GLYCOALKALOID PROFILING OF POTATO GENOTYPES FROM THE NORTH DAKOTA STATE UNIVERSITY POTATO BREEDING PROGRAM

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Irene Román Martínez

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Asunta Thompson
Chair

Senay Simsek

Edward Deckard

Deirdre Prischmann-Voldseth

Approved:

November 7, 2013 — Date

Richard Horsley — Department Chair

ABSTRACT

Gylcoalkaloids (GA) are plant secondary metabolites that offer pests and disease resistance. Studies show correlation between GA content and CPB resistance. In this study, CPB resistance was assessed in a field trial at Grand Forks, ND, during 2012 for twenty-four genotypes from the NDSU Potato Breeding Program. Two treatments were applied, a block treated with imidacloprid (Admire®), and an untreated block. The treated block showed decreased CPB damage. Presence of aglycons (non-sugar moiety of GAs) was assessed by gas chromatography in foliar and tuber tissue. Distribution of GAs in the tuber was assessed to determine variation in tuber sections and whole tuber. Potato genotypes should be developed with tuber GAs levels below 20 mg/100 g fresh weight (FW) to ensure safety for human consumption. Focus should be on GAs that are only synthesized in the tuber, which will provide pests and disease resistance, while maintaining adequate yields and decreased inputs.

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LITERATURE REVIEW

Introduction

The potato (*Solanum tuberosum* L.) is one of the most important non-cereal food crops in the world. It ranks fourth in total food production after rice, wheat, and corn (Carputo et al., 2005). Potato is cultivated worldwide, under different environmental conditions. It can be grown in temperate and tropical regions, and at elevations that range from sea level to 4000 m. An estimated 464,700.52 ha were planted at the beginning of the 2012 season in the United States (U.S.), and the total value of production for 2012 was $3.73 billion (USDA-NASS, 2013). The potato is a major carbohydrate source and a staple food worldwide. It provides significant amounts of amino acids, vitamins C and B6, and minerals such as potassium (Bethke, 2008; Carputo et al., 2005; NHS, 2009). Potato production covers the agricultural, industrial and food market sectors. Potatoes are produced for tablestock (fresh market), processing (frozen french fries, dehydrated, chips, canned products, flour, starch, and other), livestock feed, and seed (USDA-NASS, 2013).

Given the economic value of the potato, it is necessary to understand the growing conditions required to obtain a successful crop. The potato grown in the northern temperate latitudes, *Solanum tuberosum* spp. *tuberosum,* was selected for adaptation to long day conditions from *S. tuberosum* spp. *andigena*, which grows under short day conditions (Maris, 1989). As discussed by Rosen (2010), yield potential of the potato crop is determined by multiple factors: the amount of radiant energy available, number of days with no frost, adequate temperature through the growing season, and the amount and uniformity of the water supply. The potato plant grows best in a cool climate, in soil that has high nitrogen content and that is well drained

(Rosen, 2010). Temperature is a very important factor in the growth and yield potential of the crop, since it also affects the colonization of pests in the field (Lactin and Holliday, 1994; Logan et al., 1985), and development of diseases (Pérombelon, 2002). The highest yields are produced in areas where the daytime temperature is over 38°C during the hottest part of the growing season and nights are cool 18°C (Rosen, 2010).Proper field and storage management practices, as well as understanding the growing conditions of the crop, will help manage insect pests and diseases, as well as increase potential yield.

An increase in production of potato genotypes with resistance to pests and diseases is sought as a way of providing food security and higher profits for farmers (Alyokhin, 2009). However, potato is susceptible to a wide range of pests and diseases (Alyokhin, 2009; Pelletier, 2011). The Colorado potato beetle (*Leptinotarsa decemlineata* Say), flea beetles (*Epitrix tuberis* and *E. subcrinata*), leafhoppers (*Empoasca fabae*), and aphids (*Myzus persicae* Sulzer, *Macrosiphum euphorbiae* Thomas, *Aphis nasturtii* Kaltenbach and *Aphis gossypii* Glover) attack potato foliage, and wireworms (*Elateridae*) feed on the tubers (Radcliffe, 2010). Various pesticides are used to control potato insect pests (Alyokhin et al., 2008; Boiteau, 1988; Ferro, 1985; Webb, 2007). However, some potato varieties have been developed that are resistant to certain diseases (Carputo et al., 2010; Cooper et al., 2007; Dimock et al., 1986; Plaisted et al., 1992; Yencho and Tingey, 1994). In the research community, a current major interest is also identifying the genes that are responsible for insect pest resistance (Fisher et al., 2002). Genes from wild relatives are important in plant breeding, because they confer traits that improve physiological and resistance aspects of the crop (Hajjar and Hopkins, 2007). By utilizing plants natural defense mechanisms to control pests and diseases, use of inputs can be decreased and sustainable crop production may be achieved (Coombs et al., 2005; Pelletier et al., 2011).

Colorado potato beetle

Colorado potato beetle (*L. decemlineata* Say) is the most prevalent defoliator insect of the potato crop (Alyokhin, 2009). The Colorado potato beetle (CPB) is a leaf beetle (Coleoptera: Chrysomelidae) native to Southwestern U.S. and Mexico. Wild populations have been documented feeding on buffalo bur (*Solanum rostratum* Dunal), as well as other related species in the *Solanaceae* family (Grapputo et al., 2005; Pelletier, 2011). After being discovered, the CPB had little impact on agriculture (Casagrande, 1987). This changed after the area of its original distribution was occupied by farmers who produced potato on the majority of their acres (Casagrande, 1987). The potato turned out to be a suitable host plant for the CPB, and the beetles quickly switched to feeding on the new host plant (Casagrande, 1987; Weber, 2003).

The CPB has spread throughout the rest of the North American continent and has invaded Europe and Asia (Hsiao, 1985; Jolivet, 1991). It has also appeared in Western China and Iran (Jolivet, 1991; Weber, 2003). The CPB could potentially occupy much larger areas in China and Asia Minor, spread to Korea, Japan, Russian Siberia, as well as, a few areas of the Indian subcontinent, parts of North Africa, and the temperate Southern Hemisphere (Alyokhin, 2009; Boiteau, 1988; Jolivet, 1991).

The geographic distribution of the CPB indicates the ability of the insect to disperse quickly. As discussed by Weber and Ferro (1993), small-scale observations on the dispersal of adult CPB suggest a season-dependent activity. During the beginning of the season, beetles move from overwintering sites to potato fields. This is known as the post-diapause movement. In the middle of the season the beetles move mostly within the potato fields; late in the season, they move from potato fields to overwintering sites. This activity is known as pre-diapause

movement. Adults that surface during the spring often cover distances up to several hundred meters in a few days, and a small portion of adult beetles may disperse more than 500 m from the place where overwintering occurred (Boiteau et al., 2003).

The CPB is characterized by high fecundity, with one female laying 300–800 eggs (Harcourt, 1971).The eggs are usually laid on the underside of potato leaves (Coombs et al., 2002). After hatching, larvae may move over short distances within the potato canopy and start feeding within 24 hours of eclosion (Lactin and Holliday, 1994; Walgenback and Wyman, 1984). Development of CPB, from the time of oviposition to adult eclosion, takes between 14 to 56 days (Ferro et al., 1985; Logan et al., 1985; Walgenback and Wyman, 1984). The optimal temperature range for the growth of the CPB is between 25ºC to 32ºC, but this differs among populations of different geographic origins (Lactin and Holliday, 1994; May, 1981). Pupation takes place in the soil near the plants where the larval development has been completed. Because adults oviposit repeatedly for several weeks, all life stages are usually present throughout most of the growing season (Hare, 1980), making the CPB a difficult pest to control (Alyokhin, 2009).

The CPB overwinters in the soil as an adult, with the majority of the insect population aggregating in woody areas close to the fields where they have spent the previous summer (Weber and Ferro, 1993). The emergence of post-diapause beetles is, to some extent, synchronized with the emergence of the potato crop (Senanayake et al., 2000). The overwintered beetles feed from the colonized plants and then oviposit within five to six days, which varies based on the temperature (Ferro et al., 1985; Ferro et al., 1991; Lactin and Holliday, 1994; Logan et al., 1985). Fields that are not being rotated are colonized immediately by overwintered CPB that enter the field from their overwintering sites, or emerge from the soil within the field (Voss

and Ferro, 1990). If fields are rotated, the beetles have the ability to fly several kilometers to find a new host habitat (Ferro et al., 1999).

The beetles can have between one and three overlapping generations per year (Alyokhin, 2009; Alyokhin and Ferro, 1999). The newly emerged adults develop their reproductive system and flight muscles after a few days (Alyokhin and Ferro, 1999). Flight initiation is strongly related to air temperature and sunlight (Caprio and Grafius, 1990), and with favorable wind direction, the beetles are able to fly over 100 km (Wiktelius, 1981). The beetles can also fly over short distances, generally to distribute the eggs within the host habitat and in search of mates (Voss and Ferro, 1990). After development has been completed, the beetles mate and start laying eggs. Reproduction continues until diapause is induced by the short-day photoperiod (Senanayake et al., 2000). The beetles migrate to overwintering sites (mostly by flying) and enter the soil to diapause (Voss and Ferro, 1990). As discussed by Voss (1989), the beetles that emerge under short-day photoperiod do not develop their reproductive system and flight muscles during that season. They feed actively for several weeks, and enter into the overwintering sites or burrow into the soil directly in the field that was colonized (Voss, 1989). Mating status affects beetle flight activity (Alyokhin, 2009; Voss and Ferro, 1990). Unlike females from a number of other insect species (Dingle, 1985), gravid CPB females display a considerable amount of flight activity (Alyokhin and Ferro, 1999; Ferro et al., 1999), allowing them to distribute eggs within and between fields (Voss and Ferro, 1990). However, they fly significantly less than unmated females (Alyokhin and Ferro, 1999). Contrary to mated females, mated males increase their flight activity, increasing the number of mating partners (Alyokhin and Ferro, 1999).

Colorado potato beetles are insatiable feeders, causing defoliation and canopy destruction (Alyokhin, 2009; Ferro et al., 1985; Ferro et al., 1999; Logan et al., 1985). One beetle can

consume approximately 40 cm^2 of potato leaves during the larval stage (Ferro et al., 1985; Logan et al., 1985), and an additional 10 cm^2 of foliage per day as an adult (Ferro et al., 1985). If left uncontrolled, the beetles can completely destroy potato crops (Alyokhin, 2009; Ferro et al., 1985). In areas where the CPB is considered a pest, development of CPB-resistant potato cultivars is a priority in the selection process for breeding, especially since this pest is a quick colonizer and can cause considerable defoliation before the grower can initiate treatment with pesticides (Alyokhin, 2009). The economic impact of uncontrolled infestations can be substantial (Alyokhin, 2009; Grafius, 1997). As shown by Grafius (1997), after conducting a study of the economic impact of the CPB insecticide resistance on the Michigan potato industry, the estimated increased cost ranged from $44 to $69 per ha, with state-wide losses adding up to $1.4 million per year. It is important to note that defoliation of annual crops must exceed a certain threshold, usually 5% to 30%, before productivity is impaired (Mattson and Addy, 1975). Management of CPB has become increasingly difficult and expensive because the insect has quickly become resistant to various insecticides used for its control (Alyokhin et al., 2006 and 2008; Coombs et al., 2005; Forgash, 1985; Grafius, 1997). Furthermore, CPB is a fast developing insect that can withstand harsh environmental conditions, which decreases the chances of controlling the pest by the climate change (Dwyer et al., 2001).

An outbreak of CPB in 1840 led to the first large-scale use of insecticides, which continues to be the principal mechanism of crop protection against this pest (Alyokhin, 2009; Casagrande, 1987; Gauthier et al., 1981). Multiple compounds have been tested against the CPB, and various active ingredients are registered for use against this pest in the U.S. (Gauthier et al., 1981). Crop rotation, biological control, and other non-chemical tactics for regulating populations are useful in some circumstances, but are not as popular as chemical control (Alyokhin, 2009). Another

issue is that insecticide efficacy and availability vary from area to area, as well as, pesticide regulation. As a result of intensive use of insecticides, CPB has developed resistance to nearly every insecticide used for its control (Alyokhin, 2009; Alyokhin et al., 2007; Forgash, 1985).

Rapid development of pesticide resistance seems to be a natural characteristic of CPB (Hare, 1990). This may be caused by coevolution of the beetle and its host plants in the family *Solanaceae*, which are known to have high concentrations of toxins, specifically glycoalkaloids (GA) (Ferro, 1993; Tingey, 1984). Presently, CPB is resistant to a wide range of insecticides, including the arsenicals, organochlorines, carbamates, organophosphates, and pyrethroids (Alyokhin, 2009; Ioannidis et al., 1991). The resistance crisis was temporarily managed with the introduction of highly effective neonicotinoid insecticides *(*Alyokhin et al., 2006 and 2008). Various cases of beetle resistance to neonicotinoids have recently been documented in several populations (Alyokhin et al., 2006 and 2007; Mota-Sánchez et al., 2006). As discussed by Benkovskaya et al. (2008), insecticide resistance of insect pests is among the most important negative side effects of their use. Resistance, determined as a natural change in susceptibility to chemical compounds in pest populations, is a natural consequence of the severe selective pressure that results from repeated treatment with high concentrations of insecticides (Alyokhin et al., 2008). Major problem areas have been the Northeastern U.S. (Forgash, 1985), Michigan (Ioannidis et al., 1991), Canada (Stewart et al., 1997), and Europe (Boiteau, 1988; Forgash, 1985). Resistance mechanisms are highly diverse even within a relatively narrow geographical area (Ioannidis et al., 1991).

Excessive use of insecticides not only causes development of resistance, but also increases concerns about pollinators, environmental pollution, and food safety (Pariera Dinkins and Peterson, 2008). In addition to the resistance to synthetic insecticides, the beetle has the ability

to develop resistance to the *Bacillus thuringiensis* subsp. *tenebrionis* delta-endotoxin (Rahardja and Whalon, 1995). Genetically modified potato genotypes expressing *B. thuringiensis* delta-endotoxin (which is toxic to the CPB) were introduced in the U.S., but were discontinued, mainly because of consumer concerns about genetically engineered foods (Shelton et al., 2002).

Colorado potato beetle populations can be reduced through the use of relatively common cultural practices such as crop rotation, manipulation of planting time and crop varieties, use of mulches, cover and trap crops (Alyokhin, 2009; Hough-Goldstein et al., 1993). Crop rotation for CPB control has been utilized since 1872, and it has proven to be a good control strategy for CPB, as well as, for a number of potato pathogens and weeds (Casagrande, 1987). As discussed by Weber and Ferro (1994), late and early planting is utilized to suppress second generation populations of larvae. Because summer-generation adults emerge later in the season on the late-planted crop, the short-day photoperiod stimulates reproductive diapause. This eliminates the second-generation larval impact on the crop. Early planting also eliminates the second generation larvae, in this case because the crop is already being removed at the time of their emergence. As discussed by Hoy et al. (1996), trap crops may be used to attract beetles away from the main crop; it has been shown to trap both overwintered beetles colonizing a field in the spring, as well as, the beetles moving away from senescing potato plants late in the season.

In addition to cultural control, a number of physical control methods can be used to suppress CPB populations. One possible method involves digging plastic-lined trenches along a field border in order to intercept post-diapause CPB colonizing the crop in the spring (Misener et al., 1993). Other methods of physical control are propane flamers and tractor-mounted vacuum collectors (Boiteau et al., 1992; Lacasse et al., 1998). The combination of these two techniques

increases their effectiveness, making the control achieved, similar to insecticide treatments (Laguë et al., 1999).

Although CPB are fully capable of completely defoliating entire fields of potato plants, at moderate CPB densities, potato plants are somewhat tolerant. They can tolerate 30% to 40% defoliation during early growth stages, 10% to 60% defoliation during middle growth stages, and up to 100% defoliation late in the season without any significant yield reduction (Cranshaw and Radcliffe, 1980; Ferro et al., 1983; Hare, 1980). Currently, there are no truly resistant, commercially accepted, cultivars, and conventional potato breeding is complicated by tetraploidy of the potato genome (Grafius and Douches, 2008). In some cases, new varieties developed by traditional plant breeding appeared to have higher levels of GAs (Khan et al., 2013). Examples of potato varieties with increased GA content developed by traditional plant breeding include Lenape, a *Solanum tuberosum* × *Solanum chacoense* cross (Sturckow and Low, 1961). Lenape, used as a cultivar with high tolerance to CPB defoliation, was not commercially accepted due to high levels of GAs in the tubers (Akeley, 1968; Anonymous, 1970). The use of naturally occurring defense mechanisms in the potato crop is being sought, as a way to efficiently decrease damage to the crop (Alyokhin, 2009; Cooper et al., 2007; Tingey, 1984 and 1991).

Glycoalkaloids

Glycoalkaloids are nitrogen-containing steroidal glycosides (Friedman, 2006; Friedman and McDonald, 1997). These naturally occurring compounds are distributed in a variety of Solanaceous crops, such as potato, eggplant (*Solanum melongena* L.), tomato (*Solanum lycoperscum* L.), and pepper (*Capsicum annuum* L.) (Friedman, 2006). As discussed by Friedman (2006), GAs of potato possess the C_{27} skeleton of cholestane and are bound

glycosidically via the hydroxyl group at the C_3 carbon atom. They contain one or two heterocyclic rings. One or both of the latter contain a nitrogen atom. The structure of the GAs differ based on the sugar functional group and the aglycon groups (Friedman, 2006). The predominant GAs in most cultivated potato genotypes are solanine and chaconine (Figure 1) (Friedman and McDonald, 1997). Both of these GAs share the same aglycon, solanidine (Sinden et al., 1986).

