GLANDULAR TRICHOME EVALUATION THROUGH SCANNING ELECTRON MICROSCOPY AND THE MODIFIED ENZYMIC BROWNING ASSAY

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ABSTRACT

Potato (*Solanum tuberosum* L.) ranks fifth in the USA in comparison with other countries regarding potato production. Colorado potato beetle (*Leptinotarsa decemlineata* Say) is a devastating insect pest affecting potato in Canada and USA. Several researchers demonstrated that glandular trichomes on potato leaves provided CPB resistance. The experimental approach consisted of using Scanning Electron Microscope to assess the presence of glandular trichomes, the Modified Enzymic Browning Assay to screen leaves for the presence of glandular trichomes, and a two-year field trial to evaluate CPB defoliation effects on potato genotypes. The SEM provided a closer look at the morphology and quantity of glandular trichome on potato leaves. The MEBA protocol needs further refinement prior to adoption by NDSU or other potato breeding programs. The two-year field trial results indicate Ebt 6-21-3 is resistant to CPB defoliation, and combines high yield, making it an excellent genotype for parental use in cultivar development.

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LITERATURE REVIEW

Origin, Domestication, and Distribution of Potato

Potato originated in the Andes region (Peru, Chile, and Bolivia), extending as far North as the southern Rocky Mountains in the USA (Stevenson, 1951; Hijmans and Spooner, 2001; Love et al., 2003; Jerardo, 2012). Data show that potatoes were domesticated and cultivated by the Incas as early as 7000 years ago (Love et al., 2003). When the Spanish conquistadors invaded South America, they came in contact with what today is known as potato (Stevenson, 1951; Love et al., 2003). This first encounter is believed to have occurred in 1537, when they found a variety of tuber-bearing species (Jerardo, 2012). After considering its potential as a food crop, and the potato flowers being attractive to the eye, they decided to bring the potatoes to the Old World (Stevenson, 1951). Potatoes were first introduced to Spain in 1570, and to England in 1580 (Love et al., 2003). Queen Marie Antoinette made the potato flowers a fashion statement in France when she started to wear the flower clusters in her hair, and the royal court tried to imitate her (Stevenson, 1951). At first, in the Old World, potatoes were considered only for royalty, but quickly became popular with sailors suffering from scurvy, an ailment caused by the lack of vitamin C (Stevenson, 1951; Jerardo, 2012). In the late 1700s, when Europe was suffering due to crop failures and famine; the potato became accepted as a high-calorie, stable food source, that was widely adapted, and that could feed both humans and livestock (Love et al., 2003; Jerardo, 2012). By the mid-1800's, Ireland depended almost exclusively on the potato, and when for three consecutive years the potato crop failed in the field, more than 1.5 million people died of hunger (Love et al., 2003; Jerardo, 2012). It was discovered that the potatoes had a fungal disease called late blight (*Phytophthora infestans* [Montagne] de Bary), the cause for the

famous Irish Potato Famine (Stevenson, 1951; Jerardo, 2012). Since the potato famine had killed millions, and left others starving, a great wave of Irish rushed to the US (Jerardo, 2012).

The cultivated potato (*Solanum tubersoum* L.) is a heterozygous tetraploid (Stevenson, 1951). Although the potato originated in the Andes region, it now grows in various habitats from the very seasonally wet/dry climates to high altitude grasslands, and from coastlines to upland rain forests (Love et al., 2003).

Nutritional Value

The cultivated potato is the world's most important horticultural crop (Salas and Spooner, 2006). More carbohydrates per area are produced by potato than any other crop, except sugarcane (*Saccharum officinarum* L.) (Salas and Spooner, 2006). Potato has a higher quality protein than any other vegetables, with the exception of soybean (*Glycine max* (L.) Merr.), which yields more protein per acre (Salas and Spooner, 2006). One medium size potato with no additives has 110 calories, and is fat, sodium, and cholesterol free (United States Potato Board, 2013). Potato is a great source of potassium, providing 620mg, or 18%, of the recommended daily value (DV) per serving. Also, a serving of potato provides 45% of vitamin C, 8% of fiber, 10% of B_6, and 6% of iron of the recommended DV (Bohl and Johnson, 2010; United States Potato Board, 2013).

