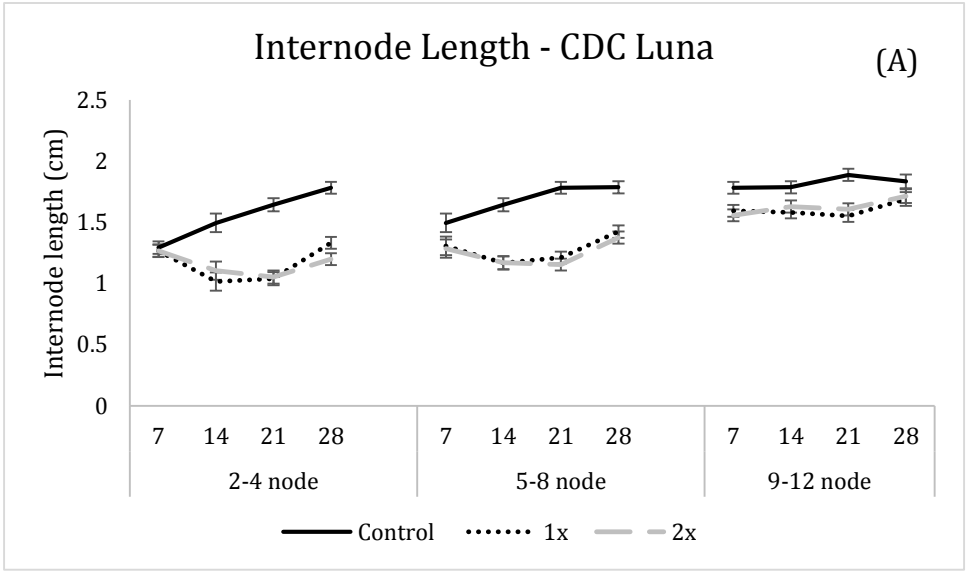
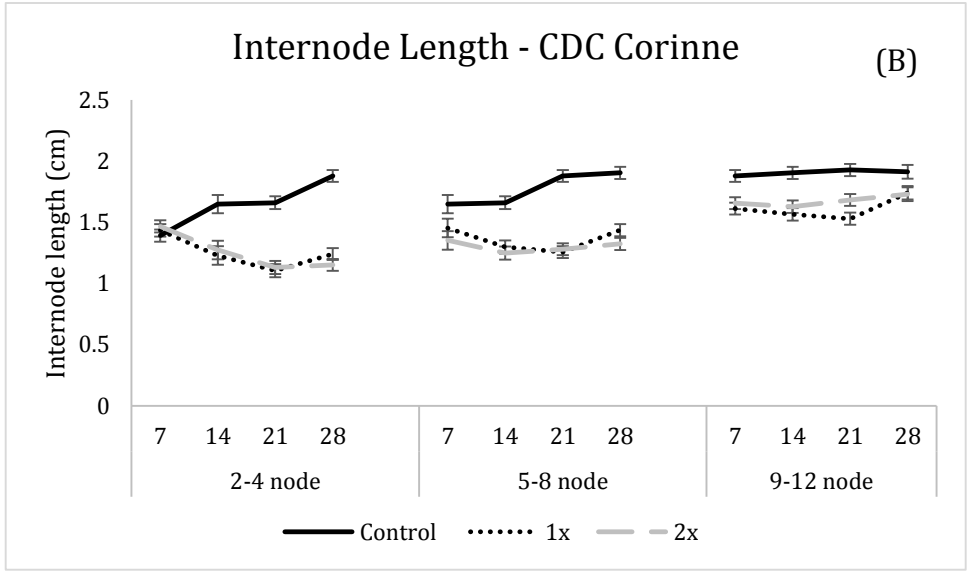
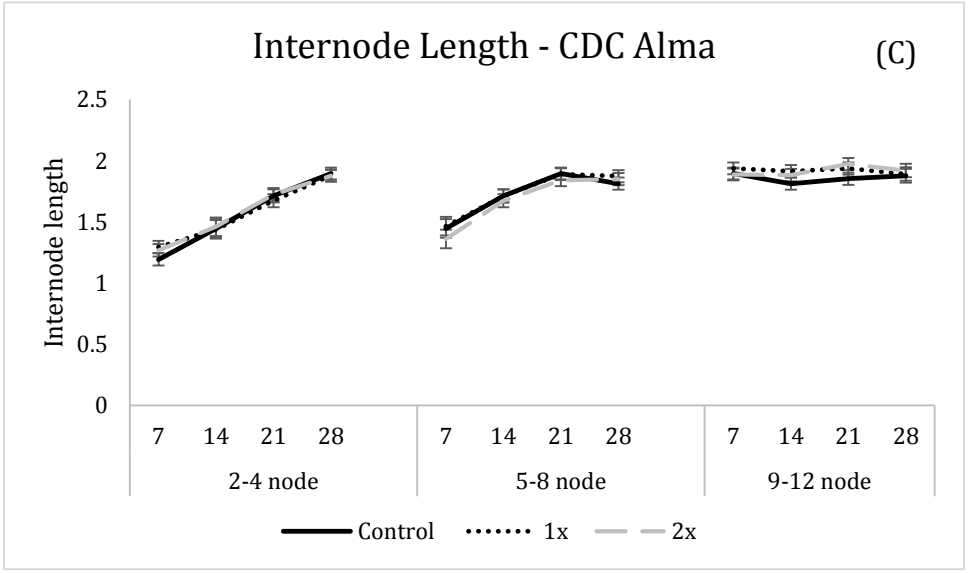


Figure 4.12 - Repeated node measurements over 7 day intervals after herbicide application at different growth stages for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d) across Saskatoon and Elrose in 2013. The interaction of herbicide and cultivar over time intervals was significant ($p<0.0001$).







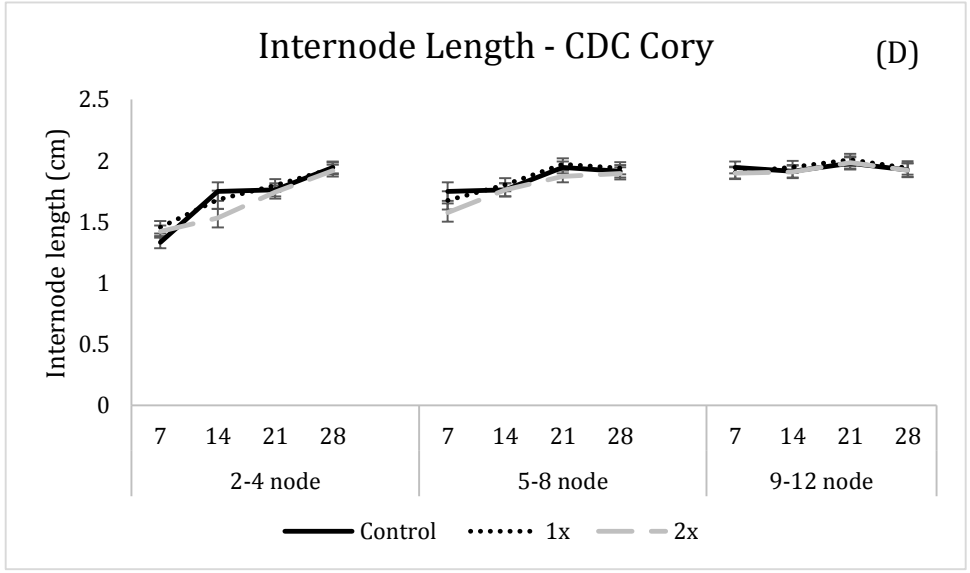


Figure 4.13 - Repeated internode length measurements over 7 day intervals after herbicide application at different growth stages for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d) across Saskatoon and Elrose in 2013. The interaction of herbicide and cultivar over time intervals was significant ($p<0.0001$).