The biosynthesis of GAs in potato is currently not fully understood. Solanidine has been proposed to be synthesized in a biosynthetic route, which includes cholesterol synthesis (Friedman, 2006; Friedman and McDonald, 1997). Glycoalkaloids are produced in all parts of the potato plant, including leaves, roots, tubers, and sprouts (Friedman and Dao, 1992). In wild potato relatives, the most common GAs are the leptines and leptidines (Grafius, 1997). Other GAs produced in wild relatives include tomatine, demissine, and commersinone (Tingey, 1984). Some GAs occurring in the foliage, such as leptines and leptinines, are not always present in the tubers (Grafius, 1997). Biosynthesis of GAs begins at germination and reaches a peak during the flowering period (Friedman, 2006). In comparison to fruits and flowers, the leaves tend to have the highest concentration of GAs (Dao and Friedman, 1996).

Glycoalkaloids in the potato plant offer resistance against some pests, and studies have shown a correlation between foliar total GA content and CPB resistance (Tingey, 1984). Additionally, some GAs inhibit the development of fungal diseases, such as *Phytophtora spp.* (Carputo et al., 2010; Friedman, 2006), and insect pests, such as the potato leafhopper (*Empoasca fabae* Harris) (Tingey, 1984). Sanford et al. (1984) listed some wild, tuber-bearing *Solanum* species that have traits conferring pest resistance, including *S. chacoense*, *S. demissum*,

S. vernei Bitt. Et. Wittm., *S. acaule* Bitt, *S. hjertingi* Hawkes, and *S. jamesii* Torr. The main focus of research has been on *S. chacoense* and *S. demissum (*Pelletier et al., 2011*)*.

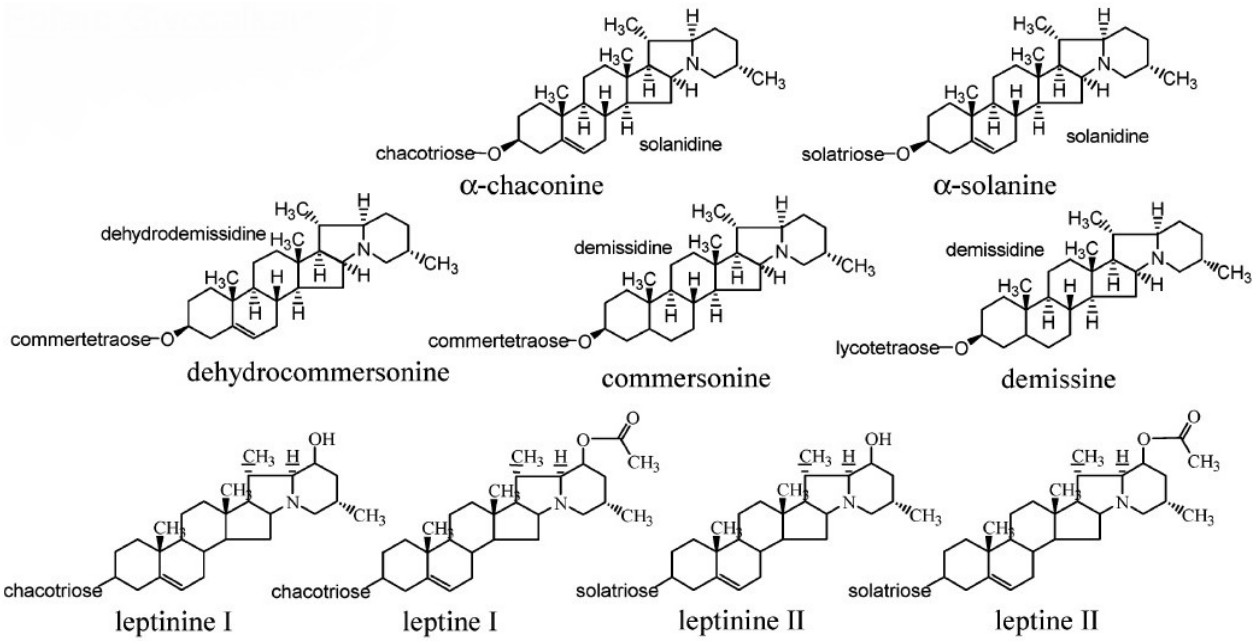

Figure 1. Glycoalkaloids in cultivated and wild potatoes (Friedman, 2006). The two common forms of GAs, solanine and chaconine, are abundant in cultivated potatoes, while the other forms of GAs are concentrated in wild potatoes.

Leptines, synthesized in genotypes with *S. chacoense* backgrounds, are potent feeding deterrents against the CPB. These compounds are only synthesized in the leaves, not the tubers (Friedman, 1997). Due to these characteristics, leptines are of major interest for developing resistance to CPB (Pelletier et al., 2011). The predominant forms of leptines, found in *S. chacoense*, are leptines I and II (Sinden et al., 1986). They share the same steroidal aglycon, and only differ by the sugar functional group (Figure 1). The steroidal aglycon of leptine is an acetylated (C_{23}) solanidine, the normal aglycon of solanine and chaconine (Figure 2). It is

possible that acetyl-leptinidine is synthesized from solanidine (Lawson et al., 1993). The conversion of solanidine to acetyl-leptinidine requires a minimum of two enzymatic activities (Lawson et al., 1993). The first enzyme adds an –OH group to the C_{23} of solanidine, which leads to the production of leptinidine (Lawson et al., 1987; Osman et al., 1987). A second enzyme synthesizes acetyl-leptinidine by adding an acetyl group to the –OH group that was added previously (Osman et al., 1987). Further acetylation may be mediated by an acetyl-transferase (Lawson et al., 1993). As discussed by Silhavy et al. (1996), considering this pathway, three phenotypic classes of GA production in potato genotypes are identified and possible. One class includes the plants that synthesize both leptinidine and acetyl-leptinidine, a second one contains genotypes that only produce leptinidine, and a third one that only produces solanidine. These three classes are observed in *S. chacoense* progenies.

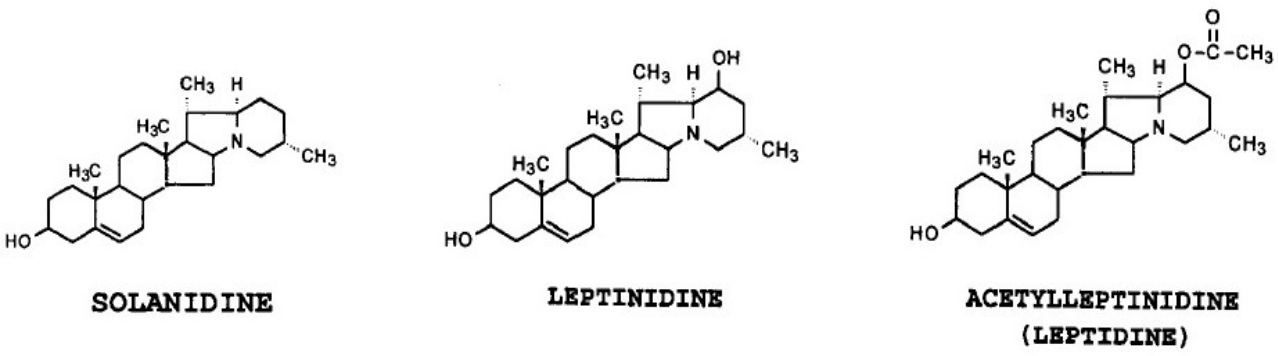

Figure 2. Solanidine, leptinidine, and acetyl-leptinidine structures (Grafius, 1997).

Glycoalkaloids can be toxic to humans and animals in high concentrations, and levels in potato tubers and products for human consumption should be below 20 mg/100 g FW (Friedman, 2006). In the potato plant, GAs are found in high concentrations in the leaves, stems, and sprouts (Friedman, 2006; Lachman et al., 2001). Relatively low concentrations of GAs can be found in

the skin of tubers and areas where sprouts emerge (Lachman et al., 2001). Dao and Friedman (1996) found that leaves had a GA concentration 10 times greater than the tubers, and a sprout GA concentration nearly 68 times greater than the tubers. The primary GA concentration in tubers is found in the first millimeter of the skin and the concentration decreases, moving towards the center of the tuber (Grafius, 1997; Nema et al., 2008). Tubers of various cultivars have uneven distribution of solanine and chaconine (Wünsch, 1989). The highest levels are found close to the eyes (Friedman, 2006). Peeling the outside tissue of the tuber before cooking removes the majority of the GAs (Pariera Dinkins et al., 2008). The greater the concentration of GAs present in tubers, the more bitter the taste (Lachman et al., 2001). Methods used to cook tubers (boiling, baking, frying, and microwaving) have variable effects on GA content (Friedman, 2006). Lack of guidelines to control processing parameters might explain the wide variation in GA content of commercial french fries (Pariera Dinkins et al., 2008), potato chips, and other processed products (Lachman et al., 2001).

Rates of GA accumulation and ratios during tuber growth and development are influenced primarily by genotype (Carputo et al., 2010; Tingey, 1984). Total GA levels generally decrease with increasing tuber size (Friedman, 2006). Therefore, desirable genotypes should possess a low rate of GA accumulation in the tuber and cease accumulation early, since some early-maturing potato plants are harvested when the tubers are small and these are often consumed unpeeled (Friedman, 1997 and 2006). Friedman (2006) found that the most pronounced increase in GA levels during potato storage occurs in the outer tuber layers. Variability among cultivars appears to depend on temperature, post-harvest management, sprouting, mechanical damage, humidity, and light (Tingey, 1984). These factors, among others, tend to induce GA synthesis in the potato tuber (Friedman and McDonald, 1997). Potato skins

tend to have high levels of GAs (Nema et al., 2008) and it may be a concern for commercial products that have high skin:flesh ratios (Friedman, 2006).

Analyses have shown that dried leaves have increased preservation of GAs compared to fresh leaves (Dao and Friedman, 1996). As discussed by Brown et al. (1999), variability in the analysis of leaf GAs is minimized by comparing leaves from the same stem position of each plant. Comparisons involving leaves from different areas of the stem showed that the GA content was not constant with respect to time or position on the stem (Brown et al., 1999). Determining GA levels on a dry weight basis instead of a fresh weight basis would be more economical and more convenient in terms of space, since freezer space to preserve the samples would not be needed (Friedman, 2006). Analysis of freeze-dried samples offers many advantages as compared to analysis of fresh samples (Brown et al., 1999; Dao and Friedman, 1996; Wünsch and Munzert. 1994). For example, the freeze-drying procedure stops compositional changes of GAs caused by enzymes, bruising, or moisture. Additionally, it allows for safe storage and facilitates transportation of samples for analysis. Finally, freeze-drying provides the chance to correlate composition to nutritional and food quality, because the same samples can be used for composition analysis and feeding studies in insects and humans (Dao and Friedman, 1996).

The GA concentration in potato is determined by specific genes (Pelletier, 2011; Sanford and Sinden, 1972). Breeding for foliar GA content is relatively difficult, due to the trait being polygenic, foliar GA content is correlated with tuber content (except for leptines), and GA production is highly influenced by environmental factors (Tingey, 1984). In efforts to enhance germplasm, resistant genotypes with a *S. chacoense* background have been developed (Akeley et al., 1968; Lorenzen and Balbyshev, 1997; Lorenzen et al., 2001; Sinden et al., 1986; Thompson

et al., 2008). The North Dakota State University (NDSU) Potato Breeding Program selected ND2858-1 as a genotype with natural pest resistance. This genotype showed reduced defoliation by CPB in the field and conferred high yield and vigor in progeny (Lorenzen and Balbyshev, 1997; Lorenzen et al., 2001). The leptines present in the foliage of this genotype are not synthesized in tubers, which is characteristic of leptines (Friedman, 2006; Friedman and Dao, 1992; Friedman and McDonald, 1997). Dakota Diamond (ND4103-2 X Dakota Pearl) (Thompson et al., 2008), a cultivar released from NDSU, is a result of crossing *S. chacoense* with *S. tuberosum* genotypes; it has reported resistance to CPB, due to GAs and an uncharacterized resistance from *S. chacoense* (Lorenzen and Balbyshev, 1997). Total GA levels for Dakota Diamond tubers, relative to check genotypes from the NDSU Potato Breeding Program, were 6.5 mg/100 g of FW (Thompson et al., 2008). This classifies Dakota Diamond as a commercially acceptable variety, since levels in potato tubers and products for human consumption should be below 20 mg/100 g FW (Friedman, 2006). Additionally, it was evaluated by Cooper et al. (2007) in a CPB field nursery and has shown resistance that may be attributed to the GAs, demonstrating that *S. chacoense* germplasm may be useful as a source to develop resistant genotypes without causing food safety issues in the market.

In field trials of potato, one or more control genotypes should be grown to assess disparities in treatments (Pelletier et al., 2011). As discussed with Dr. A.L. Thompson (personal communication, 2011), cultivars commonly used in the NDSU Potato Breeding Program for assessing GA content are Lenape, used as a high GA level check (GA concentration is unacceptably high) (Akeley, 1968; Anonymous, 1970), Russet Burbank, used as a 'barely acceptable' level check (for tubers: +17 mg/100 g FW), and Red Norland, used as a low GA accumulation check.

The diverse nature of GAs and their importance in the food industry requires the development of accurate methods to measure the content of each individual GA in fresh potato tissue (Friedman, 2006; Friedman and McDonald, 1997). Overall, there are more than 80 different GAs that have been detected in potato species, and new or undetected GAs are being reported due to the improvement of analytical methodologies (Valkonen et. al, 1996). Still, the GA levels in some crops are too low for detection, and lack of adequate measuring standards makes identification difficult (Lawson et al., 1993). Various procedures are being used for GA quantification and qualification: colorimetry (Dao and Friedman, 1996), high-performance liquid chromatography (HPLC) (Carputo et al., 2010; Pariera Dinkins et al., 2008), gas chromatography (GC) (Lorenzen et al., 2001), thin layer chromatography (TLC), mass spectrometry (MS), enzyme linked immunosorbent assay (ELISA), and biosensors (Friedman, 2006; Friedman and McDonald, 1997). Knowledge of plant secondary metabolites in potato plants is limited (Friedman et al., 1997). Additional research would lead to an improvement in understanding the GA biosynthesis and regulation in the plant, including the potential for synergy, or interaction effects among these compounds (Kowalski et al., 1999), and how their ratio in foliar tissue determines GAs effectiveness as a defense mechanism against pests and pathogens.

Justification

The potato is one of the most important non-cereal food crops in the world. A pest known to cause significant yield losses in the U.S. crop is the CPB. The economic impact of uncontrolled infestations can be substantial. As shown by Grafius (1997), after conducting a study of the economic impact of the CPB insecticide resistance on the Michigan potato industry, the estimated increased cost ranged from $44 to $69 per ha, with state-wide losses adding up to $1.4 million per year. Management of CPB has relied mainly on chemical insecticides. Due to

development of insecticide resistance, natural defense mechanisms are being sought. Gylcoalkaloids are plant secondary metabolites that offer resistance to pests and diseases, and studies show correlation between GA content and CPB resistance. Some GAs are solanine, chaconine, leptine and leptinine. Leptines, synthesized in genotypes with *S. chacoense* background, are potent feeding deterrents against the CPB. Since these compounds are only synthesized in the leaves, not the tubers, they are of major interest for CPB resistance development. Additionally, GAs can be toxic to humans and animals in high concentrations, and levels in potato tubers should be below 20 mg/100 g FW (Friedman, 2006). Focus should be on GAs that are only synthesized in the tuber, which will provide resistance against pests and diseases, while maintaining adequate yields, decreased inputs and consumer safety. Conducting a field evaluation trial will help us identify lines with potential CPB resistance and assesses yield performance under pest pressure. Profiling and assessing GA levels in potato genotypes will aid in development of selection strategies for durable resistance to CPB.

Objectives

Based on the shortcomings of our knowledge about GAs present in foliage and tuber tissue, the objectives of this research are to conduct field defoliation studies (under a naturally occurring CPB infestation) with 2 treatments (insecticide and no insecticide) at Grand Forks, ND, and to determine GA profiles in foliage and tuber tissue of 24 potato genotypes from the NDSU Potato Breeding Program.

Hypothesis

We would expect to observe differences in defoliation response and GA content between genotypes and between treatments (insecticide and no insecticide).

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CHAPTER 1: FIELD EVALUATION STUDY OF DEFOLIATION BY COLORADO POTATO BEETLE

Abstract

Potato (*S. tuberosum* L.) is a crop susceptible to many diseases and pests that affect production and overall yield. A pest that causes the highest yield losses is the Colorado potato beetle (*L. decemlineata* Say) (CPB). Management of this pest has relied primarily on chemical insecticides and, due to development of insecticide resistance, natural defense mechanisms are being sought. Gylcoalkaloids (GA) are plant secondary metabolites that offer resistance against certain pests, and studies show correlation between foliar GA content and CPB resistance. To assess CPB resistance in 24 potato genotypes from the NDSU Potato Breeding Program, a field evaluation trial was conducted at Grand Forks, ND, during the 2012 growing season. A split block arrangement in a randomized complete block design (RCBD) was utilized. The treatments consisted of a block treated with imidacloprid (Admire®) (a nicotine-based insecticide) applied in-furrow at planting, and an untreated (control) block, for assessment of defoliation through the growing season. Defoliation percentage mean across treated genotypes was 5%, and 28% for untreated. Across genotypes defoliation ranged from 2 to 15% for treated, and 5 to 48% for untreated. Yield data were collected to evaluate effects of defoliation. The average yield for treated genotypes was 33.3 tonnes/ha, compared to 19.1 tonnes/ha for untreated genotypes. At this stage of the research, we are able to identify which genotypes have potential CPB resistance in the field. This data can aid in the parental selection process for improved field resistance to CPB and in the development of a breeding scheme by selecting genotypes with good field

performance and crossing them with genotypes that can provide other quality traits, such as high yield.