Commercial Potato Production

Potato tubers come in a variety of sizes, shapes, and colors; therefore, there are different potato markets (Love et al., 2003). The various potato markets include: fresh, chip processing, frozen processing, specialty, and certified seed (Bohl and Johnson, 2010). Chip processing potatoes are used to make chips (Bohl and Johnson, 2010). Frozen processing potatoes are used to make french fries, hash browns, and more (Bohl and Johnson, 2010). The specialty market

may include organically produced potatoes and those used for home preparation; these may be yellow, red, or blue, and often have colored flesh (Stevenson, 1951; Love et al., 2003). Certified seed is produced, and certified for use as seed the next planting season (Bohl and Johnson, 2010).

Production and Economic Importance

Worldwide, potato ranks fifth for total production after sugarcane, corn (*Zea mays* L.), rice (*Orzya sativa* L.), and wheat (*Triticum aestivum* L.) (FAO, 2012). Potato is an important crop in the US, which also ranks fifth in comparison with crops (United States Potato Board, 2013). North Dakota ranks third in harvested acres, but fourth in value of production by state (USDA-NASS, 2013a). As the leading vegetable crop in the US, over 50% of potato sales are to processors for producing french fries, chips, dehydrated products, and other potato products, with the remaining for the fresh and seed markets (Jerardo, 2012). Even though potatoes are produced year round in varying states, the fall crop makes up 90% of potato production (Jerardo, 2012). The invention of irrigation systems and refrigerated rail transport made it possible to develop new potato producing areas like Idaho, Washington, and Colorado; these areas now lead US potato production, producing nearly two-thirds of the fall crop (Jerardo, 2012). The states of Idaho and Washington are responsible for over half of the US production alone (Jerardo, 2012). Over the past decade, the potato industry has significantly consolidated its operations. The Census of Agriculture reported that in 2007, there were 15,014 potato-producing farms, in comparison to 1974, when there were over 51,000 farms (Jerardo, 2012). Farmers look for ways to maximize production through larger operations, because large capital investments are required for equipment and storage facilities to maintain potato production (Jerardo, 2012).

In the US, the five-year average (2009-2013) value of production exceeded $3.8 billion, with an average yield of 4.575 Mg/ha (USDA-NASS, 2013b). The five-year average (2008-2012) value of production for North Dakota exceeded $192 million (USDA-NASS, 2013b), with an average yield of 30,375 kg/ha (USDA-NASS, 2013b).

Potatoes are an important crop in North Dakota, economically and historically. The Red River Valley is characterized by land with dark, high organic matter soils that contain almost no rocks, and that are nearly flat (SIEM and EM, 1998; NPPGA, 2014). These components are the perfect conditions for producing bright red potatoes (NPPGA, 2014). The North Dakota State University (NDSU) potato breeding program was initiated in 1930, by the North Dakota Agricultural Experiment Station, with the mission of developing improved cultivars for producers in North Dakota and the region (Thompson, 2011).

Colorado Potato Beetle

The Colorado potato beetle (*Leptinotarsa decemlineata* Say.) belongs to the order Coleoptera and the family Chrysomelidae, in which almost all members are leaf eaters (Abdelhaq, 2006). The Colorado potato beetle (CPB) feeds exclusively on foliage of cultivated and wild plants of the *Solanaceae* family (Ragsdale et al., 2007; Kuhar et al., 2009). The CPB was first recognized as a pest in 1859, when it became a crucial agricultural problem in the state of Colorado (Ragsdale et al., 2007). The beetle's original host was buffalobur nightshade (*Solanum rostratum* Dun.) (Ragsdale et al., 2007; Alyokhin, 2009). Settlers brought potato with them, and the beetle found a new host (Ragsdale et al., 2007; Alyokhin, 2009). The CPB stretched its range eastward, by approximately 136 km/year, reaching the East Coast by 1874 (Ragsdale et al., 2007).