Results for chlorophyll content based on SPAD chlorophyll meter readings (SCMR) showed that growth stage, cultivar and the interaction significantly affected SCMR across all site years. In Saskatoon 2012 (Figure 4.14) and Elrose 2012, SCMR readings were significantly lower at 7 and 14 DAA for CDC Luna and CDC Corinne. At 21 DAA, SCMR spiked dramatically to converge with control SCMRs. Measurements at 28 DAA were not significantly different from the control, except for the minor variation in CDC Corinne in Saskatoon 2012 under the 1x 2-4 node treatment.

Combined analysis of Saskatoon and Elrose 2013 demonstrated slightly different SCMRs (Figure 4.15). Different from 2012, the 1x and 2x at 2-4 node treatments were not significantly different from the control for any 7 day time interval. CDC Luna and CDC Corinne had significantly lower SCMRs under the 1x and 2x 9-12 node treatments at 7 and 14 DAA. At 21 and 28 DAA SCMRs were comparable to the control treatments. While the 5-8 node stage treatment caused no changes for CDC Luna in 2013, CDC Corinne experienced reduced SCMRs at 14 DAA and 21 DAA. Across all site years, CDC Alma and CDC Cory had only minor, if any, variation in SCMRs for all IMI treatments compared to the control.

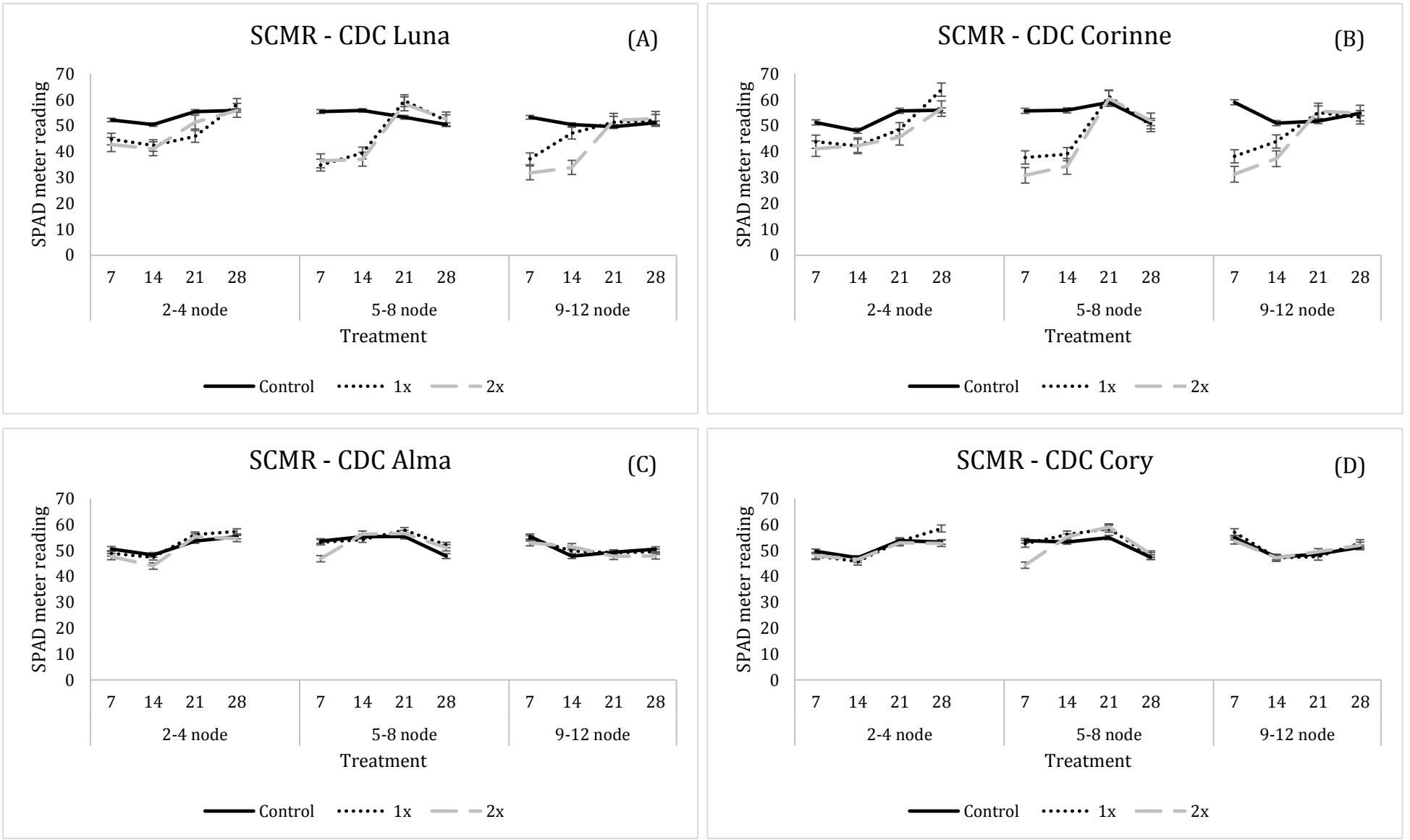


Figure 4.14 - Repeated SPAD meter readings at over 7 day intervals after herbicide application at different growth stages for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d) in Saskatoon 2012. The interaction of herbicide and cultivar over time was significant ($p<0.0001$).