Introduction

Potato (*Solanum tuberosum* L.) is a crop susceptible to many diseases and pests that affect production and overall yield (Alyokhin, 2009; Pelletier, 2011). Cultural management procedures, including application of pesticides are necessary to control these biotic stresses and diminish losses (Alyokhin et al., 2008; Boiteau, 1988; Ferro, 1985; Forgash, 1985; Hare, 1990; Webb, 2007). Field evaluation studies are conducted each season to assess damage caused by pests to crop development and production (Alyokhin, 2009; Alyokhin et al., 2006). A pest that causes some of the greatest yield losses is the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (CPB) (Alyokhin, 2009). The economic impact of uncontrolled infestations can be substantial (Alyokhin et al., 2008; Grafius, 1997; Hare, 1980; Mailloux and Bostanian, 1989). Crop rotation, biological control, and alternative tactics (manipulation of planting time and crop varieties, use of mulches, cover and trap crops, and flame trowers) for regulating populations are useful. However, management of this pest has relied primarily on chemical insecticides (Alyokhin, 2009; Hare, 1990). As result of intensive insecticide use, CPB has developed resistance to the majority of the insecticides utilized for its control (Forgash, 1985; Whalon et al., 1993).

Imidacloprid is one of the most widely used insecticides worldwide (Tomizawa and Casida, 2003), due to its systemic uptake and selective toxicity. Growers normally apply imidacloprid in-furrow to the whole field at planting (Alyokhin et al., 2006). Systemic applications in the field cause strong selection pressure on insect populations, potentially leading

to insecticide resistance development (Alyokhin, 2009; Alyokhin et al., 2006 and 2008; Coombs et al., 2005; Cooper et al., 2007; Grafius, 1997). Cases of resistance to imidacloprid have been reported in Eastern U.S. and Canada (Alyokhin et al., 2007; Olson et al., 2000; Zhao et al., 2000). As discussed by Alyokhin (2009), unless appropriate actions are taken to minimize exposure and subsequent selection pressure to vulnerable populations, failure of imidacloprid and neonicotinoid insecticides for control of the CPB is expected. Due to this history of resistance, there is a growing concern for human health and the environment, caused by the excessive use and application of pesticides (Spooner and Bamberg, 1994). It is in the best interest of potato growers to continue to use all available integrated pest management approaches to minimize the use of imidacloprid and other pesticides (Alyokhin, 2009; Dively et al., 1998).

Compared with many other staple crops, the cultivated potato has a broad pool of genetic diversity for natural host plant resistance to pests and diseases within its wild relatives (Grafius, 1997; Sinden et al., 1986). The wild species *S. demissum*, *S. polyadenium* and *S. chacoense* are known to be very resistant to CPB (Sinden et al., 1986; Flanders et al., 1992) due to synthesis of plant secondary compounds. Many plant secondary compounds have natural pesticide qualities (Lachman et al., 2001), and there is increasing interest to improve the presence of these natural pesticides for improved control of pests and diseases (Friedman and McDonald, 1997). Gylcoalkaloids are cholinesterase inhibitors, functioning much like organophosphate and carbamate insecticides (Lawson et al., 1993). These GA compounds in the potato plant offer resistance against certain pests, and studies have shown a correlation between foliar total GA content and CPB resistance (Tingey, 1984). Species which exhibit pest resistance tend to have increased levels of GAs compared to susceptible ones (Tingey, 1984). Most GAs are distributed throughout the potato plant foliage and tuber tissue. While high GA levels could be useful host

plant resistance factors, at high concentrations they impart a bitter taste in the tuber (Friedman and McDonald, 1997; Pariera Dinkins et al., 2008). *Solanum chacoense* Bitt., a wild relative of potato, has been of major interest to plant breeders because it produces an abundance of GA compounds, including leptines and leptinines, which are effective deterrents of herbivory by CPB. Leptines and leptinines are only expressed in the foliage (Lorenzen et al., 2001) and could potentially provide protection from foliar pests, while alleviating the human health concern associated with high GA content in the tuber (Sinden et al., 1986).

Plants are being bred to contain a greater diversity of natural compounds in increased quantities (Hlywka et al., 1994; Pariera Dinkins et al., 2008), but despite breeding efforts, no potato cultivars with demonstrated field resistance to CPB have been released commercially (Grafius and Douches, 2008; Yencho and Tingey, 1994). Still, host-plant resistance has been suggested as the only long term solution to control CPB (Spooner and Bamberg, 1994). A field evaluation trial was conducted at Grand Forks, North Dakota (ND), during the 2012 growing season to assess defoliation damage of genotypes from the NDSU Potato Breeding Program. The genotypes utilized in the trial have naturally occurring defense mechanisms (GAs), which are synthesized when plants are under stress (Hlywka et al., 1994). By increasing GA presence, lower defoliation damage by CPB should be observed in the field. This field trial can help identify lines with potential towards developing CPB-resistant varieties.

Materials and methods

Resistance to CPB was assessed in a field evaluation trial under natural infestations. Genotypes (Table 1) for the 2012 field evaluation trial were planted in a non-irrigated plot at Grand Forks, ND, on May 23, 2012. Seed was prepared on May 22, 2012, and Maxim MZ® was

applied as the seed piece treatment. The planting configuration consisted of five hills per plot, spaced 30.5 cm between hills; rows were spaced 91.4 cm apart. Plots were separated, within the row, by 152.40 cm of unplanted soil. Red Norland was planted as a border. Seed piece spacing was 30.50 cm and row spacing was 91.40 cm. The genotype ND2858-1 was planted in pots from (tissue culture) plantlets at the greenhouse, and transplanted to the field on July 10, 2012, because non-dormant seed tubers were not available. The main focus of the trial was on germplasm derived from *S. chacoense*. Control genotypes were grown to assess variation in treatments. Cultivars utilized as controls for assessing GA content were Lenape (Akeley et al., 1968; Anonymous, 1970) and ND2858-1 (Lorenzen and Balbyshev, 1997), used as high GA level checks, Russet Burbank, used as a 'barely acceptable' level check (for tubers: +17 mg/100 g FW), and Red Norland, used as a low GA accumulation check (Thompson, personal communication, 2011). A split block arrangement in a randomized complete block design (RCBD) was utilized. Treatments were randomized in each of two replicates. Genotypes were randomized within each treatment, and replicated. The split block arrangement is suited to analyze the two factor experiment (genotype and treatment) and the interaction effect between the two factors. Natural CPB infestation for defoliation assessment was expected to occur in the field. The split block treatments consisted of a block treated with 584.6 mL/ha of imidacloprid (Admire®) (a nicotine-based insecticide) applied in-furrow at planting, and an untreated (control) block, for assessment of defoliation through the growing season. The maximum amount of Admire® that can be applied to the field during one crop season is 609.0 mL/ha (Bayer CropScience). Standard agronomic practices were applied in the field. On June 13, three weeks after planting, fertilizer was applied as a side dress (68.5 kg of N and 62.6 kg of P). Herbicide was applied four weeks after planting, on June 20: Prowl® (2338.5 mL/ha), Sencor® (0.6 kg/ha),

and Matrix® (109.6 mL/ha). Fungicide was applied, approximately every seven days, beginning prior to row closure and continuing through the growing season: Bravo Zn® (2484.6 mL/ha) was applied on July 17 (~7 weeks after planting) and August 13 (~13 weeks after planting), and Manzate® (2.25 kg/ha) on August 6 (~12 weeks after planting), 20 (~14 weeks after planting), and September 4 (~16 weeks after planting). Vine kill occurred due to frost on September 23 (~19 weeks after planting) and harvest was conducted on September 25, 2012 (~19 weeks after planting).

Data collection began on July 10 and ended on August 17. Defoliation percentage data was collected twice a week through the growing season. The first (July 10) and last date (August 17) of data collection were not included in the analysis due to variations attributed to experimental error. The dates of data collection used for the analysis were July 13, July 17, July 20, July 24, July 27, July 31, August 3, August 7, August 10, and August 14. Defoliation percentage was determined based on visual evaluation and estimate. To minimize human error, estimations were conducted without reference to treatment or genotype.

Statistical analyses

Field defoliation data were analyzed by repeated measures analysis of variance (ANOVA) using PROC MIXED (SAS Institute 2012). Transformation of field defoliation data was not conducted. Yield data was statistically analyzed by ANOVA using PROC GLM. Treated and untreated groups were compared ($\alpha \leq 0.05$) using SAS 9.3. Possible correlation between yield and defoliation percentage, and between defoliation damage and GA content, was tested using Pearson's correlation analysis (PROC CORR, SAS Institute 2012).

Table 1. Genotypes planted in the 2012 field evaluation trial at Grand Forks, ND.

Genotype	**Parentage**	
	Female	**Male**
ND071289CAB-3	ND039104CAB-3	ND028799c-2
ND071289CAB-4	ND039104CAB-3	ND028799c-2
NDJL3C-2	ND4382-19	N142-72
NDJL3C-4	ND4382-19	N142-72
NDJL7C-1	ND4382-51	N140-201
NDJL7C-2	ND4382-51	N140-201
NDJL21C-3	ND5374-9B	Q115-24
ND060838C-3	ND028799C-3	ND860-2
ND060838C-14	ND028799C-3	ND860-2
463-4	US-W730	*S. berthaultii*
ND4100C-19	ND2858-1	Norchip
ND4100C-22	ND2858-1	Norchip
ND4382-17	ND2858-1	Norchip
ND4382-19	ND2858-1	Norchip
ND5873-53	ND4382-19	Chipeta
ND5873-21	ND4382-19	Chipeta
ND4708-6PE	ND2858-1	Norland
ND4710-10	ND2858-1	ND860-2
ND2858-1	*S. chacoense*	ND1215-1
Dakota Diamond	ND4103-2	Dakota Pearl
Dakota Pearl	ND1118-1	ND944-6
Lenape	47156	B3672-3
Red Norland	Sport of Norland	
Russet Burbank	Sport of Burbank	

Results and discussion

A field evaluation trial was conducted at Grand Forks, ND, during the 2012 growing season, to assess CPB resistance in 24 potato genotypes from the NDSU Potato Breeding Program. The main focus of the trial was on germplasm derived from *S. chacoense*, although not

all the genotypes (Table 1) produce the same types of GAs (Silhavy et al., 1996). The wild species, *S. chacoense,* is an interesting source of genetic resistance to CPB, due to production of leptine (Friedman, 1997). Common cultural practices were applied in the field during the growing season and natural colonization of CPB occurred in the field. Defoliation data was collected across the 24 genotypes, two times a week, through the growing season: July 13, July 17, July 20, July 24, July 27, July 31, August 3, August 7, August 10, and August 14.

Table 2. Analysis of variance of CPB defoliation data from the 2012 field evaluation trial at Grand Forks, ND.

Effect	Num DF	Den DF [1]	F value	Pr > F
Treatment	1	24	363.64	<0.0001*
Genotype	23	24	5.57	<0.0001*
Treatment x Genotype (error a)	23	24	3.59	0.0014*
Date	9	24	113.95	<0.0001*
Treatment x Date	9	24	35.79	<0.0001*
Genotype x Date	207	24	2.42	0.0063*
Treatment x Genotype x Date (error b)	207	24	2.02	0.0219*
Replicate	1	24	6.12	0.0209*
Replicate x Genotype (error c)	23	24	1.21	0.3249[ns]

* significant at P≤0.05, [1] = denominator DF, [ns]= not significant at P≥0.05.

Defoliation data were analyzed by repeated measures analysis of variance (ANOVA) using PROC MIXED (SAS Institute 2012) at $\alpha \leq 0.05$ significance value. Analysis of variance (ANOVA) (Table 2) showed significance for treatment by genotype by date, genotype by date, treatment by date, and the treatment by genotype interactions; the replicate by genotype interaction was not significant. Analysis of variance showed significance for all single effects: date, genotype, treatment, and replicate. The significance of replicates could be caused by the edge effect in the field. The genotype used as a border, Red Norland, is very susceptible to CPB

damage and this could have caused differences between the replicates in the field. The error a was used to calculate treatment effect and genotype effect, error b to calculate date effect, treatment by date interaction, and genotype by date interaction, and error c to calculate replicate effect.

The significance of the three way interaction (treatment x genotype x date) indicates that there is a two-way interaction that varies across levels of a third variable. Each date had an effect on the genotype defoliation percentage mean, for each treatment (treated and untreated). Due to date being a progression, defoliation percentage differences for each level of date were expected. Also, due to the nature of defoliation damage, the final date is the effective representation of the genotype response to CPB feeding. To further analyze the three-way interaction, the two-way interactions are discussed. The significance of the genotype by date interaction shows an influence of date on defoliation damage (%). Defoliation observations are additive; due to CPB damage being consistent through the growing season. This is very dependent on CPB population density (Hare, 1980). The critical period during which potatoes are most severely affected by defoliation corresponds with the emergence and oviposition of summer generation adult CPB (Hare, 1980). As mentioned by Hare (1980), beetles are present at times other than their peak abundance, causing continuous, but mild defoliation. Young plants have sufficient reserves and potential to compensate for some insect damage, but repeated damage decreases the plants ability to compensate (Hare, 1980).

The two levels of insecticide treatment (treated and untreated) and the 24 levels of genotype had an effect on CPB defoliation mean for each assessed date. This indicates that imidacloprid is currently providing effective control of CPB in the field. Because of its long residual and effective systemic activity against CPB, imidacloprid has become a widely used

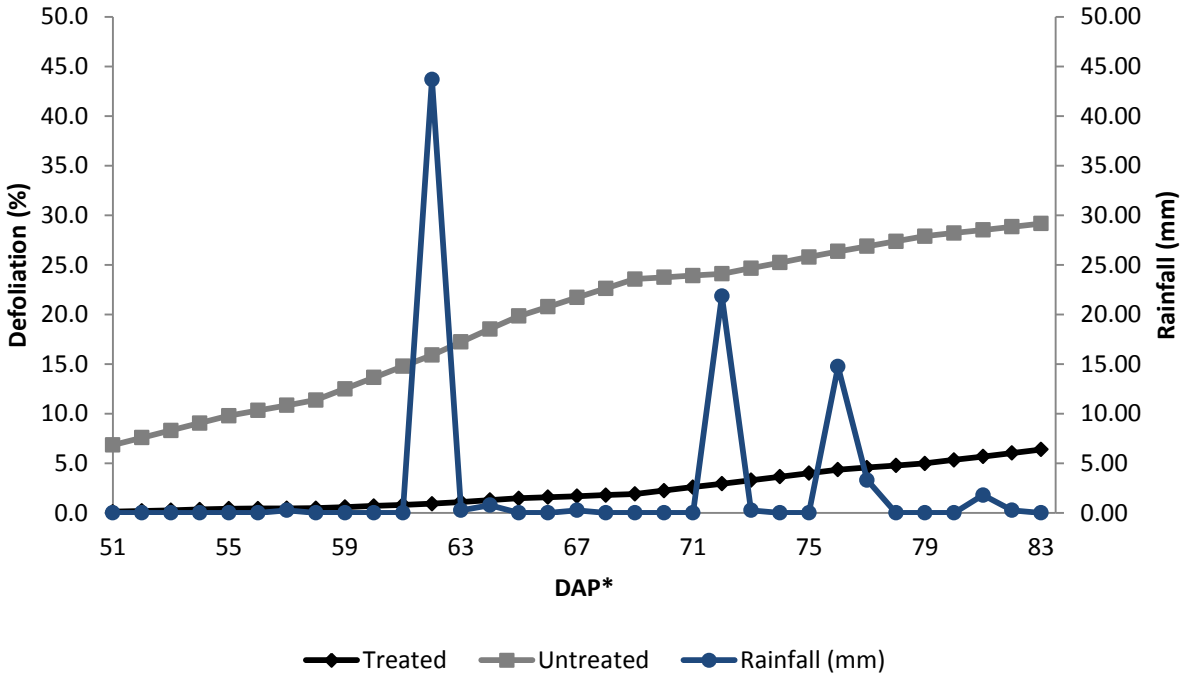
insecticide, usually in areas where populations are resistant to other insecticides (Alyokhin et al., 2006; Dively et al., 1998). It is usually applied in-furrow at planting. In-furrow applications reduce the need for foliar insecticide applications, because this could lead to increased efficacy and persistence of the toxin, which could increase selection intensity and accelerate the rate of pesticide resistance (Taylor et al., 1983). A study conducted by Alyokhin et al. (2006) showed that in-furrow applications of imidacloprid provided good control during four years of use, but efficacy declined in the years following. Knowing the history of insecticide exposure and susceptibility to individual insecticides for individual CPB populations is important in achieving good pest control (Alyokhin, 2009). Mechanisms and levels of resistance in the CPB populations may be highly diverse even within a narrow geographical area (Ioannidis et al., 1991). Consequently, results of the 2012 field evaluation trial at Grand Forks should only be applied to fields with similar histories of insecticide use and resistance, and to CPB colonies from the region (Alyokhin et al., 2006).