Numerous insect species can cause defoliation injury to potato, but none is more destructive than the CPB (Radcliffe, 1982). The CPB adults and larvae can eat entire leaves without discerning leaf tissues (Alyokhin, 2009). A single larva can consume nearly 40 cm^2/day of leaves, and a single adult can consume almost 10 cm^2/day (Alyokhin et al., 2008; Alyokhin, 2009). Potatoes can tolerate up to 30 to 40% defoliation in the vegetative stage (Ragsdale et al., 2007; Alyokhin, 2009). When the potato tubers are starting to bulk, the plant is more sensitive and can only tolerate about 10% defoliation (Ragsdale et al., 2007; Alyokhin, 2009).

The CPB annual (complete metamorphosis) life cycle starts, based on temperature and physiological state, during early spring or early summer, with the emergence of overwintering adult beetles from the ground (Anonymous, 1981). Following the adult's emergence, the beetles do short flights, or walks, to the nearest potato field to feed (Anonymous, 1981). Females lay eggs within a day or two (from 15 to 30°C), 10 to 30 at a time, placing them on the abaxial (lower) side of the leaves in several orderly rows (Anonymous, 1981). This will continue until midsummer, with a single female laying up to 2000 eggs in a two month period. Egg hatching occurs in 4 to 12 days (if temperatures are above 12°C); the hatched larvae start to feed immediately (Anonymous, 1981). Larvae will continuously feed and will only stop when molting occurs, which can occur four times during the course of 2-3 weeks (Anonymous, 1981). Larvae from the same egg batch will remain together for their first molt on the abaxial leaf surface, after which they will move on to the terminal buds (Anonymous, 1981). Matured larvae will fall to the ground and burrow in the soil at different depths, depending on environmental conditions (Anonymous, 1981). The cycle can start again if weather conditions are warm enough for the beetles to reemerge; if not, they will hibernate until the following summer (Anonymous, 1981).

The CPB has developed resistance to over 52 different compounds, belonging to all major insecticide classes, since the 1950's (Alyokhin et al., 2008). Levels of resistance can vary significantly among different populations of CPB, and life stages (Alyokhin et al., 2008). According to Alyokhin et al. (2008) there are many factors explaining the high predisposition to resistance development. One of the factors is the fact that the plants in the *Solanaceae* family have high concentrations of glycoalkaloids in their foliage (Alyokhin et al., 2008). Consequently, the beetles were required to evolve and develop a physiological capability to detoxify, or tolerate, the glycoalkaloids in the host plant (Alyokhin et al., 2008). The second factor is the high fecundity rate, increasing the probability of random mutations (Alyokhin et al., 2008). When these mutations take place, it guarantees that the new resistant (mutant) will multiple fast (Alyokhin et al., 2008). The third factor is that the larvae and beetles both feed on the same host plants, narrowing the host range (Alyokhin et al., 2008). The CPB is an oligophagous feeder native to the Americas (Hsiao, 1978). Hsiao (1978) found that oligophagous insects need geographic isolation for them to get used to the new, less preferred, host plants. Since there are no unstructured refuge plants for the CPB to escape to, there is no reduction in the size of susceptible individuals that can escape exposure to chemicals (Alyokhin et al., 2008). If some susceptible beetles survive on untreated potato, in fields that are rotated to alternate crops, or on *Solanaceous* weeds, the number is too low to reduce the frequency of resistant alleles below the economically significant level (Alyokhin et al., 2008). The fourth factor is that most farmers depend on insecticides for beetle control, because other techniques have not been practical, creating an increase in the selection pressure towards resistance (Alyokhin et al., 2008). Lastly, since the CPB is native to North America, it has not experienced the genetic bottleneck that is typical of introduced pests (Alyokhin et al., 2008). The high

genetic variability can assist in evolutionary plasticity that is necessary for the adaptation of the CPB to adverse conditions (Alyokhin et al., 2008). Nevertheless, this last factor is still unclear, since in Europe where the CPB is an introduced species, it is also a problem (Alyokhin et al., 2008).