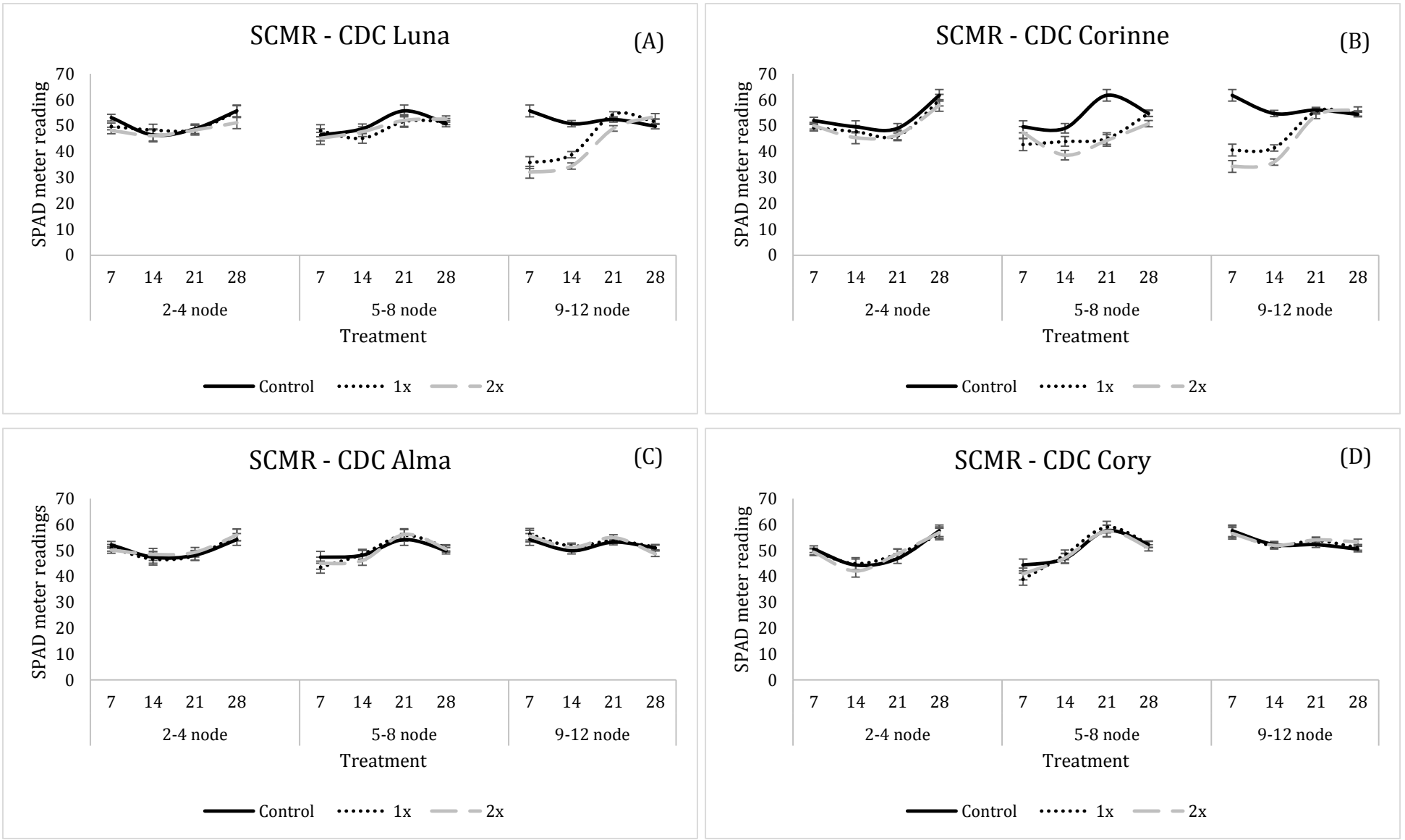


Figure 4.15 - Repeated SPAD meter readings over 7 day intervals after herbicide application at different growth stages for CDC Luna (a), CDC Corinne (b), CDC Alma (c), and CDC Cory (d) in Saskatoon 2013 and Elrose 2013 combined. The interaction of herbicide and cultivar over time was significant ($p<0.0001$).

4.3.2 Harvest Measurements

In all site years, the DTF were significantly affected by timing of herbicide application ($p<0.0001$), cultivar ($p<0.0001$), and the interaction ($p<0.0001$). CDC Luna and CDC Corinne experienced a delay in flowering from all timings of IMI application (Figure 4.16). The most drastic treatment was the 1x and 2x rate applied at the 9-12 node stage which delayed flowering by 32 and 38 days for CDC Luna and 30 and 34 days for CDC Corinne, respectively. The most tolerated treatment was the 1x rate applied at the 2-4 node stage, however the DTF were still delayed by 12 days for both susceptible cultivars. There was no difference in DTF for either CDC Alma or CDC Cory for any growth stage herbicide application compared to the controls.

Similar to DTF, DTM were delayed for both CDC Luna and CDC Corinne for all herbicide timings (Figure 4.17). The most extreme delay to maturity, 16 days, was seen with the 2x rate applied at the 9-12 node stage on CDC Corinne. CDC Alma and CDC Cory experienced no significant change of the DTM for any treatment.

Irrespective of herbicide timing, height and the number of nodes at maturity were only different among cultivars ($p<0.0001$). For all site years, desi cultivars were taller than kabuli cultivars, with the exception of Saskatoon 2012 where CDC Alma had comparable height. Following a slightly different trend, resistant cultivars CDC Alma and CDC Cory had significantly more nodes than susceptible cultivars.

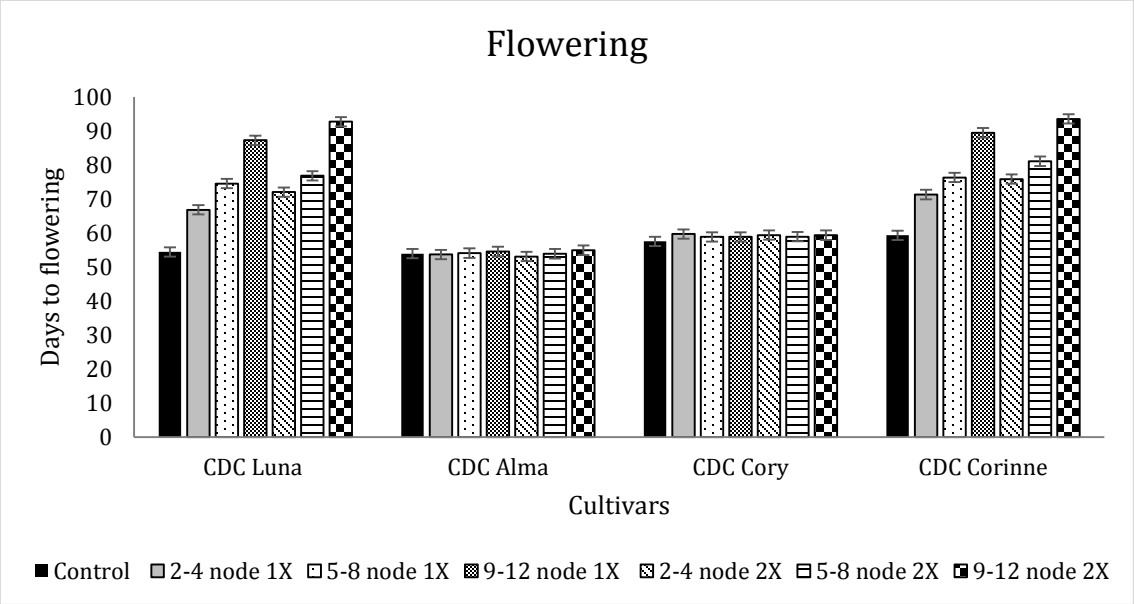


Figure 4.16 – Effects of herbicide timing of application on the number of days to flowering (DTF) for each cultivar across all site years. The herbicide timing by cultivar interaction was highly significant (p<0.0001).

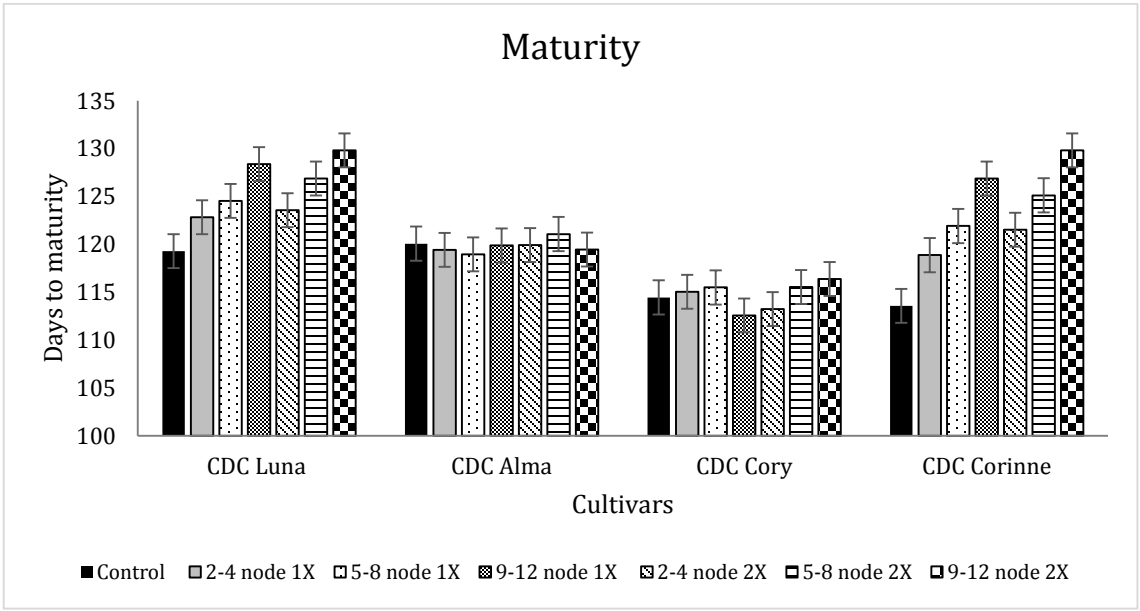


Figure 4.17 - Effects of herbicide timing of application on the number of days to maturity (DTM) for each cultivar across all site years. The herbicide timing by cultivar interaction was significant (p<0.0014).

Inconsistencies for internode length were seen across cultivars for the range of herbicide timings in all site years. There was no obvious trend for any cultivar or any treatment, but rather a random fluctuation in internode length across the board.