The CPB has been inadequately managed for many years (Alyokhin, 2009; Casagrande, 1987). Improved and alternative methods for control are needed to reduce pest pressure in the field, as well as, insecticide resistance development. As discussed by Alyokhin (2009), timing of insecticide application is critical to successful control. It is recommended to apply early in the season, since small larvae are easier to kill than larger ones. Most defoliation damage is caused by late-instar larvae, but adults can cause considerable damage by feeding as well (Alyokhin, 2009). The recommendation for CPB management in ND is to spray at first egg hatch (Knodel et al., 2012). Good results are achieved by flagging the first egg masses that are located in the field, monitoring the population levels, and spraying at 15% to 30% hatch. If the insecticide used is effective, but not persistent, a second application should be made five to ten days later. For a

second application, the use of an insecticide with a different mode of action and chemistry is recommended, to decrease development of insecticide resistance (Knodel et al., 2012). This approach should provide control of the first generation CPB larvae. Also, adequate insecticide coverage on the crop canopy is a critical factor to insure that the CPB population is exposed. Larvae that are not killed by the first application could be resistant to the insecticide and will not be affected by a second application of the same insecticide. Populations that are too low to cause economic damage should not be treated. For the 2012 field evaluation trial at Grand Forks, insecticide was applied in-furrow at planting to treated rows, providing control of young larvae (first generation). Controlling the first generation diminishes pest damage at the beginning of the growing season but, as previously mentioned, if any other insects are left behind it could be due to insecticide tolerance or resistance. Still, CPB presence in all life stages was consistent in our trial throughout the growing season, mostly in the untreated block. The beetles that resurfaced in the untreated block (which were not exposed to the chemical) dispersed in the field, leading to colonization of the treated block as well. Soil half-life for imidacloprid ranges from 40 days in soil that has not been amended to 124 days for soil amended with organic fertilizers (Rouchaud et al., 1994). Also, imidacloprid presence in the soil is affected by rain, among other environmental factors. Precipitation data (Figure 3) collected from North Dakota Agricultural Weather Network (NDAWN) shows the amounts for the 2012 growing season at Grand Forks (NDAWN, 2012).

The allowed use of (imidacloprid) per crop season is 609.0 mL/ha, which is the amount applied at planting. If any other pesticide would have been needed it would have to be of a different mode of action to prevent resistance development (Knodel et al., 2012). In the treated block, colonization was expected to be less. At the beginning of the growing season CPB were

not present, but towards the middle of the season, the pest dispersed to the treated block. The dispersal from the untreated block could have been caused by the decrease of food availability in the untreated block, which promotes the movement of the pest to another field containing the host plant. Even though treatments were significantly different at an $\alpha \leq 0.05$ (Table 2), under visual assessment, both groups (treated and untreated) had CPB presence. This could be caused by high population densities of the CPB, small size of the field, and lack of chemical barrier in the untreated block, which served as a refuge and allowed the pest to develop and disperse over to the treated block.



*DAP , days after planting (July 13 – August 14)

Figure 3. Rainfall (mm) (NDAWN, 2013) and progression of CPB defoliation (%) in treated genotypes and untreated genotypes in the 2012 field evaluation trial at Grand Forks, ND.

The trial at Grand Forks was moderately defoliated towards the end of the growing season; no genotypes had damage above 50%. The defoliation mean of the treated block was 7%

and 30% for the untreated block. If the CPB were able to overcome the first application of imidacloprid, a second application would have failed (Knodel et al., 2012), and may have increased resistance development of CPB colonies in the region. The differences in defoliation percentage could be associated with insecticide treatment and GA concentration in genotypes. In our field trial we expected to see decreased damage in the treated block through the growing season. Genotypes and treatment were highly significant in the analysis (Table 2). The genotypes had varied responses to treatment in terms of CPB defoliation; this is presumed to be due to variation of GA content among genotypes. This variation in response could also be related to many external factors, such as planting layout and CPB dispersal through the field. In a study conducted by Dively et al. (1998), genotypes located close to heavily damaged plots or border rows were highly affected by CPB due to movement inside the plot. Also, CPB colonizing from field edges that increasingly encounter treated rows are most likely to not cause damage to the rest of the field due to insecticide exposure (Dively et al., 1998). As discussed by French et al. (1993), regardless of whether potatoes are planted adjacent to, or isolated from previous year fields, a significant number of diapausing beetles overwinter in surrounding non-host areas and colonize fields along the outside edge. Untreated Red Norland was used as a border and this could have increased the edge effect by increasing CPB pressure in plants located closer to the edge of the field.

Germplasm with *S. chacoense* background were expected to have decreased defoliation, even without insecticide. Due to stress caused by defoliation, an increase of GA levels in the potato plant tissue is expected (Coombs et al., 2005: Hlywka et al., 1994), as well as, a decrease in tuber yield and grade. Correlation analysis was conducted between defoliation damage (%) and GA concentration; this data will be discussed in Chapter 2. Density of CPB and defoliation

percentage should decrease in rows treated with imidacloprid (Dively et al., 1998). The treatment by genotype interaction indicates that the difference in defoliation between treated and untreated conditions is much greater for some genotypes than others (for example, ND2858-1 compared to Dakota Pearl). This suggests that the naturally occurring resistance mechanism derived from *S. chacoense*, in this case the GAs, are effective in controlling CPB in the field.

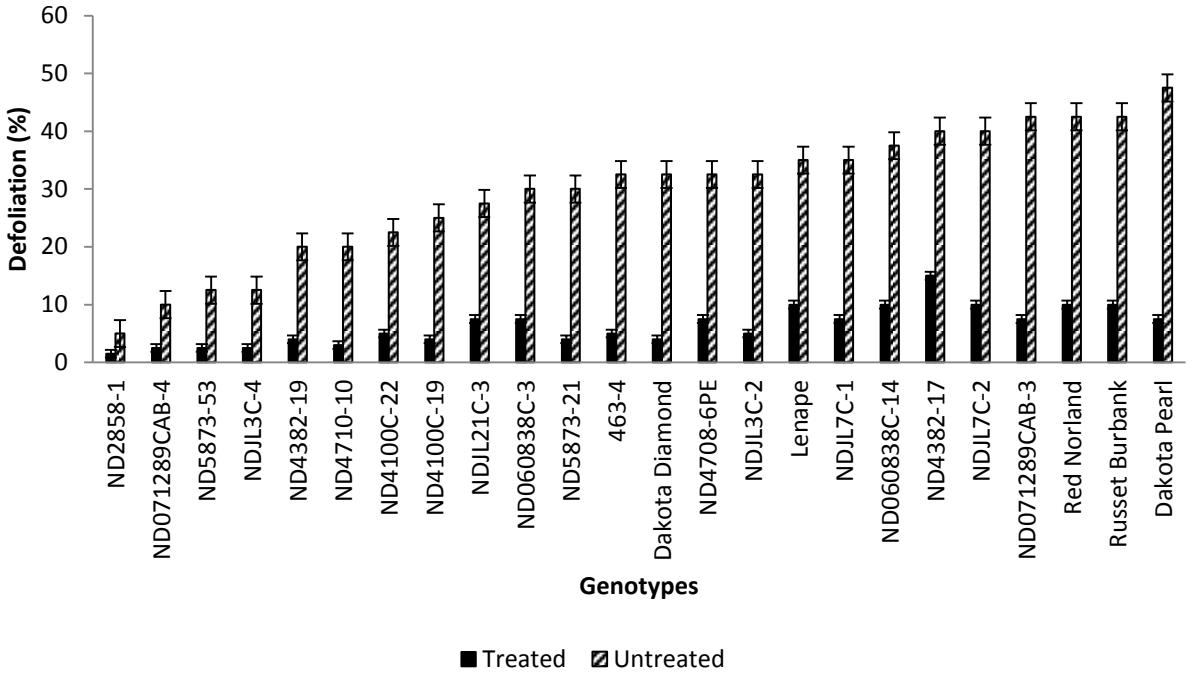


Figure 4. Colorado potato beetle defoliation (%) for 24 genotypes under both treated conditions and untreated conditions in the 2012 field evaluation trial at Grand Forks, ND. Error bars represent ±SE. LSD for defoliation damage (%) comparison under treated and untreated conditions = 7.6.

The treated genotypes had less CPB defoliation damage through the season compared to the untreated genotypes, which were considerably defoliated (Figure 4). Defoliation percentage mean across treated genotypes was 5% (SD = 2.68) and for untreated genotypes was 28% (SD =

11.11); defoliation percentage across genotypes ranged from 2 to 15% for treated, and from 5 to 48% for untreated genotypes. As seen in Figure 4, in the untreated block the genotypes with the highest defoliation values were Dakota Pearl (48%), Russet Burbank, Red Norland, and ND071289CAB-3 (all with 43%); the genotypes with the lowest defoliation values were ND2858-1 (5%) and ND071289CAB-4 (10%). In the treated block, the genotypes with the highest defoliation values were ND4382-17 at 15%, and Russet Burbank, Red Norland, NDJL7C-2, and ND060838C-14, all with 10% defoliation; the genotypes with the lowest defoliation values were ND2858-1 at 2%, and ND071289CAB-4, ND5873-53, and NDJL3C-4, all with 3%. Although CPB are fully capable of completely defoliating entire fields of potato plants, at moderate CPB densities, potato plants are somewhat tolerant of defoliation (Hare, 1980). Based on our one-year data, the genotypes ND2858-1, ND071289CAB-4, ND5873-53, and NDJL3C-4 could be rated as resistant to CPB. The genotypes ND4382-19, ND4710-10, ND4100C-19, ND4100C-22, NDJL21C-3, ND060838C-3, and ND5873-21 would be rated as moderately resistant. The genotypes Dakota Diamond, 463-4, ND4708-6PE, NDJL3C-2, Lenape, NDJL7C-1, ND060838C-14, ND4382-17, and NDJL7C-2 would be rated as moderately susceptible, and ND071289CAB-3, Dakota Pearl, Red Norland, and Russet Burbank are highly susceptible. High defoliation damage was expected in Red Norland due to its susceptibility to CPB, caused by low GA content (Thompson et al., 2008), which are known to provide protection against CPB (Friedman, 2006; Tingey, 1984). The genotype 463-4 is known to have glandular trichomes and CPB resistance (Novy and Helgeson, 1994), which should lead to good pest feeding deterrence, although our one-year data showed otherwise. The genotype ND2858-1, known for its *S. chacoense* background (Lorenzen and Balbyshev, 1997), had CPB damage mainly towards the end of the growing season (Figure 4), but the damage was minimal compared

to the rest of the genotypes. This genotype has shown consistent response under various levels of CPB colonization, but in this trial we were not able to measure overall field performance due to the genotype being planted later in the field and not having yield data due to lack of tuberization under long day conditions.

Dakota Pearl is a genotype known to have moderate resistance to CPB damage (Thompson et al., 2005). In the trial at Grand Forks, under untreated conditions, Dakota Pearl was very susceptible to CPB with 48% defoliation; under treated conditions it had good performance against the CPB with 5% defoliation. As discussed by Thompson et al. (2008), Dakota Diamond exhibits preferential avoidance by CPB in feeding trials. A replicated trial with 24 genotypes conducted in 1998 at Park River, ND, showed a wide difference between Dakota Diamond and the rest of the genotypes; Dakota Diamond had 9% defoliation damage and the trial mean was 55%. The 1998 Park River trial showed that Red Norland, Russet Burbank, and Dakota Pearl had high defoliation rates, 70%, 52%, and 62%, respectively. Dakota Diamond is known to require reduced applications of chemicals to control CPB (Thompson et al., 2008), which explains the good performance under CPB colonization in treated (4%) conditions (Figure 4) in our trial. The mechanism for resistance could be related to the presence of high levels of GAs in the leaves conferred by its *S. chacoense* background (Lorenzen et al., 2001). Dakota Diamond field resistance to CPB has also been observed in small plot choice trials (Coombs et al., 2005), but during the field trial at Grand Forks its defense mechanisms did not deter CPB feeding. The genotype ND4382-19 is known to have high resistance to CPB, low GA content, and good agronomic performance (Sagredo Diaz et al., 2009; Lorenzen et al., 2001). In the 2012 field evaluation trial at Grand Forks, defoliation damage to ND4382-19 under treated conditions was 4%, while in untreated genotypes the defoliation was 20%, which is good performance

considering the defoliation damage of other genotypes (for example: Dakota Pearl) was considerably high.

Through the growing season, beetles were found on all genotypes, regardless of host plant resistance mechanisms (GA nature), although near the end of the season the beetles were not actively feeding. Similar results were seen by Coombs et al. (2005), where a field evaluation trial was conducted and various plant resistance mechanisms were assessed for control of CPB. The resistance mechanisms of the genotypes consisted of *S. chacoense* background, glandular trichomes, *Bt-cry3A*, *S. chacoense* + *Bt-cry3A*, glandular trichomes + *Bt-cry3A*, and a susceptible control. The CPB did not show any preference for genotypes and were present in all genotypes across the field. Still, the genotype with *S. chacoense* derived resistance demonstrated strong resistance and deterrence when compared to the rest of the genotypes (Coombs et al., 2003 and 2005). This shows that the defense mechanism of GAs could only serve as an avoidance strategy that, as the growing season develops, decreases in effectiveness. This compares to what we observed in our trial; at the end of the growing season, genotypes that were expected to have good performance against the CPB had average performance. An example of this is Lenape, a variety that is considered to deter CPB feeding due to high GA levels (Akeley et al., 1968); in our trial, Lenape was classified as moderately susceptible, which was not expected for this variety. This could be due to high insect pressure, or other environmental factors.

Alternative mechanisms of control should be combined to obtain a higher level of control for CPB and decrease losses. Also, differences in variety, geographical location, cultural practices, timing of defoliation, microclimate, soil type, soil fertility, photoperiod, and rain water, are possible sources of variation in defoliation studies (Mailloux and Bostanian, 1989). These differences should be assessed to accurately detect field resistance in genotypes. It is

common to conduct screening under laboratory conditions, which is useful for development of initial recommendations for potato growers in areas impacted by neonicotinoid resistance. True field resistance can only be identified and measured under field conditions, since genotype performance could vary when exposed to environmental factors (Friedman and McDonald, 1997).

Multiple environmental factors affect CPB colonization in the field (Lactin and Holliday, 1994; Logan et al., 1985). Day length at the time of adult emergence from the soil has a decisive influence on reproductive behavior (de Wilde et al., 1959; de Wilde and Hsiao, 1981), and on diapause induction. Colorado potato beetle requires a long photoperiod and enters diapause after exposure to critically short photoperiod. In addition to photoperiod, low temperatures, senescing leaves, or absence of foliage at the end of the growing season, can result in diapause induction at relatively long photoperiods (de Wilde et al., 1959; Hare, 1990; May, 1981; Lactin and Holliday, 1994). As discussed by Jansky et al. (2009), during field seasons in temperate regions, adult CPB emerge from overwintering sites adjacent to cultivated fields in late spring. They colonize emerging potato plants in early summer and lay eggs. Larvae emerge from the eggs in mid-summer and pass through four instar stages over a period of about three weeks.

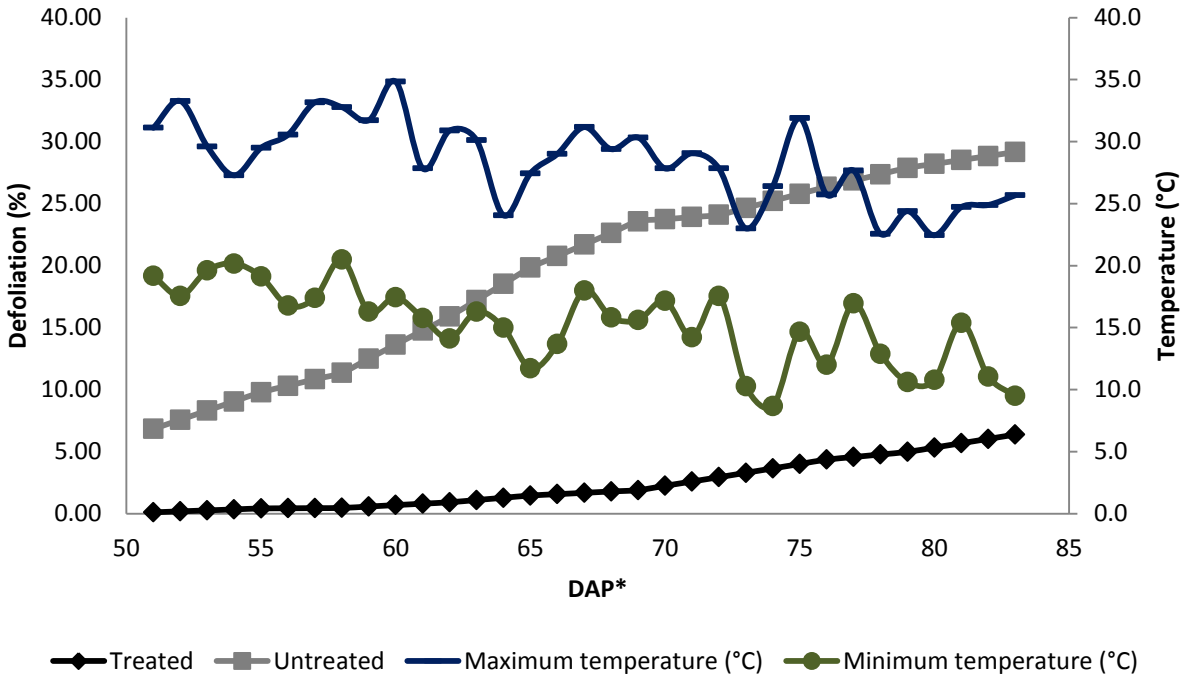
In the trial at Grand Forks, CPB began colonizing the field right after crop emergence (mid-June). Figure 5 shows that damage was very high in early August, when the CPB second generation usually hatches and larval feeding is at its highest (Radcliffe and Ragsdale, 1993; Senanayake et al., 2000). Colorado potato beetle populations should be constantly monitored during the growing season (Knodel et al., 2012). It is recommended that producers be familiar with the life cycle of the insect and be able to identify the emergence of the first field generation and treating beforehand to prevent losses. Density of summer adults can be reduced by control of

larvae early in the season and by control of the previous CPB generation. Currently, CPB control in the Red River Valley area is obtained by systemic insecticides at planting and applications during the remaining growing season (Senanayake et al., 2000). In our trial, chemical application achieved adequate control of the CPB through the entire season. If we observe Figure 4, the defoliation percentage in both the treated and untreated blocks did not reach 50% of damage. The insecticide Admire can only be applied once during the crop season, indicating that the active ingredient was present through the season. Still, the CPB located in the untreated block were able to disperse and colonize the treated block as well.