Plant Resistance Mechanisms to Insects

Plant resistance is defined by Snelling (1941) as "the characteristics which enable a plant to avoid, tolerate, or recover from the attacks of insects under conditions that would cause greater injury to other plants of the same species". Painter (1958), on the other hand, defined plant resistance as "the relative amount of heritable qualities possessed by a plant which influence the ultimate degree of damage done by the insect." In other words, it represents the ability of certain cultivars to produce a larger crop of good quality, than do ordinary cultivars at the same level of insect population. Resistance is a relative measurement that utilizes susceptible cultivars of the same plant species as checks, and this degree of resistance varies between two extremes, immunity and high susceptibility, as defined by Singh et al. (2005). Degrees of resistance can be classified according to the following scale by Painter (1958):

a) Immunity: "An immune cultivar is one that a specific insect will never consume or injure under any known condition."

b) High resistance: "A variety with high resistance is one which possesses qualities resulting in small damage by a specific insect under a given state of conditions."

c) Low resistance: "A low level of resistance indicates the possession of qualities which cause a cultivar to show less damage or infestation by an insect than the average for the crop under consideration.

d) Susceptibility: “A susceptible cultivar is that showing average or more than average infestation or damage by an insect.”

e) High susceptibility: “A cultivar shows high susceptibility when much more than average damage is done by the insect under consideration.”

According to Singh et al., (2005), host-plant resistance is the result of a series of plant-insect interactions, which can influence plant selection as hosts, and the plants effect on the insect survival and multiplication. Painter (1958) proposed mechanisms of resistance that can be grouped into three categories:

a) Non-preference: for oviposition, shelter, or food, because of absence of a certain quality. Several factors can affect the preference and non-preference of the insects (Singh et al., 2005):

- “The basis for preference depends on the insect behavior like orientation, feeding and oviposition.”
- “Preference depends on the response to color or intensity of light.”
- “Preference depends on the response to mechanical stimuli from physical structure and surface of the plant for example trichomes, surface wax, silication or sclerotization of tissues.”
- “Preference depends on response to chemical stimuli.”

b) Antibiosis: can affect the biology of the insect unfavorably (Singh et al., 2005). Painter (1958) suggested four physiological explanations for antibiosis.

- “The toxic effects of specific chemicals including toxins, for example glycoalkaloids.”
- “Food present, but for one reason or another, is not accessible to the insect.”

- "Portion of the plant eaten was absent of specific plant nutrients or materials."
- "Presence of repellent materials making it impossible for insects to feed and starve to death."

c) Tolerance: is a plant response to an insect pest (Teetes, 2013). The plants resistance that is able to tolerate or regain from the damaged cause by the insect in the same quantity as a plant that does not contain the resistance characters (susceptible) (Teetes, 2013).

Genetic Sources of Resistance to the Colorado Potato Beetle

Many wild potato species have resistance to insect pests (Vallejo et al., 1994; Jansky et al., 2009). *Solanum chacoense* Bitter, *S. berthaultii* Hawkes, *S. tarijense* Hawkes, *S. demisssum*, *S. vernei* Bitter et Wittm., *S. acaule* Bitter, *S. hjertingi* Hawkes, *S. jamesii* Torr., *S. polyadenium* Greenm, *S. demissum*, and *S. neocardenasii* Hawkes and Hjert are known wild potato species with resistance to CPB (Gibson, 1976; Sanford et al., 1984; Dimock et al. 1986; Sinden et al., 1986; Dimock and Tingey, 1988). The resistance identified in *S. berthaultii* Hawkes, *S. tarijense* Hawkes, and *S. polyadenium* Greenm is based on glandular trichomes, whereas, the resistance in *S. chacoense* Bitter and *S. neocardenasi* Hawkes and Hjert depends on glycoalkaloids (Gibson, 1976; Dimock et al., 1986; Sinden et al., 1986; Dimock and Tingey, 1986).

There are several other control methods that can be used to manage CPB, which can include sanitation, cultural practices, physical, biological, microbial, and chemical controls (Ragsdale et al., 2007). It has been found that a combination of control methods should be used to ensure the management of CPB (Ragsdale et al., 2007); therefore, plant breeders have been

investigating various approaches. Glycoalkaloids and glandular trichomes are two of the mechanisms plant breeders have been researching (Coombs et al., 2005; Jansky 2009).