With all site years combined in analysis, the number of branches were significantly influenced by the herbicide and cultivar interaction (Table 4.10). For both CDC Luna and CDC Corinne, 1x and 2x IMI application at 9-12 nodes increased branching most significantly. Herbicide applied at the 2-4 node stage was most tolerated. In general, branching was unaffected in CDC Alma and CDC Cory cultivars. The one exception was observed with the 2x rate of IMI applied at the 5-8 node stage on CDC Cory. Branching was slightly increased compared to the control.

Table 4.10 - P values from mixed model analyses investigating height, branching, dry weight, seed weight, pods per plant, seeds per plant, and seeds per pod on chickpea cultivars treated with IMI herbicides applied at different growth stages, in Elrose and Saskatoon.

	Height	Branching	Dry wgt	seed wgt	pods/pl	seed/pl	seed/pod
Saskatoon 2012							
Herbicide	0.0243	<.0001	0.0239	.	0.4603	0.0279	0.0010
Cultivar	<.0001	<.0001	<.0001	.	0.2208	<.0001	<.0001
H x C	0.0708	<.0001	0.2127	.	0.0002	0.0010	<.0001
Saskatoon 2013							
Herbicide	0.6450	<.0001	0.0239	0.2731	0.6972	0.0636	0.3833
Cultivar	<.0001	<.0001	<.0001	<.0001	0.2840	<.0001	<.0001
H x C	0.1558	<.0001	0.2127	0.2597	0.7918	0.1695	0.4995
Elrose 2012							
Herbicide	0.1311	<.0001	0.0612	0.3662	0.2866	0.2620	0.7063
Cultivar	<.0001	<.0001	0.0001	0.7372	0.1904	<.0001	<.0001
H x C	0.0978	<.0001	0.9381	0.1585	0.9150	0.3334	0.2966
Elrose 2013							
Herbicide	0.1311	<.0001	0.0612	0.0371	0.4031	0.7848	0.3225
Cultivar	<.0001	<.0001	0.0001	0.4515	0.3633	0.1407	0.6924
H x C	0.0978	<.0001	0.9381	0.0202	0.0184	0.0118	0.0032

. Obscure data influenced by disease removed from analysis

Locational differences existed for final above ground dry weight measurement with Saskatoon having higher dry weights than Elrose. At both locations, IMI timing had no effect on dry weight (Table 4.10). At Saskatoon, CDC Cory had the highest dry weight at 43.4 g/plant while CDC Luna had the lowest at 25.0 g/plant. At Elrose, CDC Alma and CDC Cory had the highest dry weights of 21.1 g/plant and 20.4 g/plant, respectively.

The number of seeds per plant (Table 4.11) and the ratio of seeds per pod (Appendix 2) had obscure results for all site years. There was unaccountable variation among all cultivars for all timings of IMI applications.

Table 4.11 – The number of seeds per plant after imazamox (35%) + imazethapyr (35%) applied at different growth stages on the four cultivars at all measured site years.

Location (year)	Treatment	Seeds per Plant			
		CDC Luna	CDC Corinne	CDC Alma	CDC Cory
Saskatoon 2012	Control	2.3	68.9	2.8	51.8
	2-4 node 1X	0.2	22.4	2.7	63.4
	5-8 node 1X	0.3	36.2	1.7	58.3
	9-12 node 1X	0.1	14.0	1.8	48.2
	2-4 node 2X	1.2	17.4	1.6	38.0
	5-8 node 2X	0.9	32.9	1.8	68.8
	9-12 node 2X	0.4	7.4	1.6	61.9
	LSD (0.05)	16.1			
Saskatoon 2013	Control	51.7	65.7	39.7	70.5
	2-4 node 1X	29.3	53.9	38.0	61.6
	5-8 node 1X	30.8	53.7	41.2	67.6
	9-12 node 1X	23.4	36.3	36.5	57.0
	2-4 node 2X	24.4	39.3	44.1	56.0
	5-8 node 2X	27.4	49.9	41.2	58.7
	9-12 node 2X	23.4	47.7	34.4	68.6
	LSD (0.05)	19.2			
Elrose 2012	Control	33.1	48.9	24.6	34.8
	2-4 node 1X	18.8	34.0	25.5	41.3
	5-8 node 1X	26.9	34.3	36.0	32.5
	9-12 node 1X	48.3	31.5	29.3	31.2
	2-4 node 2X	26.7	21.3	39.4	35.6
	5-8 node 2X	31.9	29.2	33.7	35.9
	9-12 node 2X	21.5	32.0	33.3	44.0
	LSD (0.05)	10.6			
Elrose 2013	Control	19.6	32.1	21.8	23.6
	2-4 node 1X	25.3	28.3	23.7	23.3
	5-8 node 1X	16.3	29.3	21.0	25.7
	9-12 node 1X	21.0	35.3	20.5	29.9
	2-4 node 2X	30.5	35.0	20.6	24.9
	5-8 node 2X	23.0	32.4	21.9	25.2
	9-12 node 2X	36.7	32.6	27.3	31.7
	LSD (0.05)	15.7			

4.3.3 Yield Results

Saskatoon 2012 plots were not harvested due to snowfall before plots reached maturity. Yield data reflects all other site years. Apart from Elrose 2012 where cultivar was the only significant factor (Figure 4.18), the other three site years, Saskatoon 2013, Elrose 2013 and Moose Jaw 2013 demonstrated significant effects of the interaction of herbicide timing of application and cultivar (Table 4.12). At all 2013 locations there was a reduction in yield for all the of the IMI timings on CDC Luna and CDC Corinne (Figure 4.19). At Saskatoon 2013, yield of CDC Luna decreased from the control of 3961 kg/ha to 2241 kg/ha with the 2x rate of IMI applied at the 9-12 node stage. A more mild reduction to 3254 kg/ha was experienced for the 1x 2-4 node stage application. Similarly, CDC Corinne experienced the most intense yield reduction of 2135 kg/ha from 2x 9-12 node timings. This trend was comparable across 2013 sites. Yields for CDC Alma and CDC Cory were more ambiguous for IMI timings. Using CDC Alma in Saskatoon 2013 as the most extreme example of observed fluctuation, yields increased by 363 kg/ha from the 1x at 9-12 node application and decreased by 676 kg/ha from the 2x at 5-8 node application, compared to the control. In contrast, no IMI timings affected CDC Alma yield in Elrose 2013. Accounting for the number of green seeds in the samples and adjusting yields did not change the overall yield results for any site year (Table 4.12).

Table 4.12 - P values from mixed model analyses of yield, adjusted yield, 1000 seed weight and harvest index (H.I.) for all site years.

	Yield	Adjusted yield	1000 seed weight	H.I.
Saskatoon 2012				
Herbicide	n/a	n/a	0.4512	0.0016
Cultivar	n/a	n/a	<.0001	<.0001
Herbicide x Cultivar	n/a	n/a	0.572	<.0001
Saskatoon 2013				
Herbicide	<.0001	0.0014	0.2547	0.1482
Cultivar	<.0001	<.0001	<.0001	<.0001
Herbicide x Cultivar	<.0001	<.0001	0.4988	<.0001
Elrose 2012				
Herbicide	0.6953	0.6947	0.1573	0.3128
Cultivar	<.0001	0.0247	<.0001	0.0468
Herbicide x Cultivar	0.2712	0.1847	0.0029	0.0027
Elrose 2013				
Herbicide	0.0147	0.0145	<.0001	0.8143
Cultivar	<.0001	<.0001	<.0001	0.0253
Herbicide x Cultivar	0.0025	0.0019	<.0001	0.2376
Moose Jaw 2013				
Herbicide	<.0001	<.0001	0.0111	n/a
Cultivar	<.0001	<.0001	<.0001	n/a
Herbicide x Cultivar	0.0058	0.0017	0.0266	n/a