Temperature is an environmental factor that influences the colonization and spread of CPB in the field (Lactin and Holliday, 1994; Logan et al., 1985). Colorado potato beetle will emerge earlier in the season (Radcliffe and Ragsdale, 1993; Senanayake et al., 2000) if the temperatures are high enough (Radcliffe and Ragsdale, 1993). On the other hand, if temperatures are low, the insect will take longer to resurface from the soil and colonization might take place later in the growing season. In a study conducted by Cooper et al. (2007), the lower temperatures during the growing season slowed CPB development rate and affected survival to adult stage. Temperatures above 27°C increase hatching and development rate of CPB (Lactin and Holliday, 1994; Ferro et al., 1985; May, 1981; Radcliffe and Ragsdale, 1993). Figure 5 shows daily temperature data obtained from NDAWN (http://ndawn.ndsu.nodak.edu/, 2013), compared with the damage progression in the field. During July and August the overall temperature was above 27°C. Temperatures at the beginning of the season were 30°C/20°C (maximum/ minimum), and decreased towards the end of the season. As the temperature increases the insects leave the soil, and begin colonizing the plants, leading to plant damage.

The plants emerged approximately three weeks after planting, by the week of June 11. The CPB resurfaced from the soil soon after plant emergence, approximately by the week of June 18. Damage increase was observed around 70 days after planting (DAP). The damage reached a plateau at the end of the season, which usually is a response to lower temperatures (Lactin and Holliday, 1994; Logan et al., 1985). The decrease in temperature triggers diapause, causing the CPB to burrow back into the soil, and leading to cessation of field defoliation damage. In our case, this could be due to decrease of plant canopy or another external factor, since we stopped collecting data on August 17 (DAP). Temperatures were still high (Figure 5) towards the end of the data collection. As discussed by Lafta and Lorenzen (2000), temperature in the growing environment influences resistance of potato plants to CPB by affecting leptine levels in the foliage. High temperatures might stimulate GA accumulation in leaves. Awareness of environmental conditions is necessary when assessing potato plants for CPB resistance.

Yield data for treated and untreated genotypes was recorded as tonnes/ha (Figure 6). Analysis of variance was conducted for yield data (Table 3). The replicate, treatment, and genotype effect were significant, as well as, the genotype by treatment interaction. Treated genotypes had higher yield compared to untreated genotypes. The average yield for treated genotypes was 33.3 tonnes/ha (SD =11.70), compared to 19.1 tonnes/ha (SD =9.50) for untreated genotypes. Based on observation while collecting yield data, tubers from the untreated block were significantly smaller in comparison to tubers from the treated block. The genotype ND2858-1 was not considered in the yield ANOVA, because no yield data was obtained from treated or untreated plots. As previously mentioned ND2858-1 does not set tubers under long day conditions, which characterize summers in North Dakota.



*DAP , days after planting (July 13 – August 14)

Figure 5. Maximum and minimum daily temperature (°C) (North Dakota Agricultural Weather Network, 2013) and progression of CPB defoliation (%) in treated genotypes and untreated genotypes in the 2012 field evaluation trial at Grand Forks, ND. *DAP, days after planting (July 13 – August 14).

In the 2012 field evaluation trial at Grand Forks, a decrease in yield was expected, most of all in the untreated block (Figure 6) due to increased defoliation and susceptibility to CPB since the chemical barrier was not established. Correlation analysis was conducted between defoliation percentage and yield of the treated genotypes ($r = -0.02$, $n = 23$, and $p = 0.93$) and of the untreated genotypes ($r = -0.15$, $n = 23$, and $p = 0.48$). It showed that there was no correlation between the two variables under the two treatments (treated and untreated). The genotype ND2858-1 was not considered for correlation due to the lack of yield data.

Table 3. Analysis of variance for yield data (tonnes/ha), of treated and untreated genotypes, from the 2012 field trial at Grand Forks, ND.

Effect	DF	Type III SS	Mean square	F value	Pr > F
Replicate	1	187.074088	187.074088	6.13	0.0171*
Treatment	1	4620.837392	4620.837392	151.45	<.0001*
Genotype	22	8222.296924	373.740769	12.25	<.0001*
Genotype*Treatment	22	1769.252233	80.420556	2.64	0.0029*
Error	45	1373.01366	30.51141		

* significant at $P<0.05$, ^{ns}= not significant at $P\geq0.05$.

The genotype ND4382-19 was selected for high tolerance to CPB, reasonably low tuber GA, and good agronomic performance (Lorenzen et al. 2001). Even with GAs as a defense mechanism, all genotypes had CPB damage. Dakota Diamond is known to have good field performance and high yield (Thompson et al., 2008), but under treated and untreated conditions it had a yield of 36.62 tonnes/ha and 28.64 tonnes/ha, respectively. Exposure to high density levels of CPB is known to decrease production (Alyokhin, 2009; Ferro et al., 1985). As seen in Figure 6, NDJL3C-2 and ND060838C-3 had the highest yield under treated conditions at 52.24 tonnes/ha and 51.27 tonnes/ha, respectively. Yields in the untreated block decreased significantly; NDJL3C-2 had a yield of 17.09 tonnes/ha and ND060838C-3 had 35.64 tonnes/ha.

Lenape, Red Norland, and Russet Burbank were utilized as checks in the trial. Lenape had defoliation damage of 8% (treated), and 35% (untreated), and a yield of 27.5 tonnes/ha (treated) and 26.0 tonnes/ha (untreated), Red Norland had defoliation damage of 10% (treated) and 43% (untreated), and a yield of 30.9 tonnes/ha (treated) and 9.6 tonnes/ha (untreated), and Russet Burbank had defoliation damage of 8% (treated) and 40% (untreated), and a yield of 20.2 tonnes/ha (treated) and 11.7 tonnes/ha (untreated), respectively.

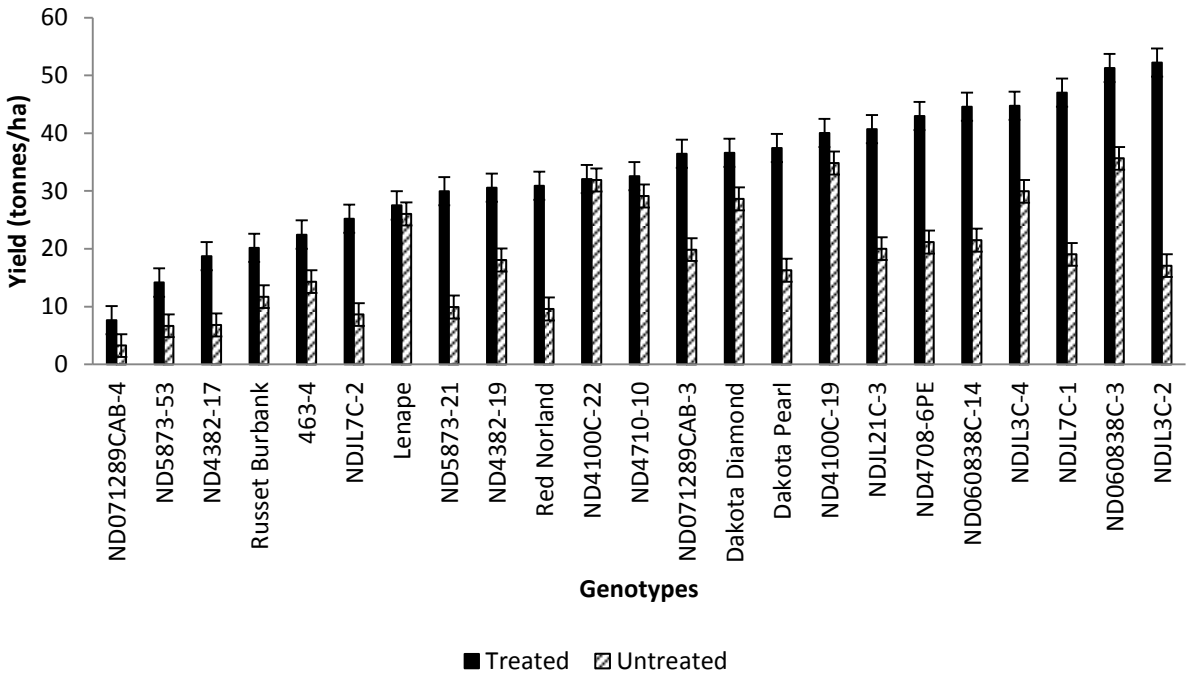


Figure 6. Yield (tonnes/ha) of treated genotypes and untreated genotypes harvested from the 2012 field evaluation trial at Grand Forks, ND. Error bars represent ±SE. LSD for yield (tonnes/ha) comparison under treated and untreated conditions = 7.87.

The defoliation damage and yield results for the trial showed that Lenape had good yield performance under both treatments, even though the defoliation levels for this genotype were among the highest in the trial under treated and untreated conditions, thus, it could be considered as tolerant to feeding by CPB. The genotypes ND4100C-22, ND4710-10, and ND4100C-19 had a very similar response in terms of yield, production under both treated and untreated conditions was similar. Although foliar feeding occurred, they show tolerance in terms of yield potential under pest pressure (Figure 7). Perhaps they could be utilized in organic field trials or based on subsequent data they must be suitable for commercial production in similar scenarios.

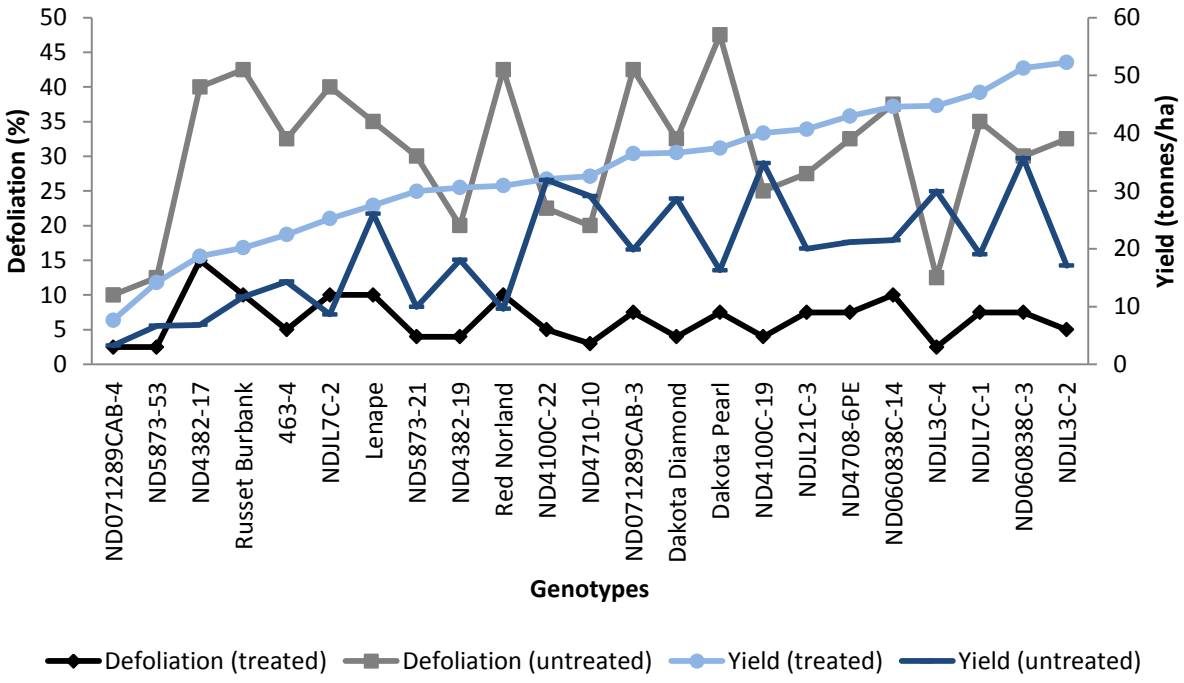


Figure 7. Yield (tonnes/ha) and CPB defoliation (%) in treated genotypes and untreated genotypes in the 2012 field evaluation trial at Grand Forks, ND.

Similar to the results obtained by Coombs et al. (2005), and Cooper et al. (2007), field resistance was expected in our trial, but *S. chacoense* derived resistance did not deter feeding completely. Cooper et al. (2007) suggests that the majority of the resistance, if any, could be due to adult preference in the field. Also, Sinden et al. (1986) found that the GA nature (solanine, chaconine, leptinine, or leptine) was more important than the amount. They also found that the leptines in *S. chacoense* were more effective than other potato GAs against the CPB. At this stage of the research, we are able to identify genotypes with potential for CPB tolerance and good yield performance under stress conditions. This data could aid in the selection process of parents for crossing and improve field tolerance to CPB. This will also help decrease substantial losses by pest damage.

The field evaluation trial was only conducted during one season, but the data collected shows that under high CPB population density, the use of insecticides or naturally-occurring defense mechanisms of the potato plant will not be effective enough to reduce damage. Field evaluation trials must be conducted multiple years in various locations to obtain an adequate assessment of genotype field performance. Cooper et al. (2007) determined that future CPB studies should be conducted to evaluate the behavior of the pest under resistance mechanisms derived from *S. chacoense*, in laboratory and field; neither field trial nor laboratory trial closely mimics all aspects of commercial field conditions, but they provide insight into tolerance factors under diverse conditions. Small plot choice field trials, similar to the one conducted at Grand Forks, are critical in identifying potentially tolerant lines, to evaluate the level of tolerance, and to determine if the tolerance will be effective in large-scale fields for commercial production.

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CHAPTER 2: GLYCOALKALOID PROFILES OF GENOTYPES FROM THE NORTH DAKOTA STATE UNIVERSITY POTATO BREEDING PROGRAM

Abstract

Colorado potato beetle (*Leptinotarsa decemlineata* Say) (CPB) is a prevalent defoliator insect of the potato crop. The use of naturally occurring defense mechanisms in the potato crop is an effective alternative to decrease CPB damage. Genetic resistance from *S. chacoense*, associated with high glycoalkaloid (GA) content, is one of the most effective mechanisms of resistance against this pest. Glycoalkaloids are secondary plant metabolites that serve as defense mechanisms against pests and diseases. They can be toxic for humans, with a threshold of 20 mg/100 g FW for human consumption. Three aglycons (non-sugar component of a glycoside molecule) were analyzed, using gas chromatography (GC), to determine presence of GAs in foliar and tuber tissue of 24 genotypes from the NDSU Potato Breeding Program. Solanidine is the aglycon to chaconine and solanine. Leptinidine is the aglycon for leptinine I and leptinine II, and acetyl-leptinidine is the aglycon for leptine I and leptine II. Solanidine presence was assessed in foliage and tuber tissue; leptinidine, and acetyl-leptinidine only in foliage, since they are not synthesized in the tubers. Solanidine was present in both foliar and tuber tissue of all genotypes analyzed; mean solanidine concentration of foliar tissue from treated genotypes was 9962.60 µg/g, and for the untreated was 13771.16 µg/g. In tuber tissue, mean solanidine content for treated genotypes was 1861.01 µg/g and for untreated genotypes was 1654.72 µg/g. Mean leptinidine content of treated genotypes was 2311.70 µg/g, and the mean of untreated genotypes was 3847.78 µg/g. Mean acetyl-leptinidine content for treated genotypes was 1639.35 µg/g and for untreated was 1620.01 µg/g. Distribution of GAs in tuber tissue was assessed in three genotypes from the untreated block (Red Norland, Lenape, and Russet Burbank). The section

with the highest concentration of solanidine across the three genotypes was in the bud end, and the section with the lowest concentration was the center. Since knowledge of plant secondary metabolites in potato plants is limited and the lack of adequate measuring standards makes identification difficult, additional research would lead to an improvement in understanding GA biosynthesis and their regulation in the plant.