Glycoalkaloids

European scientists noticed that several wild *Solanum* species, such as *S. demissum* Lindl., *S. chacoense* Bitter, and *S. polyadenium* Greenm, had defensive properties due to the presence of glycoalkaloids (Tingey, 1984; Sinden et al., 1986). Early studies proposed a positive correlation between total (leaf) glycoalkaloid content (TGA) of the wild potato species and resistance to CPB (Tingey, 1984).

There are several considerations for glycoalkaloids in potato breeding programs as a means of deploying insect resistance. Initially, the TGA content of potato foliage is controlled by polygenic mechanisms, and the TGA content of foliage and tubers is highly correlated (Tingey, 1984). Botanically speaking, potato tubers are modified stems including all the internal structures (Dwelle, 2003). In a cross section of a tuber, you can find an outer skin tissue (periderm), an outer ring of storage tissue (the cortex), a ring of vascular tissue and inner storage tissue (the pith) just like a stem (Dwelle, 2003). Solanine and chaconine are found in all potato cultivars as part of normal tuber components; however, if a tuber has total glycoalkaloids levels exceeding 20 mg/100 g fresh weight, it is considered unsafe (Sinden and Sanford, 1981). Glycoalkaloids vary quantitatively and qualitatively, depending on photoperiod, wavelength, intensity of light, soil moisture, stage of plant growth, tuber storage conditions, and wounding of tuber tissue (Tingey, 1984). Potato poisoning due to glycoalkaloids in humans have included mild gastrointestinal effects that began within 8 to 12 hours after ingestion and were resolved after a day or two (Lawley, 2013). Further reported symptoms include nausea, vomiting,

diarrhea, stomach cramps, headaches (Lawley, 2013). Some serious cases have involved neurological problems including hallucinations, paralysis, and fatalities (Lawley, 2013).

Glandular Trichomes

Another approach for providing protection from the CPB is the use of glandular trichomes. *Solanum berthaultii*, *S. tarijense*, and *S. polyadenium* possess glandular trichomes, which provide insect resistance (Gibson, 1976; Dimock et al., 1986). *Solanum berthaultii* Hawkes, a wild Bolivian potato, has shown resistance to aphids, leafhoppers, flea beetles, spider mites, and CPB (Neal et al., 1989; Kowalski et al., 1992; Hare, 1999). Thus *S. berthaultii* has become the center of attention in plant breeding programs investigating resistance to the CPB (Casagrande, 1982; Wright et al., 1985). Insect resistance in *S. berthaultii* is due to understated influences glandular trichomes have upon insect feeding, survival, growth rate and oviposition (Casagrande, 1982; Dimock and Tingey, 1987), rather than direct immobilization of larvae and/or adult beetles upon contact with the glandular trichome exudate (Dimock and Tingey, 1987). Research has shown that hybrids between *S. berthaultii* (wild parent) and *S. tuberosum* L. (cultivated potato) have demonstrated resistance to CPB (Neal et al., 1989). This resistance is possibly due to the high density of foliar glandular trichomes (Neal et al., 1989; Kowalski et al., 1992).

Trichomes are uni- or multicellular appendages that originate from epidermal cells and grow outward on the surface of various plant organs (Werker, 2000). There are two main types: glandular and non-glandular trichomes (Vallejo et al., 1994). Glandular trichomes come in a variety of shapes and structures, but they have in common, metabolically active cells, and the ability to store or secrete great quantities of specialized metabolites (Tissier, 2012). These

insecticidal mixtures of chemicals are often used in the pesticide industry (Peter and Shanower, 1998).

The plant kingdom has a vast distribution of glandular trichomes, suggesting a fairly ancient evolution (Tissier, 2012). Species from the angiosperm dicotyledonous families like *Lamiaceae, Solanaceae, Asteraceae,* and *Cannabanae* are rich in glandular trichomes (Tissier, 2012). When domesticated crop species were compared with their wild progenitors it was discovered that they were more vigorous, and the glandular trichome-derived specialized metabolites were lost during the domestication process (McDowell et al., 2011). This loss of metabolites may partially explain the susceptibility in domesticated crops to pathogen and herbivore attacks, compared with their wild progenitors (McDowell et al., 2011). By reintroducing these compounds into domesticated crop species, crop loss caused by insect attacks and disease may be avoided in a natural, effective manner (McDowell et al., 2011).