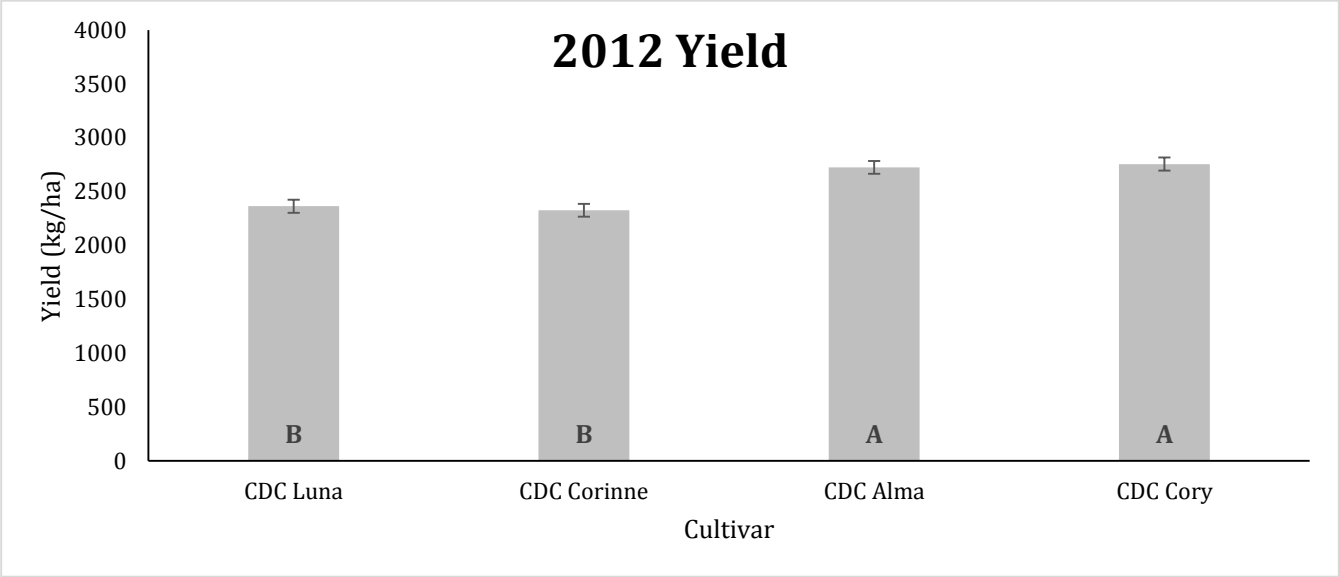


Figure 4.18 – Grain yield of four chickpea cultivars across different rates and timing of herbicide application at Elrose in 2012.

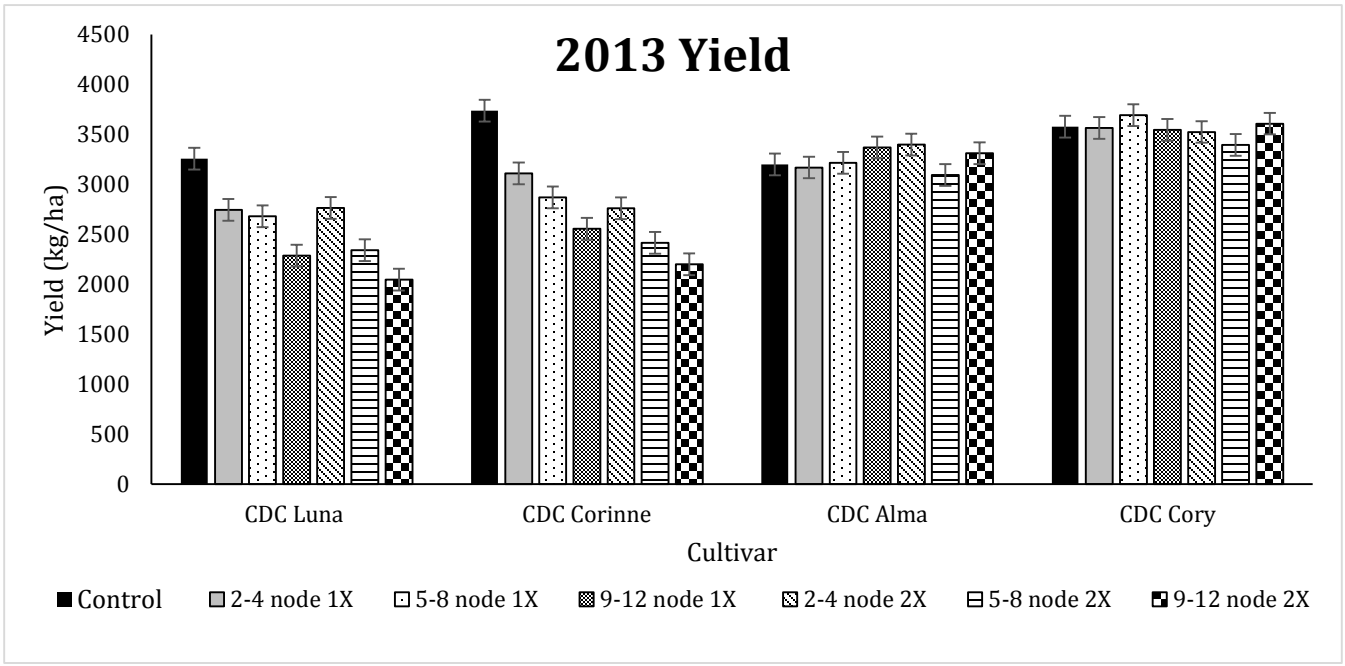


Figure 4.19 – Grain yield of four chickpea cultivars at different rates and timing of herbicide application combined from Saskatoon 2013, Elrose 2013 and Moose Jaw 2013. The interaction of cultivar and timing was significant (P<0.0001)

In general, the 1000 seed weight of kabuli cultivars, CDC Luna and CDC Alma, was greater than that of desi cultivars. Although analyses of variance demonstrated significant effects of the herbicide and cultivar interaction, seed weight did not correspond between locations and years making treatment effects inconclusive.

Harvest index was affected by the interaction of timing and cultivar, except in Elrose 2013 where cultivar was the only significant factor (Table 4.12). Increases or decreases in harvest index were not consistent across herbicide timings or cultivars.

5.0 Discussion

Identifying chickpea cultivars with resistance to IMI herbicides would expand the currently limited broadleaf weed control options. Further, distinguishing growth stages that can tolerate IMI herbicides would allow for applications at appropriate timing for maximal weed control. This study reported the reaction of four chickpea cultivars to IMI herbicides and tested the reaction at different growth stages. The results of this research clearly demonstrated that conventional cultivars CDC Luna and CDC Corinne are susceptible to IMI herbicide and near-isogenic lines CDC Alma and CDC Cory are resistant to IMI herbicides.

5.1 Response of Susceptible Cultivars

5.1.1 Physiological Responses

Susceptibility of CDC Luna and CDC Corinne to IMI herbicides was apparent from visual injury ratings and other physiological changes after IMI application. Imidazolinone herbicides bind to the ALS enzyme, restricting its catalytic function. The pathway for BCAA synthesis is interrupted, reducing protein synthesis. Cell division slows as a consequence, and cell death resulst (Zhou et al., 2007). The symptoms of chlorotic and necrotic tissues observed in visual injury ratings, as well as the stunted growth of CDC Luna and CDC Corinne, clearly demonstrate the symptoms of ALS inhibiting herbicides.

The negative effects of IMI herbicide were observed as high visual injury scores and stunted growth. Injury was unacceptable for all IMI herbicides across all growth stages. Imidazolinone applied at the 5-8 node stage produced the most severe injury, while both the 2-4 and 9-12 node stage applications were slightly less damaging. Young, actively growing plants at a 2-4 node growth stage may have a faster metabolism. These plants may be able to metabolically deactivate the herbicide at a faster rate. The later application at the 9-12 node stage produced less injury as well. Protein reserves found in mature tissue of established plants can be catabolized for BCAA (Zhou et al., 2007), therefore when the ALS enzyme is inhibited, less injury may result on mature plants compared to younger plants with less protein stores.