Introduction

Colorado potato beetle (*Leptinotarsa decemlineata* Say) (CPB) is the most prevalent defoliator insect of the potato crop (Alyokhin, 2009). It has spread throughout North America and has invaded Europe and Asia (Ferro et al., 1985; Hsiao, 1985; Jolivet, 1991). Management of CPB has become difficult and expensive because the insect has had the ability to develop resistance to various insecticides used for its control (Alyokhin et al., 2006 and 2008; Coombs et al., 2005; Forgash, 1985; Friedman and McDonald, 1997; Grafius, 1997). The principal mechanism of crop protection against this pest is insecticides (Alyokhin, 2009; Casagrande, 1987; Gauthier et al., 1981). Multiple compounds have been tested against CPB, and various active ingredients are registered for use against this pest in the U.S. (Gauthier et al., 1981). Crop rotation, biological control, and other non-chemical tactics for regulating populations are useful, but are not as popular as chemical control (Alyokhin, 2009). An issue is that insecticide efficacy and availability vary from area to area, as well as, pesticide regulation. Excessive use of insecticides not only causes development of resistance, but also increases concerns about environmental pollution and food safety (Pariera Dinkins and Peterson, 2008). In the research community, a current major interest is identifying genes that are responsible for pest resistance (Fisher et al., 2002). Genes from wild relatives are important in plant breeding, because they confer traits that improve physiological and resistance aspects of the crop (Hajjar and Hodgkins,

2007). By utilizing the plants' natural defense mechanisms to control pests and diseases, use of inputs can be decreased and sustainable crop production can be achieved (Coombs et al., 2005; Pelletier et al., 2011).

Solanum has good potential genetic diversity for supplying host plant resistance. Many wild *Solanum* species, including *Solanum berthaultii* Hawkes, *Solanum chacoense* Bitter, *Solanum polyadenium* subsp. *Orizabae* Bitter, and *Solanum tarijense* Hawkes, are thought to have genetic traits providing insect resistance (Pelletier et al., 1999). The most promising genetic resistance mechanisms against the CPB are glandular trichomes and glycoalkaloids (GA) (solanine, chaconine, leptine, and leptinine) (Sagredo Diaz, 2000). Glycoalkaloids are secondary plant metabolites that are toxic to bacteria, fungi, viruses, insects, animals, and humans (Maga, 1994). The GA biosynthetic pathway in potato is still not fully understood, but it is thought to be via the mevalonate/isoprenoid pathway (Friedman, 2006; Khan et al., 2013). Most GAs are distributed throughout the plant, including the tubers (Friedman and McDonald, 1997). High levels of GA in the tuber impart a bitter taste and may be toxic to humans (van Gelder, 1990). Due to their toxicity to humans, guidelines limiting the GA content of new cultivars, before they can be released for commercial use, have been established. Levels in excess of 20 mg/100 g FW are not permitted (Friedman, 2006; Valkonen et al., 1996; van Gelder, 1990). In most cases, the GA content in whole tubers ranges from 10 to 150 mg/100 g FW (van Gelder et al., 1988). Following harvest, GA content can increase during storage when under the influence of light, heat, cutting, slicing, sprouting, and due to exposure to pathogens (Friedman and McDonald, 1997; Tingey, 1984).

Currently, there are no truly resistant, commercially acceptable, cultivars, and conventional potato breeding is complicated by tetraploidy of the genome (Grafius and Douches,

2008). In some cases, the new varieties developed by traditional plant breeding appeared to have good resistance to CPB, perhaps due to higher levels of GAs (Khan et al., 2013), but these were later removed from production due to tuber GA levels being above those acceptable for human consumption. Examples of potato varieties developed by traditional plant breeding that showed increased GA content include Lenape, a *S. tuberosum* × *S. chacoense* cross (Stürckow and Löw, 1961) for pest resistance. Lenape, used as a variety with high resistance to CPB defoliation, was not commercially accepted due to high levels of GAs in the tubers (Akeley, 1968; Anonymous, 1970) and release was rescinded for general planting (Zitnack and Johnson, 1970). Another conventionally bred potato variety (Magnum Bonum) was removed from the market for similar reasons (Hellenas et al., 1995). The use of naturally occurring defense mechanisms of potato plants (GAs and trichomes) is being researched as a way to efficiently decrease damage to the crop (Alyokhin, 2009; Cooper et al., 2007; Tingey, 1984). Varieties with host plant resistance could have a positive impact on the potato industry by reducing the use of insecticides for CPB control (Alyokhin, 2009; Tingey, 1984). Reduced use of insecticides will lead to a decrease in production costs and in the risk for human health and environmental concerns (Tingey, 1984).

The two most common GAs found in potatoes are chaconine and solanine, which make up as much as 95.0% of the total GAs present in the potato (Lachman et al., 2001; Matthews et al., 2005). The GAs structures differ according to the sugar moiety and the aglycon group (Figure 8) (Friedman and McDonald, 1997; Sagredo Diaz, 2000). An aglycon is the non-sugar (non-carbohydrate) moiety of a glycoside. The aglycon of leptines is a form of solanidine, which is the aglycon of *S. tuberosum* GAs (Sagredo Diaz, 2000). It is possible that acetyl-leptinidine is synthesized from solanidine; this process requires two enzymatic activities (Lawson et al., 1993; Osman et al., 1987). The first enzyme adds an –OH group on the C-23 of solanidine to produce

leptinidine, and a second enzyme to synthesize acetyl-leptinidine by the addition of an acetyl group on the –OH group previously added by the first enzyme. Considering this pathway, three phenotypic classes are possible. One class includes the plants that synthesize both leptinidine and acetyl-leptinidine, a second one contains genotypes that only produce leptinidine, and a third one that only produces solanidine. These three phenotypic classes have been observed in *S. chacoense* progenies (Silhavy et al., 1996), where all plants contain solanidine, some have leptinidine, and only a small number have acetyl-leptinidine. Solanidine is the aglycon to chaconine and solanine, the most common GAs in potato (Lachman et al., 2001). Leptinidine is the aglycon for leptinine I and leptinine II, and acetyl-leptinidine is the aglycon for leptine I and leptine II. Leptine I is known to be a feeding deterrent against the CPB (Lorenzen et al., 2001). Leptinidine and acetyl-leptinidine are not synthesized in tubers, only in foliage (Lorenzen et al., 2001).

Leptines I and II, found in a few accessions of the wild species *S. chacoense*, are a potent factor for host-plant tolerance to CPB (Sinden et al., 1986; Stürckow and Löw, 1961). *S. chacoense* Bitter tolerance has been associated with high leptine content (Sinden et al., 1986; Lorenzen et al., 2001). Leptines are rare GAs synthesized by accessions of *S. chacoense,* and are only produced in the leaves, but not the tubers (Sanford et al., 1996). This alleviates the human health concern associated with high GA content in the tuber (Sinden et al., 1986). The diverse nature of GAs and their importance in the food industry requires the development of accurate methods to measure the content of each GA in fresh potato tissue (Friedman, 2006; Friedman and McDonald, 1997).

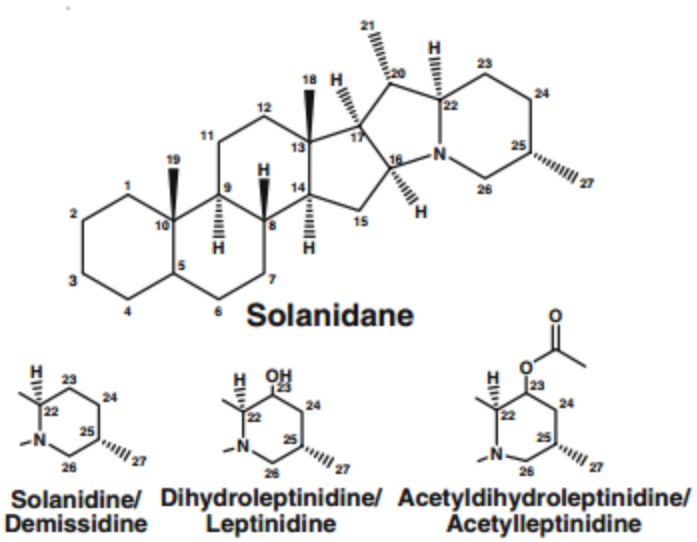

Figure 8. Structures of common *Solanum* glycoalkaloid (GA) aglycons. The complete structural formula for solanidane is shown with structural features of individual aglycons below. (Ginzberg et al., 2009).

Overall, there are more than 80 different GAs that have been detected in potato species, and new or undetected GAs are being reported, due to the improvement of analytical methodologies (Valkonen et. al, 1996). Still, the GA levels in some crops are too low for detection, and a lack of adequate measuring standards makes identification difficult (Lawson et al., 1993). Various procedures are being used for GA quantification and qualification: colorimetry (Dao and Friedman, 1996), high-performance liquid chromatography (HPLC) (Carputo et al., 2010; Pariera Dinkins et al., 2008), gas chromatography (GC) (Lorenzen et al., 2001), thin layer chromatography (TLC), mass spectrometry (MS), enzyme linked immunosorbent assay (ELISA), and biosensors (Friedman, 2006; Friedman and McDonald, 1997). Since knowledge of plant secondary metabolites in potato plants is limited (Friedman, 1997), additional research would lead to an improvement in understanding the GA biosynthesis and regulation in the plant. This can include the potential for synergy and interaction effects

among these compounds (Kowalski et al., 1999), and how their ratio in the tissue determines GA effectiveness as a defense mechanism against pests and pathogens. This research was conducted to assess, by GC, aglycon (solanidine, leptinidine, and acetyl-leptinidine) presence in foliar and tuber tissue of genotypes from the North Dakota State University (NDSU) Potato Breeding Program.

Materials and methods

Twenty-four genotypes from the NDSU Potato Breeding Program were grown at Grand Forks, ND, during the 2012 growing season using standard cultivation practices; these practices are repeated in the Materials and Methods section of Chapter 1. Aglycon presence was determined by gas chromatography (GC) in both leaf and tuber tissue. The protocol in our study, for GA extraction and assessment, was modified from Lawson et al. (1992). This procedure combines the extraction and hydrolysis of GAs into one step and uses capillary GC to quantitate aglycons (solanidine, leptinidine, and acetyl-leptinidine) from *S. tuberosum* and *S. chacoense* foliar and tuber tissue. The benefits of the procedure are that it requires a small amount of plant tissue (≥100 mg DW), prevents ammonia precipitation during sample preparation, and uses an accesible internal standard (tomatine) for quantitation (Gregory et al., 1981; Jellema et al., 1981; Morris and Lee, 1981; Sinden et al., 1986). The foliar and tuber tissue samples collected were lyophilized prior to being analyzed. Freeze drying the samples allows more time for subsequent analysis. Analysis of freeze-dried samples offers many advantages when compared to analysis of fresh samples (Brown et al., 1999; Dao and Friedman, 1996; Wünsch and Munzert. 1994). For example, the freeze-drying procedure stops compositional changes of GAs caused by enzymes, bruising, or moisture, it allows for safe storage, and facilitates transportation of samples for analysis. Also, freeze-dried samples do not undergo browning during handling. A major

disadvantage of freeze-drying a large number of samples is that it adds time, effort, and equipment costs.

The first modification to the Lawson et al. (1992) protocol was increasing the HCL molarity. The molarity of HCl was increased from 1.0 M to 3.5 M to increase the hydrolysis rate and to obtain a higher amount of aglycon in the sample. Extracting with strong acid such as 3.5 M sulfuric acid (Coxon et al., 1979; Blincow et al., 1982) allows extraction and hydrolysis, simultaneously. For potato GAs, acid hydrolysis rates increase with higher acid concentrations and temperatures, and decreases with increasing proportions of water in mixed organic solvent-water solutions (Friedman et al., 1993 and 1998; Friedman and McDonald, 1995; Van Gelder, 1984). Friedman et al. (1993) carried out a detailed study on the effects of time, temperature, and acid concentration on hydrolysis. In general, he noted that hydrolysis rates increase with increased HCL concentration and temperature and decrease with the amount of water in organic solvent-water solutions. Under conditions of strong acid and high temperatures, solanidine formed from the hydrolysis of chaconine or solanine will further react to form solasodiene. The partition with benzene was done two times to increase the recovery of aglycons from the sample. Aglycons, instead of GAs, were analyzed because GAs are quickly hydrolyzed and easily lost; aglycons are easy to recover and give an idea in terms of GA presence.

Samples of potato leaves for chemical analyses were taken from each treatment on August 14. Samples of potato tubers (4 to 6 tubers from each replicate; two replicates per treatment) were taken from each of the plots following harvest on September 25, 2012. Variability in the analysis of foliar GAs was minimized by sampling and comparing single leaves from the same stem position of each plant (Brown et al., 1999). Leaves were collected randomly from below the first fully expanded leaf of each plant from the individual plots. Foliar and tuber

samples were maintained in storage at 4°C until lyophilized. Freeze-dried foliar and tuber samples were ground to a fine powder using a Waring commercial blender. About 20 mg of freeze-dried tissue was placed in a 10 mL screw top vial and 200 µL of tomatine (internal standard) was added, along with 3 mL of 3.5 mol·L-1 HCl in methanol. Headspace was purged with N_2 gas. The vials were sealed and placed on a hot plate, at 70°C, for 4 h. The vials were vortexed every 30 minutes. At the end of the extraction-hydrolysis period, the vials were cooled to 25°C. After cooling, 2 mL concentrated NH_4OH (ammonium hydroxide) was added to increase the pH> 10. Vials were centrifuged at 1800 xg for 10 min. Supernatants were partitioned against 3 mL of benzene and this step was repeated once more to ensure adequate aglycon extraction. Afterwards, 2 mL aliquots of the benzene phase were placed in screw cap tubes and evaporated to dryness at 50°C under N_2 gas. The residue containing the aglycon was re-dissolved in 0.5 mL of chloroform and subjected directly to GC. Samples were injected onto a 15 m' 0.53 mm i.d. '0.25 mm RTx1 fused silica column, installed in an Agilent 7890A gas chromatograph. Helium carrier gas was at a linear flow rate of 45 cm·s-1, the injector temperature was 270°C and column temperature was 210°C, increasing 2°C·min-1 to 260°C. The flame ionization detector was at 280°C. For each sample, the mean aglycon concentration was based on two GC injections.

The amount of potato aglycons was calculated based on the standard curve of tomatine (internal standard). The Response/Calibration Factor and External Standard equation is CF=(Ax)/(Cx); the Internal Standard equation is RF=[(Ax)(Cis)]/[(Ais)(Cx)], and the Internal Standard equation is Cx=[(Ax)(Cis)]/[(Ais)(RFAVE)], where: Ax = area of the compound, Cx = concentration of the compound, Ais = area of the internal standard. The analysis results are given as µg per 1 g of dried tissue.

Statistical analyses

Foliar and tuber data was statistically analyzed using analysis of variance (ANOVA). The field defoliation data was analyzed by repeated measures ANOVA. Control and treated groups were compared ($\alpha \leq 0.05$) using SAS 9.3 (PROC GLM, SAS Institute 2012). For leptinidine and acetyl-leptinidine foliar data evaluation, data points with a value of zero for both replicates were not considered and were not accounted for in the analysis. Pearson's correlation was conducted to determine the relationship between defoliation damage and aglycon content (CORR, SAS 2012).

Results and discussion

Three aglycons (non-sugar component of a glycoside molecule) (solanidine, leptinidine, and acetyl-leptinidine) were analyzed, using GC, to determine the presence of GAs in foliar and tuber tissue of 24 genotypes from the NDSU Potato Breeding Program (Table 1). Solanidine is the aglycon to chaconine and solanine, the most common GAs in the potato plant (Lawson et al., 1992; Sagredo Diaz, 2000). Leptinidine is the aglycon for leptinine I and leptinine II, and acetyl-leptinidine is the aglycon for leptine I and leptine II. Leptine I is known to be a feeding deterrent against CPB (Lorenzen et al., 2001). Leptinidine and acetyl-leptinidine are not synthesized in tubers, only in foliage (Sinden et al., 1986).

Foliar and tuber tissue samples were collected, lyophilized, and ground prior to being analyzed. Foliar samples were analyzed to detect presence and content of solanidine. Analysis of variance (ANOVA) was based on an $\alpha \leq 0.05$. Based on the ANOVA (Table 4), the genotype and treatment effects are significantly different. This indicates that there are significant differences between genotypes in terms of solanidine concentration.

Table 4. Analysis of variance of solanidine concentration in foliar samples, untreated and treated with insecticide, collected from the 2012 field evaluation trial at Grand Forks, ND.

Effect	DF	Type III SS	Mean square	F value	Pr > F
Genotype	23	8834062202	384089661	8.31	<0.0001*
Treatment	1	348123029	348123029	7.54	0.0085*
Replicate	1	11419481	11419481	0.25	0.6214^{ns}
Genotype X Treatment	23	2221492060	96586611	2.09	0.0161*
Error	47	2171222839	46196231		

* significant at P≤0.05, ^{ns} = not significant at P≥0.05.

Solanidine was consistently present in all genotypes analyzed (Silhavy et al., 1996). This is expected, since solanidine is the aglycon of chaconine and solanine (Sinden et al., 1986), the predominant GAs present in the potato plant (Friedman and McDonald, 1997). The replicate effect was not significant, which means that there were no differences between replicates in terms of solanidine content. The genotype by treatment interaction is significant, which indicates that genotypes responded different under each level of treatment.