Glandular trichomes in potato can be divided into two types: Type A and Type B (Vallejo et al., 1994). Type A glandular trichomes are short trichomes, around 120 to 210 μm long, and their apex contains a tetralobulate membrane-bound gland (50 to 70 μm in diameter) (Vallejo et al., 1994). Type B glandular trichomes are longer, around 600 to 950 μm long, and their tips have an ovoid gland that constantly releases a clear, sticky, viscous exudate (Vallejo et al., 1994).

The sequential glandular trichome activity against green peach aphid can be divided into six steps: 1) the insect lands on the foliage and comes in contact with the tall Type B trichomes; 2) the exudates of the Type B trichomes, an adhesive coating, and sesquiterpenes, attaches to the insects' tarsi, producing a disturbance behavior; 3) as the insect fights to escape breaks off the Type A trichome heads; 4) broken Type A trichome heads release α and/or β–

polyphenoloxidases (PPO) and phenolic substrates (chlorogenic acid) and the oxidative process begins; 5) enzymatic oxidation of phenols, or the browning reaction, produces quinones; and 6) the insect is unable to move, stops feeding and dies (Gregory et al., 1986; Peter and Shanower, 1998).

Some methods for developing resistance to CPB in potato have been a) the production of resistant somatic hybrids through electrofusion of protoplasts of *S. chacoense* and *S. tuberosum* subsp. *tuberosum* (Cheng et al., 1995), b) the introgression of germplasm from other species of *Solanum* haploids such as *S. berthaultii* (Plaisted et al., 1992), *S. fendleri ssp. fendleri* (Lorenzen and Balbyshev, 1997), or *S. chacoense* (Sanford et al. 1984), and c) transgenic insertions of *Bacillus thuringiensis* (Bt) genes (Ebora and Sticklen, 1997). Questions have arisen if these protocols can ensure long-term resistance; research has supported both sides of the argument (Fischer et al., 2002). According to Groden and Casagrande (1986), the Rhode Island CPB population was maintained without the introduction of new insects to ensure purity. After only two years of quarantine, the CPB populations laid eggs and survived at the same rates as if they were on conventional potato cultivars (Groden and Casagrande, 1986). Cantelo et al., (1987) discovered that CPB began to adapt to the feeding of *S. chacoense* (glycoalkaloid mediated resistance) after only 12 months of continuous rearing. However, Franca (1991) discovered that after 10 generations of feeding on *S. berthaultii* (glandular trichome mediated resistance), the beetles could not adapt.

Neal et al. (1989) investigated the effects of the different types of glandular trichomes in potato. When Type A or Type B trichomes were not present on the leaves, the proportion of larvae that fed increased. Both situations resulted in decreased mortality. The research revealed that the active compounds of Type B trichomes are only effective in the presence of Type A

trichomes (Neal et al., 1989). Therefore, Type A trichomes are essential in the plant insect resistance mechanism.

Other experiments have investigated the effects of different resistance mechanisms employed by plants. Coombs et al. (2005) reported choice and no choice field study evaluations for resistance mechanisms against the CPB. In this study, host plants with different resistance characters were used: natural (glandular trichomes or glycoalkaloids), engineered (transgenic Bt), and both combined. The natural host plants were NYL235-4 (glandular trichomes), ND5873-15 (*S. chacoense* derived) known to have field CPB resistance, and 'Norwis' (susceptible control). The engineered host plant was NORc3.8 (Bt-cry3A), and the combined host plants were NYLc3.3 (Bt-cry3A and glandular trichome), and ND5c.1 (Bt-cry3A and *S. chacoense* derived). In the choice field study, ND5873-15, NORc3.*8*, ND5c3.1 and NYLc3.3 proved to be effective in controlling defoliation by CPB (Coombs et al., 2005). Coombs et al. (2005) stated that NYL235-4 suffered less defoliation than 'Norwis', although it had greater defoliation than NORc3.8. In the no-choice study, only the NORc3.8*,* ND5c3.1 and NYLc3.3 were effective in controlling defoliation (Coombs et al., 2005). However, Coombs et al. (2005) reported that ND5873-15 and NYL235-4 were more effective than 'Norwis'. This study showed that a combination of host plant resistance mechanisms could help breeders achieve the development of CPB resistant clones.