Days to flowering and maturity of the conventional cultivars were also negatively affected by IMI herbicides. The indeterminate growth habit of chickpea is already problematic in Saskatchewan's short growing season. Combined with the delay of flowering and maturity caused by IMI herbicide, there is higher risk for low quality and reduced yields. Days to flowering were delayed, in extreme cases, by up to 20 days with imazamox at 40 g a.i/ha. While not as significant, maturity was also delayed under IMI herbicide treatments across most site years. In Saskatoon 2012, treated plots did not mature before the end of season, causing a complete loss in yield. This site year particularly demonstrates the unacceptability of IMI applications on susceptible cultivars. Favourable environmental conditions at all other site years allowed for susceptible cultivars to mature despite required additional growing days. When testing herbicide application across different growth

stages, it was evident that later applications prolonged DTF and DTM more drastically. A 9-12 node stage application of IMI herbicide would therefore be most threatening for immature chickpea seed at harvest on the conventional cultivars. With IMI herbicide causing delays in DTF and DTM, production risks are elevated on an already vulnerable crop.

Morphological characteristics such as the number of branches from the primary stem, final dry weight, and height at maturity of the susceptible cultivars were relatively unaffected by IMI herbicide. It was observed that lateral branching tends to increase after IMI treatments. Although all tested IMI herbicides did not increase primary branching at the 2-4 node stage, minor increases were observed with the combination imazamox + imazethapyr applied at later growth stages. Imidazolinones inhibit branched chain amino acid synthesis in young tissue causing symptoms to first appear in meristematic regions (Zhou et al., 2007). If cell function in the primary shoot apical meristem is compromised, axillary buds may be stimulated, therefore promoting lateral branching (Shimizu-Sato et al., 2009). A developed plant with mature tissues would have more protein and amino acid reserves than a young, immature plant (Zhou et al., 2007). Therefore, when herbicide is applied at a 9-12 node stage, the plant can catabolize protein reserves for amino acids, lessening injury and creating the potential for faster recovery through new development of lateral branches. As well, a developed plant would have more potential sites for axillary growth, compared with a small, immature plant. Data collection of lateral branching could be altered to include secondary and tertiary branching which may present stronger results.

The dry weight and final height at maturity of CDC Luna and CDC Corinne were unaffected by herbicide treatment and timings. These measurements are indicators to the continual recovery of susceptible cultivars over the growing season.

The level of leaf greenness was measured after IMI application using a SPAD-502DL Plus meter. Although IMI herbicides do not directly target photosynthesis, it has been suggested that treated plants may have a chlorophyll fluorescence response (Riethmuller-Haage et al., 2006). The intent to capture herbicide damage of possible chlorophyll content reduction and general chlorosis was unsuccessful in this study however. There was large variation in the SPAD chlorophyll meter readings (SCMRs) across 7DAA intervals without obvious trends. This variation can be explained through the general mechanisms of ALS inhibiting herbicides and sampling techniques employed. Imidazolinones impede new tissue development and cause chlorosis with foliar applications. Due to arrested development, the first fully expanded leaf remained the same over many sampling intervals for numerous IMI treatments. In contrast, lower rate applications such as 1x imazethapyr, allowed for faster recovery and development of new pine-like leaves. Therefore, two leaf responses were being measured incorrectly in tandem, leading to confounding SCMR results. This sampling inconsistency explains some of the variation in SCMRs.

5.1.2 Recovery from IMI treatment

Despite initial debilitating injury after IMI applications, recovery of susceptible cultivars CDC Luna and CDC Corinne was apparent. First signs of

recovery were established at 28 DAA. Visual injury was scored approximately 5% less severe at 28 DAA compared to ratings at the pinnacle at 21 DAA. While vertical growth was initially arrested after IMI treatments, significant increases in height were recorded again at 28 DAA. The initial signs of recovery at 28 DAA were a preface for the continued recovery throughout the season, leading to no yield loss of susceptible cultivars.

The recovery mechanism of susceptible chickpea to overcome IMI herbicide injury is currently not understood. It can be hypothesized that over time the herbicide is metabolized, allowing the ALS enzyme to regain its activity. Imidazolinone resistant soybean is evidence of rapid metabolic detoxification of IMI herbicide (Tecle et al., 1993). The herbicide selectivity is based on the plants' ability and rate of metabolism. Susceptible chickpea may be able to metabolize IMI's at an extremely low rate, accounting for initial severe injury after application, succeeded by slow recovery. Increasing the dose of IMI herbicide would eliminate the opportunity for recovery of susceptible chickpea cultivars.

The double copy of the ALS gene in chickpea may also contribute to the recovery process. The first gene copy, and the gene responsible for IMI resistance in chickpea, is found on Chromosome 5 (Thompson and Tar'an, 2014). This mutation restricts herbicide binding, allowing for the continuation of branched chain amino acid production, conferring herbicide resistance. While this mechanism was recently confirmed, the role of the second ALS gene copy which is located on chromosome 1 in resistant and susceptible cultivars is still unknown. In the instance of IMI resistant hard red wheat, the level of resistance was dependent on genome location,

gene number, and growth habit (Hanson et al., 2006). Hanson et al. (2007), also studied the amount of enzyme produced from the susceptible gene as part of the total extractable ALS enzyme. Susceptible enzyme regained maximum levels 3 days after treatment indicating rapid recovery. This research elucidates the role a second gene copy may have in the level of IMI resistance and the speed of recovery of susceptible cultivars.

5.1.3 Environmental Constraints Affecting Yield

In most site years, susceptible cultivars CDC Luna and CDC Corinne were able to recover after initial injury from the application of IMI herbicides. The results showed no yield difference between treated plants and untreated controls. Therefore, if conditions are conducive, early application of IMI herbicides may not diminish yield. However, environmental conditions tend to be highly variable between years and locations. In Elrose 2013, yield reductions on the susceptible cultivars were observed from 2x rates of imazamox, imazethapyr and the combination imazamox + imazethapyr. The conditions at the end of the season in Elrose 2013 were warmer and drier than normal. Limited moisture and heat stress may have forced maturity earlier in the reproductive phase causing fewer pods to set seed. Susceptible cultivars treated with IMI herbicide may not have had enough moisture for full vegetative and reproductive recovery, therefore yield decreased. Additionally, the complete loss of Saskatoon 2012 plots demonstrates the potential severity of unfavourable conditions.

The growth stage at which the IMI herbicide was applied also affected the yield. Although generally the IMI application at the 9-12 node stage was less injurious, yields of CDC Luna and CDC Corinne were lowest at this herbicide application timing. The length of recovery time before the end of season was shorter and therefore, complete recovery was not possible and yields were compromised.

While seed traits of susceptible cultivars were unaffected by IMI herbicide in all other site years, in Saskatoon 2012 differences in seed weight per plant, pods per plant, seed per plant, and seeds per pod were evident. This may be because in addition to herbicide damage, ascochyta blight infested Saskatoon 2012 plots. Deduced from the negative correlation to all seed traits, ascochyta blight amplified the negative effects of IMI treatments on the susceptible cultivars. Disease incidence was not a factor in other site years (Moose Jaw 2013 did not include seed trait measurements) and did not show herbicide cultivar interactions. Therefore, without disease pressure, susceptible cultivars can recover from initial herbicide damage resulting in no seed trait differences. Growers cannot risk application of IMI herbicides on susceptible cultivars, however, because depending on biotic and abiotic stressors, seed traits and reductions or complete loss of yields are possible.

5.2 Response of Resistant Cultivars

Field research demonstrated minimal to no visual injury and no changes in physiological response from any herbicide treatment on resistant cultivars CDC Alma and CDC Cory. Concurrent research had located the point mutation on the ALS gene at base pair 675 leading to the amino acid substitution of 205 alanine to 205 valine (Thompson and Tar'an, 2014). Substitutions cause a conformational change in the ALS enzyme, altering the herbicide-binding site (Tranel and Wright, 2002). The catalytic function of ALS is maintained with several substitutions in the conserved amino acids, suggesting a separate herbicide-binding site from the active site. Imidazolinones are not able to bind to the enzyme, therefore, the ALS enzyme continues to function normally. This mode of IMI resistance in CDC Alma and CDC Cory corresponds to the lack of response from IMI herbicide treatment observed in the field. Visual injury was not apparent and growth factors were unaffected in the presence of IMI herbicide due to continued enzymatic activity.