Mean separation was conducted for solanidine concentration values obtained by GC analysis (Table 5). The mean of the treated block was 9962.60 µg/g, and for the untreated was 13771.16 µg/g. Percentage defoliation observed in the field was compared with the solanidine concentration, since it is expected that as solanidine concentration increases, defoliation by CPB should decrease. Additionally, as defoliation increases, solanidine content increases. The content of GAs in the plant is known to increase, mainly in damaged tissue, under insect pest pressure (Hlywka et al., 1994).

Table 5. Mean foliar solanidine content of genotypes, treated with imidacloprid and untreated, in the 2012 field evaluation trial at Grand Forks, ND.

	Treated	Untreated
Genotype	Solanidine	Solanidine
	µg/g	µg/g
ND2858-1	28579.62	22700.12
ND071289CAB-4	20877.81	21720.31
NDJL3C-4	1547.46	274.32
ND5873-53	17597.7	10864.91
ND4382-19	10530.85	8577.68
ND4710-10	6287.01	25723.58
ND4100C-22	24481.55	59536.94
ND4100C-19	14381.04	11092.33
NDJL21C-3	411.03	440.12
ND060838C-3	9110.84	16436.96
ND5873-21	23815.38	15579.55
NDJL3C-2	642.51	1755.64
463-4	2911.8	1669.66
ND4708-6PE	7192.33	27306.99
Dakota Diamond	11457.91	8785.92
NDJL7C-1	142.83	628.12
Lenape	16285.68	22765.34
ND060838C-14	10627.83	18553.97
NDJL7C-2	961.03	419.44
ND4382-17	4109.47	4170.86
ND071289CAB-3	7072.56	11617.69
Red Norland	7837.33	12321.99
Russet Burbank	7844.16	21274.36
Dakota Pearl	4396.71	6291.08
Mean	9962.60	13771.16
[1]LSD	13587.69	13587.69

[1]LSD for solanidine content comparison under two treatments ($\alpha \leq 0.05$).

The values presented in Table 5 showed that there is wide variation in solanidine concentration for all the genotypes. For example, ND2858-1 had low defoliation damage (2%) in the field, but had the highest content of solanidine (28579.62 µg/g) under treated conditions. The genotype ND4100C-22 had the highest content of solanidine (59536.94 µg/g) under untreated

conditions, and relatively low defoliation damage (23%). This variation could be addressed by conducting additional field trials to evaluate GA content under CPB pest pressure. Additional data could provide a trend in terms of GA content, and we may be able to draw conclusions as to which genotypes are consistent in GA presence and content. Improved methods of detection are needed to obtain accurate quantification and qualification of all the GAs (Friedman and McDonald, 1997).

There was a slight negative correlation between defoliation damage and solanidine content in the foliar tissue under treated conditions ($r = -0.43$, $n = 24$, $p = 0.037$), and no correlation under untreated conditions ($r = -0.23$, $n = 24$, $p = 0.28$). The slight negative correlation means that as solanidine content increases, damage by CPB decreased. Some values of solanidine detected in the foliar samples, 20000.0 µg/g or more (threshold is 20 mg/ 100 g FW) (Table 5), were above the maximum levels for human consumption (Friedman, 2006; Valkonen et al., 1996; Van Gelder, 1990), but these are not a concern for humans, since we do not consume potato foliage. Many external factors also influence GA content. High temperatures in the field, irradiation, and exposure to chemicals affect leaf and tuber GA content, while harvesting and storage have a big effect on tuber GA content (Friedman and McDonald, 1997). Conditions to determine safety for consumption have to be controlled to diminish effect of external factors on GA content.

North Dakota State University (NDSU) released a cultivar, Dakota Diamond (ND5822C-7), with insect resistance attributed to GAs (Thompson et al., 2008). In a study conducted by Thompson et al. (2008), Dakota Diamond total GAs were measured on tubers harvested in ND during 2005. The total GA level for Dakota Diamond was 2.5 mg/100 g FW, compared to 6.1 mg/100 g for Lenape and 5.1 mg/100 g for Russet Burbank, common check cultivars. Tuber total

GA levels were high at certain locations in trials during previous years. However, most of the tubers were recorded as damaged and/or green, and evaluation took place several months after harvest, after tubers were subjected to light during storage (Thompson et al., 2008), which leads to variability in GA content (Tingey, 1984). Dakota Pearl total GA levels were low, averaging 1.5 mg/100 g fresh tuber tissue (Thompson et al., 2005). In our research Dakota Pearl showed low values of GA, in comparison to the rest of the genotypes (Table 5).

Leptinidine, the aglycon for leptinine I and leptinine II, was analyzed to determine its presence in foliar samples collected at Grand Forks, ND. Analysis of variance (Table 6) shows that genotype and treatment effects were significantly different; this means that there are significant differences between genotypes and treatments in terms of leptinidine concentration and that application of imidacloprid (Admire®) may have had an effect on GA content. Leptinidine is known to be synthesized in genotypes with a *S. chacoense* background (Silhavy et al., 1996). The replicate factor is not significant, indicating that there was no difference between replicates. The genotype by treatment interaction was not significant, indicating that genotypes responded similar under both levels of treatment.

Table 6. Analysis of variance of leptinidine concentration in foliar samples (untreated and treated with insecticide) collected from the 2012 field evaluation trial in Grand Forks, ND.

Effect	DF	Type III SS	Mean square	F value	Pr > F
Genotype	23	2167597022	94243349	7.73	<0.0001*
Treatment	1	56628472	56628472	4.64	0.0363*
Replicate	1	47628	47628	0.00	0.9504^{ns}
Genotype*Treatment	23	402004446	17478454	1.43	0.1465^{ns}
Error	47	573277808	12197400		

* significant at P≤0.05, ^{ns} = not significant at P≥0.05.

Mean separation for leptinidine was conducted (Table 7). The mean of the treated block was 2311.70 µg/g, and of the untreated was 3847.78 µg/g. The highest leptinidine content was observed in 463-4 under treated conditions (20031.57 µg/g) and under untreated conditions (19664.12 µg/g). The values of leptinidine were relatively high across treatments and this aglycon was only present in a few genotypes (Table 7). Some exceptions were NDJL3C-2 and NDJL21C-3, which showed aglycon values under untreated conditions, but under treated conditions aglycons were not detected by GC. This could be due to variation in the samples, sample size, experimental error, or aglycon quantities being too small for detection. For leptinidine, the values should not be of concern, since this aglycon is not present in the tuber. There was no correlation between defoliation damage and leptinidine content in the foliar tissue under treated conditions ($r = -0.40$, $n = 24$, $p = 0.05$), and under untreated conditions ($r = -0.35$, $n = 24$, $p = 0.10$).

Acetyl-leptinidine is the aglycon of leptine I and leptine II. Leptine I is known to be a strong feeding deterrent against the CPB (Lorenzen et al., 2001). Foliar samples were analyzed to quantify and qualify acetyl-leptinidine. Acetyl-leptinidine is synthesized in genotypes with *S. chacoense* background (Silhavy et al., 1996). Usually, this aglycon is known to be present only in the foliage, not in the tuber tissue, and is considered to be low risk for human toxicity (Sinden et al., 1986; Sturckow and Low, 1961).

Based on the ANOVA (Table 8), the genotype effect is significantly different, meaning that there are significant differences between genotypes for acetyl-leptinidine concentration. Treatment was not significantly different, indicating that the application of insecticide had no direct influence on the aglycon concentration from field foliar samples. The genotype by treatment interaction was not significant, meaning that genotypes responded similar under both

treatments. The replicate effect was not significant, indicating that there was no difference between replicates.

Table 7. Mean foliar leptinidine content of genotypes in the 2012 field evaluation trial at Grand Forks, ND.

Genotype	Leptinidine
	µg/g
463-4	19848
ND2858-1	9794
NDJL3C-2	8952
NDJL3C-4	8012
ND4100C-22	6967
ND4710-10	6473
ND4382-19	4918
ND4100C-19	3089
ND5873-53	2671
NDJL7C-1	1672
ND060838C-3	1006
NDJL7C-2	513
ND071289CAB-3	0
ND071289CAB-4	0
NDJL21C-3	0
ND060838C-14	0
ND4382-17	0
ND5873-21	0
ND4708-6PE	0
Dakota Diamond	0
Dakota Pearl	0
Lenape	0
Red Norland	0
Russet Burbank	0
Mean	3030
[1]LSD	5337

[1]LSD for leptinidine content comparison ($\alpha \leq 0.05$).

Table 8. Analysis of variance of acetyl-leptinidine concentration in foliar samples (untreated and treated with insecticide) collected from the 2012 field evaluation trial in Grand Forks, ND.

Effect	DF	Type III SS	Mean square	F value	Pr > F
Genotype	23	8.65E+08	37599002	9.18	<0.0001*
Treatment	1	8983	8983	0.00	0.9628^{ns}
Replicate	1	2284867	2284867	0.56	0.4588^{ns}
Genotype X Treatment	23	55095445	2395454	0.59	0.9172^{ns}
Error	47	1.92E+08	4094562		

* significant at P≤0.05, ^{ns} = not significant at P≥0.05.

Mean separation was conducted for acetyl-leptinidine values (Table 9). The mean for treated was 1639.35 µg/g and for untreated was 1620.01 µg/g. The highest value of acetyl-leptinidine was for NDJL7C-2 (12798.10 µg/g) under treated conditions, and under untreated conditions, NDJL3C-4 had the highest levels (10914.08 µg/g). Levels of acetyl-leptinidine across all genotypes were relatively low compared to the threshold value for GAs. There was no correlation between defoliation and acetyl-leptinidine content in the foliar tissue under treated conditions (r = 0.03, n= 24, p= 0.88) and untreated conditions (r = -0.14, n= 24, p= 0.50).

Core samples from tubers harvested at Grand Forks were taken with a potato corer. Afterwards, the core samples were stored and lyophilized. Glycoalkaloids were extracted and analyzed by GC (as previously described in the Materials and Methods section). The ANOVA (Table 10) shows that the effect of genotype is significant, meaning that there are significant differences between genotypes for solanidine concentration. The treatment effect, the genotype by treatment interaction, and replicate, were not significant.

Table 9. Mean foliar acetyl-leptinidine content of genotypes in the 2012 field evaluation trial at Grand Forks, ND.

Genotype	Acetyl-leptinidine
	µg/g
NDJL7C-2	10831
NDJL3C-4	8931
NDJL21C-3	5959
ND4100C-19	4263
NDJL7C-1	3976
ND4382-19	2796
NDJL3C-2	2356
ND071289CAB-3	0
ND071289CAB-4	0
ND060838C-3	0
ND060838C-14	0
463-4	0
ND4100C-22	0
ND4382-17	0
ND5873-53	0
ND5873-21	0
ND4708-6PE	0
ND4710-10	0
ND2858-1	0
Dakota Diamond	0
Dakota Pearl	0
Lenape	0
Red Norland	0
Russet Burbank	0
Mean	1630
[1]LSD	2626

[1]LSD for acetyl-leptinidine content comparison ($\alpha \leq 0.05$).

For tubers, the only GA analyzed was solanidine. The aglycons leptinidine and acetyl-leptinidine were not presented in the chromatograms for each genotype. Leptinidine and acetyl-leptinidine are not synthesized in the tubers, only in the foliage (Friedman, 2006). However, Shakya and Navarre (2008), using LC–MS, a very sensitive technique, detected trace content of leptinines and leptinidines in tubers of *S. chacoense, S. bulbocastanum, S. stenotomun* and *S.*

spegazzini. In some cases the progenies contained GAs not detected in the parental species (Väänänen et al., 2005).

Table 10. Analysis of variance of solanidine concentration in tuber samples (untreated and treated with insecticide) collected from the 2012 field evaluation trial at Grand Forks, ND.

Effect	DF	Type III SS	Mean square	F value	Pr > F
Genotype	22	350871884	15948722	2.68	0.0026*
Treatment	1	978733	978733	0.16	0.6873^{ns}
Replicate	1	22252837	22252838	3.73	0.0597^{ns}
Genotype X Treatment	22	72353334	3288788	0.55	0.9332^{ns}
Error	45	268268825	5961529		

* significant at P≤0.05, NS = not significant at P≥0.05.

Mean separation and comparison to field evaluation damage was conducted (Table 11). The mean solanidine concentration for treated genotypes was 1861.01 µg/g, and for the untreated was 1654.72 µg/g. The genotype 463-4 had the highest solanidine concentration (10876.98 µg/g) under both treated and untreated conditions (8010.89 µg/g). The lowest concentration was for Red Norland (279.36 µg/g) in treated conditions; and for untreated conditions, ND5873-21 had the lowest concentration (206.86 µg/g). Data for ND2858-1 was not available; this genotype develops tubers under a short photoperiod and thus, tuber set had not occurred. Potato plants stressed by CPB are known to produce tubers with a higher GA concentration than unstressed plants (Hlywka et al., 1994). These results imply that potatoes from plants stressed by CPB may not be as safe to consume as those protected by synthetic pesticides if significant defoliation occurs during the growing season. There was no correlation between defoliation damage and solanidine content in the tuber tissue under treated conditions ($r = -0.16$, $n = 23$, $p = 0.47$) and under untreated conditions ($r = 0.05$, $n = 23$, $p = 0.83$).

Table 11. Mean tuber solanidine content of genotypes in the 2012 field evaluation trial at Grand Forks, ND.

Genotype	Solanidine
	µg/g
ND071289CAB-3	1031
ND071289CAB-4	2324
NDJL3C-2	788
NDJL3C-4	790
NDJL7C-1	812
NDJL7C-2	1038
NDJL21C-3	1426
ND060838C-3	3227
ND060838C-14	807
463-4	9444
ND4100C-19	2583
ND4100C-22	3670
ND4382-17	837
ND4382-19	1491
ND5873-53	596
ND5873-21	473
ND4708-6PE	912
ND4710-10	765
Dakota-Diamond	486
Dakota-Pearl	375
Lenape	2121
Red-Norland	308
Russet-Burbank	4126
Mean	1758
[1]LSD	3239

[1]LSD for solanidine content comparison under ($\alpha \leq 0.05$).

Solanidine was present in all genotypes, which was expected since solanidine is the aglycon of the two most common GAs in potato (solanine and chaconine) (Matthews et al., 2005). Additionally, all solanidine concentrations in the tuber tissue were below safety limits for human consumption. Even Lenape, a variety removed from the market due to high tuber GA content, was below safety limits, 2626.66 µg/g and 1615.17 µg/g, for treated and untreated,

respectively. Glycoalkaloid levels of leaves are generally much higher than those in tubers (Friedman, 2006). Phillips et al. (1996) observed a greater concentration of GAs in leaves compared to tubers from the same plants; however, there was a great deal of variability among leaf GA concentrations within the same genotype.

The CPB is the main insect pest reducing potato yields. It can be effectively controlled by an application of chemical insecticides which, however, can affect *S. tuberosum* chemical composition (Fidalgo et al., 2000). Data obtained from previous studies showed that application of insecticide influences the content of GAs present in the plant (Zarzecka et al., 2013), but this has not been clearly determined for neonicotinoid insecticides. In research conducted by Zarzecka et al. (2013), the insecticides applied to control CPB increased (Calypso 480 SC), or decreased (Actara 80 WG, Regent 200 SC), total GA content in leaves as compared with the control. The literature available lacks information on the impact of insecticides on changes in GA content of potato leaves (Zarzecka et al., 2013). Also, as mentioned by Friedman and McDonald (1997), the role of pesticides on GAs in general is difficult to evaluate if the control plants are subjected to stress due to pathogen or insect pest attack; if the control plants are relatively healthy, there may be little observable effect.

Glycoalkaloid distribution assessment

Distribution of GAs in the tuber was assessed to determine variation in different tuber sections (bud end, center, and stem end), as well as the whole tuber. For this part of the research three genotypes from the untreated block were assessed. These genotypes were utilized as controls in the field evaluation trial at Grand Forks. The genotypes were Red Norland, Lenape,

and Ruset Burbank. These cultivars are commonly used in the NDSU Potato Breeding Program as controls to assess GA content; Lenape, is used as a high GA level check (GA concentration is unacceptably high) (Akeley, 1968; Anonymous, 1970), Russet Burbank, is used as a 'barely acceptable' level check (for tubers: +17 mg/100 g FW), and 'Red Norland', is used as a low GA accumulation check. Based on the ANOVA (Table 12), the genotype effect is significantly different, meaning that there are significant differences between genotypes for solanidine concentration. Treatment was not assessed for this part of the research, due to the focus being assessment of GA distribution in tubers from untreated block. The replicate, section, and the genotype by section interaction were not significant. Only solanidine was assessed for this part of the research. Leptinidine and acetyl-leptinidine are not synthesized in the tubers, only in the foliage (Friedman, 2006; Silhavy et al., 1996).

Table 12. Analysis of variance of solanidine concentration in whole tuber samples (untreated and treated with insecticide) collected from the 2012 field evaluation trial at Grand Forks, ND.

Effect	DF	Type III SS	Mean square	F value	Pr > F
Genotype	2	24246726	12123363	14.84	<0.0001*
Replicate	2	1203128	601564	0.74	0.4903^{ns}
Section	3	4455779	1485259	1.82	0.1733^{ns}
Genotype X Section	6	4796128	799354	0.98	0.4630^{ns}
Error	22	17972288	816922		

* significant at P≤0.05, NS = not significant at P≥0.05.