These two studies presented above (Neal et al., 1989; Coombs et al., 2005) can help us better appreciate the importance of glandular trichomes on crop species. If glandular trichome populations can be measured, it would help breeders select genotypes with a higher quantity. Type A glandular trichomes release polyphenoloxidase (PPO), an important asset in the insect

resistance mechanism (Neal et al., 1989). Two tests can be used to analyze the presence of PPO, the Enzymic Browning Assay (EBA), and the Modified Enzymic Browning Assay (MEBA).

The EBA protocol is based on utilizing the Type A trichome exudates from three potato leaflets collected in a test tube (Avé et al., 1986). The reagent solution in the tube changes from pink to violet, indicating the level of biochemical products in the trichomes. The biochemical products include polyphenoloxidase and phenolic substrates; when combined, they create a viscous substance that hardens (Avé et al., 1986). It has been demonstrated that there is a positive correlation between color intensity of the EBA, and green peach aphid resistance (*Myzus persicae*) (Ryan et al., 1983).

The MEBA is an indirect measurement of the concentration of glandular trichomes present on potato leaves (Avé et al., 1986). The MEBA uses a filter paper soaked with distilled water and a rubber stopper to press the leaves; this filter paper is submerged into the reagent solution (70 mM sodium phosphate buffer with 0.075% p-phenylenediamine) (Avé et al., 1986). The optical densities of the samples are measured on a spectrophotometer (Avé et al., 1986).

There are differences between the EBA and the MEBA protocols. For the EBA, leaves are submerged in the reagent solution (Avé et al., 1986). For the MEBA, the pressing of the leaves to the filter paper guarantees a complete rupture of Type A glandular trichomes (Avé et al., 1986). The paper disks in the MEBA protocol rupture the glandular trichomes over a specific area, and when combined with trichome density counts, provide a more precise assessment of enzymic browning in potato trichomes (Avé et al., 1986). Observations through the microscope demonstrated that leaves used in the EBA had little evidence of massive Type A glandular trichome rupture (Avé et al., 1986). The MEBA is dependent of the density of the Type A glandular trichomes, and instead monitors the presence of PPO and the substrates

(Kalazich and Plaistead, 1991). Tingey and Sinden (1982) demonstrated that potato cultivars containing both Type A and B trichomes appear to be more resistant against green peach aphid and potato leafhopper (*Empoasca fabae*), than the cultivars with only Type A glandular trichomes alone.

Kalazich and Plaistead (1991) demonstrated that the MEBA protocol is mostly influenced by the density of Type A trichomes, and not by the diameter of the Type A trichomes head. This information allows us to conclude that Type A trichome density is more useful than Type A head diameter when doing preliminary screening. The volume of Type B exudates has a higher correlation with diameter of the droplet, than with the Type B density (Kalazich and Plaistead, 1991). The Type A and B density on the abaxial side (bottom, lower surface) of the leaf is highly correlated with the adaxial side (top, upper surface), regardless of the type of cross (backcrossing, etc.) (Kalazich and Plaistead, 1991).

Another assay available to measure trichomes was developed by Zhang and Oppenheimer (2004). This assay is different from the MEBA because the main objective is to remove the trichomes from the plant surfaces. Leaves are collected, fixed, and treated with ethylene glycol tetra-acetic acid. Individual leaves or leaf pieces are placed in a Petri dish, and trichomes are removed by gently rubbing the samples using a small paintbrush. With this method, Zhang and Oppenheimer (2004) could acquire the trichomes, and could generate downstream applications such as cell wall analysis, immunolocalization of trichome proteins, analysis of DNA content, and proteomics.

This literature review discloses plant breeder's efforts to comprehend and introgress wild potato species insect resistance mechanisms into cultivated potato. Integrated crop management practices including host-plant resistance could provide for a high yielding, high quality crop,

with minimal environmental impacts. The goal of this research was to evaluate the presence of glandular trichomes as an insect resistance mechanism against CPB, and identify clones in the North Dakota State University potato breeding program for use as parental genotypes and/or release as named cultivars for adoption by the potato industry.