Imidazolinone resistance in CDC Alma and CDC Cory was sustained across all growth stages tested. No visual symptoms nor growth alterations were observed for any growth stage that IMI herbicides were applied. This allows for residual control of weeds later in the season with no damage to the chickpea crop. Current chemical broadleaf weed control in chickpea cannot be applied past a 3-node stage. The current options leave the crop vulnerable to weed pressure through the critical weed free period (Al-Thahabi et al., 1994; Mohammadi et al., 2005). This study confirms a high level of IMI resistance at the 9-12 node stage for CDC Alma and CDC

Cory. Therefore, a higher level of weed control can be achieved later in the season allowing a higher productivity of the chickpea crop.

Regardless of the lack of response of resistant cultivars to IMI herbicides for most measurements, yield in Elrose 2013 was positively affected. Both the 1x and 2x rates of each IMI herbicide caused a slight increase in yields compared to untreated controls. This result is similar to the phenomenon described by others as hormesis (Duke et al., 2006; Cedergreen, 2008). Hormesis occurs when a low dose of toxicant is stimulatory. A well-known example is the use of low dose glyphosate on sugarcane to increase sucrose (Belz et al., 2011). Although exact mechanisms of hormesis are unknown and unquestionably species-specific, theories involve chemicals eliciting a stress response or induction of defense systems. The "escape" mechanism proposed by Duke et al. (2006) could explain the increasing chickpea yield with IMI herbicide. The plant may increase seed production in a chemically stressed environment, increasing the chance of germination and survival of the following generation in more favourable conditions. This is one possible explanation for higher yields of resistant chickpea under IMI herbicide treatment.

Unintentional damage from vigorous hand weeding is another explanation of control plots of IMI resistant cultivars yielding less than IMI treated plots. Control plots did not receive herbicide application, therefore weed density, before manual removal, would be higher than herbicide treated plots. Entry into control plots was more frequent and robust weeding may have caused minor damage. The absence of intensive hand weeding in IMI treated plots might have caused higher yield compared to controls.

6.0 Conclusions

This study examined four cultivars of chickpea for their reaction to IMI herbicides. The level of resistance and physiological responses of CDC Luna, CDC Corinne, CDC Alma and CDC Cory to IMI herbicides were measured across three growth stages. Information generated from this field research allows for expansion of broadleaf weed control options for use in chickpea.

This research confirmed CDC Luna and CDC Corinne as susceptible cultivars. Visual injury scores were severe, growth was stunted, and flowering and maturity were delayed under IMI herbicide treatment. Applications at all growth stages produced unacceptable injury and the later applications reduced yield. Despite initial severe injury, CDC Luna and CDC Corinne were able to recover from IMI herbicides applied at the 2-4 node stage. Unfavourable conditions due to weather or disease can amplify negative responses from herbicides. Therefore, IMI use on susceptible cultivars is not recommended and could result in complete yield loss.

CDC Alma and CDC Cory, on the other hand, were confirmed as IMI resistant cultivars. No adverse response was observed from any of the herbicide treatments. Additionally, all growth stages of herbicide application were highly tolerated. In certain conditions, IMI herbicide may actually have a stimulatory effect on resistant cultivars causing increased yield.

The results from this research are very promising for the future use of IMI herbicide on resistant cultivars CDC Alma and CDC Cory. Not only will broadleaf weed control options expand to include IMIs, but weed control later in the season will also be possible. Chickpea breeding programs can be enhanced by the inclusion

of IMI resistance in future chickpea cultivars. These advancements will improve chickpea production in Saskatchewan.

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Appendices

Appendix 1: Component 1 – IMI Resistance Additional Data

Table 7.1 – ANOVA table comparing location and year for the level of injury over 7 day intervals in herbicide trial component 1

Day interval	*Source of variation*	*df*	*SS*	*MS*	*F Value*	*P value*
7 DAA	location	2	343.38	171.69	0.47	0.6252
	year	1	31691.57	31691.50	86.77	<.0001
	location*year	1	69.14	69.14	0.19	0.6637
	loc*year*herb*cult	18	459.70	25.54	1.17	0.2810
14 DAA	location	1	1538.25	1538.25	1.45	0.2291
	year	1	3803.25	3803.25	3.59	0.0590
	location*year	0	0	.	.	.
	loc*year*herb*cult	0	0	.	.	.
21 DAA	location	2	9243.51	4621.76	4.45	0.0121
	year	1	4.72	4.72	0.29	0.5909
	location*year	1	92.89	92.89	0.09	0.7649
	loc*year*herb*cult	18	245.10	13.62	0.83	0.6598
28 DAA	location	2	6969.16	3484.58	3.83	0.0224
	year	1	23.68	23.68	0.03	0.8719
	location*year	1	643.68	643.68	0.71	0.4009
	loc*year*herb*cult	18	364.99	20.28	1.36	0.1450

Table 7.2 – ANOVA table comparing location and year for height at maturity after chickpea cultivars were treated with IMI herbicide.

Source of variation	*df*	*SS*	*MS*	*F Value*	*P value*
location	1	2728.83	2728.83	341.81	<.0001
year	1	203.65	203.65	25.51	<.0001
location*year	1	1092.14	1092.14	136.80	<.0001
loc*year*herb*cult	18	96.03	96.03	0.67	0.8421

Appendix 2: Component 2 – Timing of IMI Applications Additional Data

Table 7.3 - ANOVA table comparing location and year for the level of injury of 7 day intervals in component 2 - timing trial

Day interval	*Source of variation*	*df*	*SS*	*MS*	*F Value*	*P value*
7 DAA	location	2	5635.28	2817.64	3.99	0.0191
	year	1	448.41	448.41	0.63	0.4261
	location*year	1	1759.93	1759.92	2.49	0.1152
	loc*year*herb*cult	12	1031.02	85.92	2.45	0.0043
14 DAA	location	2	372.86	186.43	0.19	0.8255
	year	1	6096.99	6096.99	6.27	0.0126
	location*year	1	2784.00	2784.00	2.86	0.0913
	loc*year*herb*cult	9	59.25	6.58	0.26	0.9840
21 DAA	location	2	4623.28	2311.63	2.10	0.1233
	year	1	2135.00	2135.00	1.94	0.1642
	location*year	1	190.32	190.32	0.17	0.6776
	loc*year*herb*cult	18	1780.55	98.92	5.86	<0.0001
28 DAA	location	2	14565.30	7282.63	9.52	<0.0001
	year	1	2010.27	2010.27	2.63	0.1056
	location*year	1	512.14	512.14	0.67	0.4137
	loc*year*herb*cult	18	4937.91	274.32	11.54	<0.0001

Table 7.4 - The number of seeds per pod after imazamox (35%) + imazethapyr (35%) applied at different growth stages on the four cultivars at all measured site years.