Three potato cultivars (Red Norland, Lenape, and Russet Burbank) were analyzed to assess GA distribution in the whole tuber and in three tuber sections (bud end, center, stem end). Figure 9 represents the values of solanidine for the whole tuber and tuber section samples. The ANOVA showed that the three genotypes were different from one another. The genotype with the highest solanidine concentration was Lenape (3216.13 µg/g), which was expected, since this

variety is known to have high GA levels, making it unsafe for human consumption (Akeley, 1968; Anonymous, 1970). Red Norland (536.97 μg/g) had the lowest solanidine values, and Russet Burbank ranked in between (1816.21 μg/g). Apart from the whole tuber, the three sections analyzed were stem end, bud end, and center. There were no significant differences between the sections of the tuber in terms of aglycon concentration. Even though the section effect was not significant, it is important to point out that levels of GAs tend to be higher at the bud end than at the stem end of the tuber; this could be due to the majority of the eyes being located at the bud end. The eyes are the areas where the sprouts develop and sprouts are known to have the highest content of GA in the plant (tuber) (Friedman, 2006).

The majority of GAs in the tuber are located within the first 1.0 mm of skin from the outside surface and decrease toward the center of the tuber (Friedman and McDonald, 1997). Various cultivars usually present uneven distribution of GAs in the tuber. Accumulation rate of GAs during tuber growth and development are strongly influenced by genotype (Sadowska et al., 2007; Van Gelder et al., 1987), and total levels generally decrease with increasing tuber size. Because naturally occurring pesticides often are synthesized when plants are under stress, it is expected that injury to plant tissue would instigate synthesis of higher concentrations of these compounds in the injured, versus uninjured, plant tissue. Hlywka et al. (1994) found that tubers from plants subjected to CPB defoliation contained higher GA concentrations than tubers from plants not affected by the pest.

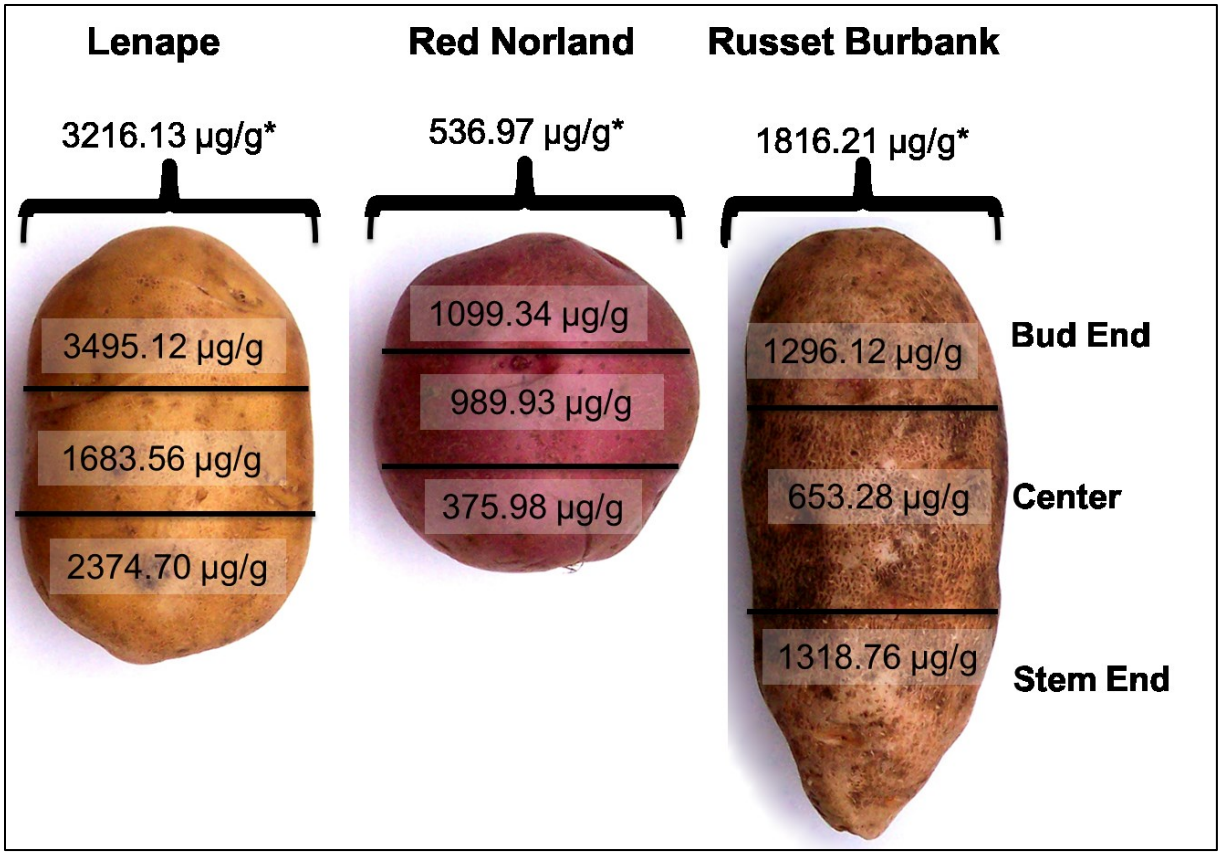

Figure 9. Solanidine (aglycon) content of whole tuber and tuber section samples (bud end, center, stem end). *Solanidine (aglycon) content of whole tuber samples.

Various procedures are being used for GA quantification and qualification: colorimetry (Dao and Friedman, 1996), high-performance liquid chromatography (HPLC) (Carputo et al., 2010; Pariera Dinkins et al., 2008), gas chromatography (GC) (Lorenzen et al., 2001), thin layer chromatography (TLC), mass spectrometry (MS), enzyme linked immunosorbent assay (ELISA), and biosensors (Friedman, 2006; Friedman and McDonald, 1997). Methods for quantifying the GAs, like most analytical methods, consist of three parts. Coxon (1984) identified the steps necessary before analysis of GAs and called them extraction, clean-up, and quantification. Jadhav et al. (1981) referred to them as extraction, separation, and analysis. The steps consist of extracting all compounds of interest, eliminating all other compounds that will interfere with the

chosen method of analysis, and determining the amount of metabolite present in the sample. Some of the analytical methods may include another step, such as derivatization or hydrolysis, which can also be called modification. Gas chromatographic (GC) methods require conversion of GAs to aglycons before the analysis.

The GC technique has several disadvantages. It is relatively expensive compared with colorimetry and TLC. Due to high temperatures involved (around 300°C or higher), the columns can run 100 or fewer samples, before needing to be changed (Herb et al., 1975). Run times are often long, and, as with all hydrolysis methods, no information on individual glycoside content is available. However, there is good separation of all compounds. Gas chromatography has no carrier solvents, therefore there are no solvent disposal problems, detection is simple, and it is suited to direct coupling with other instruments such as mass spectrometers (MS).

Figure 10 shows the chromatogram from the study conducted by Lawson et al. (1992). This chromatogram only represents a foliar tissue sample from *S. chacoense*. Figures 11 and 12 are the chromatograms obtained with foliar and whole tuber tissue, respectively, from the current study. The separation obtained is very similar, as well as, the peak time of each compound. By modifying the run time from the Lawson et al. (1992) protocol, we were able to obtain better compound separation, facilitating aglycon identification, because of improved peak resolution.

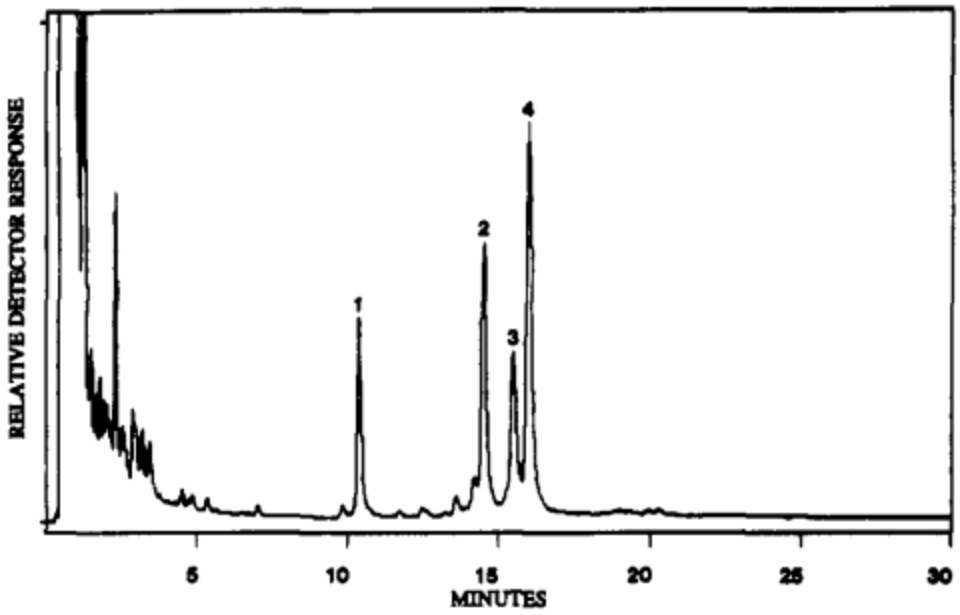

Figure 10. Chromatogram of *S. chacoense* leaf extract. Peaks: 1 = solanidine; 2 = leptinidine; 3 = tomatidine (internal standard); 4 = acetyl-leptinidine (Lawson et al., 1992).

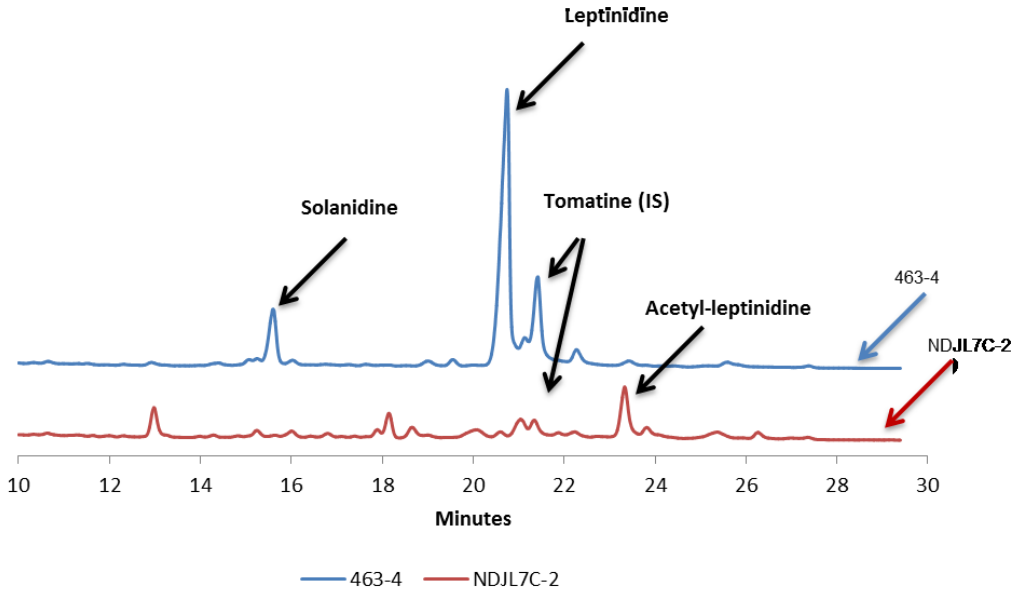

Figure 11. Chromatogram from 463-4 and NDJL7C-2 foliar tissue samples analyzed by GC. IS: internal standard (tomatine)

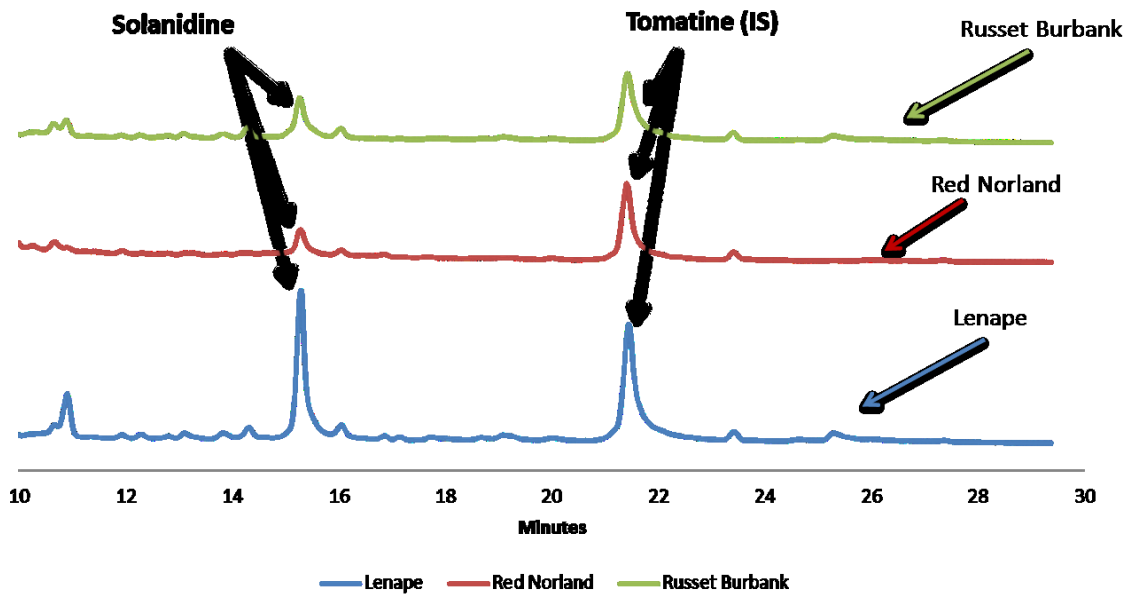

Figure 12. Chromatogram from Lenape, Red Norland, and Russet Burbank whole tuber samples analyzed by GC. IS: internal standard (tomatine)

Glycoalkaloids have evolved in nature to protect the plant against pests and diseases. In addition to needed research on this topic, scientists need to define the genetic mechanisms and control of the biosynthesis, metabolism, and degradation of GAs, as well as, to assess possible synergistic effects of different ratios of chaconine and solanine found in different genotypes against pests. It could be that specific ratios of chaconine to solanine that exhibit synergism are more relevant than total levels in protecting plants against pests and diseases. Potato genotypes should be developed with reduced amounts of GAs, while maintaining its resistance traits. Because many factors, such as light, temperature, and mechanical injury, can induce GA synthesis in tubers, it is important to reduce the sources of variability when comparing the GA levels of different genotypes. Samples should be analyzed quickly after harvest or stored in controlled environments to decrease or prevent changes in GA content.

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CONCLUSION

Our study shows the diverse GA contents and profiles found in different cultivated and wild *Solanum* species. Some genotypes utilized in the research have been used in breeding programs to improve resistance against several pests and diseases. Still, the need for accurate assessment of GAs (qualitative and quantitative) should be emphasized, principally when implementing wild species for breeding and releasing new cultivars (varieties). The diverse nature of GAs and their importance in the food industry requires the development of accurate methods to measure the content of each GA in fresh potato tissue (Friedman, 2006; Friedman and McDonald, 1997). The development of new cultivars with low GA levels in tubers, while retaining high levels in leaves to protect the crop against insects and fungal diseases it's being sought after as an alternative. Varieties with host plant resistance could have a positive impact on the potato industry by reducing the use of insecticides for CPB control (Alyokhin, 2009; Tingey, 1984).

The one-year data collected from this research presented showed that certain genotypes had good potential for CPB tolerance in terms of yield. Examples are ND4100C-22 and ND4100C-19. Both genotypes had average performance against CPB damage, but showed similar yield under treated and untreated conditions. Implementing these genotypes in a breeding scheme by crossing them with genotypes that confer other mechanisms (trichome or other types of GAs) or quality traits will benefit the grower by decreasing inputs in the field while maintaining good yield. Reduced use of insecticides will lead to a decrease in production costs, in the risk for human consumption, and environmental concerns (Tingey, 1984). Our current knowledge of GA biosynthesis and regulation is incomplete and further studies are needed to accomplish the goal of understanding these compounds.

At this stage of the research, we are able to identify genotypes with potential for CPB resistance, which could aid in the selection process of parents for crossing and improve field resistance to CPB. Also, we were able to compare defoliation damage and GA content in the genotypes. The results showed that, although some genotypes were expected to show high levels of GAs due to their genetic background and the damage in the field, they had low levels of GAs in the foliage and tuber, which shows how variable the nature of GAs can be. Since many factors, such as light, temperature, and mechanical injury, can induce GA synthesis in tubers, it is important to reduce the sources of variability when comparing the GA levels of different genotypes. Samples should be analyzed quickly after harvest or stored in controlled environments to decrease or prevent changes in GA content.

The field evaluation trial was only conducted during one season, but the data collected shows that under high CPB population density, the use of insecticides or naturally-occurring defense mechanisms of the potato plant will not be effective enough to reduce damage. Field evaluation trials must be conducted multiple years in various locations to obtain an adequate assessment of genotype field performance. Cooper et al. (2007) determined that future CPB studies should be conducted to evaluate the behavior of the pest under resistance mechanisms derived from *S. chacoense*, in laboratory and field; neither field trial nor laboratory trial closely mimics all aspects of commercial field conditions, but they provide insight into resistance factors under diverse conditions. Small plot choice field trials, similar to the one conducted at Grand Forks, are critical in identifying potentially resistant lines, to evaluate the strength of resistance, and to determine if the resistance will be effective in large-scale fields for commercial production. In addition to needed research on this topic, scientists need to define the genetic mechanisms and control of the biosynthesis, metabolism, and degradation of GAs. Potato

genotypes should be developed with reduced amounts of GAs, while maintaining resistance traits.