Location (year)	Treatment	Seeds per Pod			
		CDC Luna	CDC Corinne	CDC Alma	CDC Cory
Saskatoon 2012	Control	0.09	1.18	0.09	0.91
	2-4 node 1X	0.04	0.78	0.13	1.01
	5-8 node 1X	0.03	0.85	0.08	0.89
	9-12 node 1X	0.03	0.46	0.09	0.93
	2-4 node 2X	0.11	0.64	0.08	0.88
	5-8 node 2X	0.04	0.66	0.07	1.04
	9-12 node 2X	0.04	0.28	0.08	0.93
	LSD (0.05)	0.22			
Saskatoon 2013	Control	0.88	1.31	0.79	1.24
	2-4 node 1X	0.82	1.27	0.80	1.25
	5-8 node 1X	0.89	1.30	0.81	1.17
	9-12 node 1X	0.91	1.23	0.82	1.11
	2-4 node 2X	0.92	1.24	0.83	1.27
	5-8 node 2X	0.92	1.31	0.82	2.26
	9-12 node 2X	0.91	1.30	0.79	1.22
	LSD (0.05)	0.17			
Elrose 2012	Control	0.80	1.27	0.75	1.06
	2-4 node 1X	0.85	1.14	0.70	1.05
	5-8 node 1X	0.75	1.16	0.71	1.12
	9-12 node 1X	0.82	1.11	0.65	1.19
	2-4 node 2X	0.86	1.15	0.69	1.13
	5-8 node 2X	0.89	1.17	0.84	1.11
	9-12 node 2X	0.90	1.15	0.79	1.16
	LSD (0.05)	0.16			
Elrose 2013	Control	0.94	1.19	0.78	0.85
	2-4 node 1X	0.68	1.44	0.67	1.08
	5-8 node 1X	1.04	1.05	1.38	1.08
	9-12 node 1X	1.89	1.21	0.61	1.57
	2-4 node 2X	0.94	0.64	1.64	1.26
	5-8 node 2X	0.98	0.98	1.05	1.41
	9-12 node 2X	0.87	1.35	1.12	0.99
	LSD (0.05)	0.65			

Appendix 3 – Segregation of IMI resistance

The objective of this component was to determine whether resistance to imidazolinone is controlled by one locus or whether more than one locus contributes to IMI resistance in chickpeas.

Materials and Methods: Producing F1 populations

The level of resistance to IMI application under controlled environment was previously established for plant material used in this study. ICCX860047-9, a desi type, selected from germplasm originating from ICRISAT, Patancheru, India showed high resistance (no injury) to IMI application. Two other genotypes (ILC531 and ILC1493) showed minimum to moderate injury with good plant appearance and minor chlorosis. ILC531 is a small seeded (17g/100 seeds) kabuli type originating from Egypt. ILC1493 is a small-medium seeded (30g/100 seeds) kabuli originating from Afghanistan. Two kabuli cultivars (CDC Leader and CDC 494-9) susceptible to IMI were also used in crosses.

Six F2 populations were used in this segregation study (Table 7.5).

Table 7.5 – Crosses used to produce F2 populations

Cross	Pedigree number	Female Parent	Male Parent
1	1785	CDC Leader (S)	MM-9 (R)
2	2032	ILC531 (MR)	CDC Leader (S)
3	2086	CDC 494-9 (S)	ILC1493 (MR)
4	2041	ILC531 (MR)	MM-9 (R)
5	2042	ILC 531 (MR)	MM-9 (R)
6	2100	ILC1493 (MR)	MM-9 (R)

To produce F1 plants, crosses were performed in the Agriculture Greenhouse in March and April 2012. Average air temperature for the duration of crossing was

23.5°C and relative humidity was 47.94%. One-gallon pots were filled with Sunshine mix no. 4 and washed 5 times. MM-9 seeds were scarified with tweezers. 12 seeds of MM-9, 12 seeds of CDC Leader, 12 seeds of ILC 1493, and 12 seeds of ILC 531 were treated with mefenoxam + fludioxonil (Apron Maxx®, Syngenta Canada). Three seeds of the same cultivar were planted into each pot totaling 16 pots per cultivar. Pots were labeled appropriately. At the 5-8 node stage, plants were thinned to one healthy plant per pot. Pots were watered as needed (roughly every 2 days). They received tap fertilizer once a week until flowering at which time fertilizer was applied once every 2 weeks.

To perform crosses, young, unopened flowers were selected on the female parent and anthers were analyzed. If anthers were low in the flower and had yet to exude pollen, the flower was selected for crossing. Flowers were emasculated using fine tipped tweezers. Every anther was removed. Tweezers were sterilized in ethanol. Open flowers from the male parents were used as a source of pollen. Bright yellow/orange pollen was collected onto the tweezers tip and transferred onto the stigma of the emasculated flower. Using a pipette, 0.4ml of mix hormone was deposited into the manually pollinated flower. Fine cotton was dipped into PGR hormone and wrapped around the abscission layer. Flowers were labeled with parental cultivars as well as date of pollination. After plants matured, seeds from the labeled pods were harvested and used as the F1 plants in the segregation study.

Materials and Methods: Screening F1 and F2 populations

Square 4-inch pots were filled with Sunshine mix no. 4 and washed 5 times. Desi type seed was scarified using tweezers. One hundred seeds of each F2 cultivar

and all of available F1 seeds were pre-germinated in petri dishes on wet filter paper. Germinated seeds were planted into prepared pots and grown under 16h light, 8h dark, 22°C/18°C conditions in growth chamber 1-33. Plants were watered approximately every two days. Once plants reached the 2-4 node growth stage, they were subjected to herbicide application.

Plants were transferred to a cabinet sprayer for application. Imazamox was weighed up at 0.029g/100mL. Imazamox and 1.0 mL of Merge surfactant was added to 200ml of distilled water. The cabinet sprayer was set at 40 PSI for a spray pressure of 35 PSI. The speed setting was 3.21, which is equivalent to 4.230 km/hr in the field. The machine was run once to ensure even spray pattern with 8001 EVS nozzles. Trays of 10 pots were placed three at a time in the center of the cabinet sprayer. The height was adjusted so that the top of the plant would be 12 inches from the spray. Plants were returned to growth chamber after herbicide application was complete.

Visual injury ratings were conducted at 7, 14 and 21 DAA. Plants were categorized as either susceptible (S), moderately resistant (MR), or resistant (R) (Figure 7..1). Susceptible plants had severely stunted growth, chlorosis, and necrotic tissue. Plants were classified as moderately resistant if any morphologically changes were apparent. Symptoms could include stunted growth, minor chlorosis, needle like leaves, and increased branching (Figure 7.7.2). Resistant plants had no visible signs of injury. The number of plants in each category were totaled and subjected to chi-square tests.

Figure 7.1 - Select plants from population 2041 at 28 days after application. Susceptible (A), moderately resistant (B), and resistant (C) plants are represented above

Figure 7.2 - Example of needle like leaves and increased branching of a moderately resistant plant.

F_1 Results

Limited seed existed for F1 populations. One seed of population 2032, two seeds of population 2041, and two seeds of population 1785 were available for screening. All F1 plants of 2032, 2041, and 2041 populations had a moderately resistant phenotype.

F_2 Results

Populations 1785 (S x R), 2041 (MR x R), and 2042 (MR x R) followed a single-gene incomplete dominance 1:2:1 segregation ratio (Table 7.6).Populations 2032 (MR x S) and 2086 (S x MR) did not follow 1:2:1 segregation. Population 2100 (MR x R) had no emergence so could not be analyzed.

Table 7.6 –Analysis of each pedigree, using chi-square statistics, to test 1:2:1 segregation ratios for IMI herbicide resistance.

Pedigree Number	Number Resistant Plants	Number Moderately Resistant Plants	Number Susceptible Plants	X^2 Value	P Value
1785 (S x R)	7	21	9	0.8918	0.6402
2041 (MR x R)	19	27	11	2.4029	0.3011
2042 (MR x R)	13	21	10	0.4999	0.7788
2032 (MR x S)	0	21	6	11.0000	0.0040
2086 (S x MR)	0	20	10	9.1667	0.0102
2100 (MR x R)	.	.	.	.	.

Conclusions

Six F2 populations were assessed for their segregation pattern based on IMI herbicide application. Plants were classified into one of three categories susceptible (S), moderately resistant (MR), or resistant (R). Based on chi-square analyses of each segregating population, no conclusive results were apparent. Three

populations, 1785 (S x R), 2041 (MR x R), and 2042 (MR x R), followed a single-gene incomplete dominance 1:2:1 segregation ratio. However, two populations, 2032 (MR x S) and 2086 (S x MR), did not follow 1:2:1 segregation. One population, 2100 (MR x R), had no emergence.