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CHAPTER 1. ASSESSMENT OF POTATO GERMPLASM FOR THE PRESENCE OF GLANDULAR TRICHOMES USING THE SCANNING ELECTRON MICROSCOPE

Abstract

The Colorado potato beetle (*Leptinotarsa decemlineata* Say.) is one of the most devastating insect pests in North America of potato (*Solanum tuberosum* L). Several wild potato species are insect resistant due to glandular trichomes on the foliage. The research objective was to assess the presence of glandular trichomes in six cultivars and advanced selections using the Scanning Electron Microscope. Micrographs revealed a range of densities (ratio of Type A: Type B) on both sides of potato leaflets. ND059804C-13 had the most glandular trichomes (9:13). Its parents, ND2858-1 (1:3) and King Harry (3:8), were intermediate in the quantity of glandular trichomes present. Russet Burbank (6:16) and ND060496-1 (8:5) came second behind ND059804C-13. ND059804C-7 had the least amount of glandular trichomes overall (1:2). This information is valuable to potato breeding programs in developing breeding strategies employing multiple defense mechanisms against insect pests, including the Colorado potato beetle.

Introduction

The potato center of origin lies in the Andes region (Peru, Chile, and Bolivia), extending as far north as the southern Rocky Mountains in the USA (Stevenson, 1951; Hijmans and Spooner, 2001; Love et al., 2003; Jerardo, 2012.). The US ranks fifth in comparison with other countries producing potato (FAO, 2012a). Within the US, potato ranks seventh in production, compared to other crops (FAO, 2012b).

Several insect species can cause injury to potato, but none are more damaging than the Colorado potato beetle (CPB) (Radcliffe, 1982). The CPB feeds solely on foliage of cultivated

and wild plants of the *Solanaceae* family (Ragsdale et al., 2007; Kuhar et al., 2009). Since the 1950's, CPB has developed resistance to over 52 different compounds, belonging to all major insecticide classes (Alyokhin et al., 2008).

There are various control methods that can be used to manage CPB, including cultural practices, physical and biological methods, and chemical controls (Ragsdale et al., 2007). A combination of control methods should be used to ensure the management of the CPB (Ragsdale et al., 2007). Glycoalkaloids and glandular trichomes are two approaches plant breeders have been researching to provide durable host plant resistance.

Solanum berthaultii Hawkes, *S. tarijense* Hawkes, and *S. polyadenium* Greenm are insect resistant due to glandular trichomes (Gibson, 1971; Gibson, 1974; Tingey and Laubengayer, 1981; Gregory et al., 1986; Dimock and Tingey, 1988). *S. berthaultii* and *S. polyadenium* have Type A glandular trichomes present on their foliage (Avé et al., 1986). *S. berthaultii* has shown resistance to aphids, leafhoppers, flea beetles, spider mites, and CPB (Gibson, 1971; Gibson, 1974; Tingey and Laubengayer, 1981). Research has shown that hybrids between *S. berthaultii* (wild parent) and *S. tuberosum* L. (cultivated potato) have confirmed resistance to CPB due to the high density of glandular trichomes (Neal et al., 1989; Kowalski et al., 1992).

Potato has two types of glandular trichomes, Type A and Type B (Vallejo et al., 1994). Type A glandular trichomes are short trichomes (around 120 to 210 μm long) and their apex contains a tetralobulate membrane-bound gland (50 to 70 μm in diameter) (Vallejo et al., 1994). Type B glandular trichomes are longer, around 600 to 950 μm long, and their tips have an ovoid gland that constantly releases a clear, sticky, viscous exudate (Vallejo et al., 1994). Glandular trichomes have metabolically active cells and the ability to store or secrete specialized metabolites (Tissier, 2012).

An objective of the North Dakota State University (NDSU) potato breeding program is the identification and introgression of genetic resistance to insect pests that cause economic losses in potato production. The goal of this research was to visually assess the presence of glandular trichomes on several potato cultivars and selections.

Materials and Methods

Six cultivars and advanced selections were used for this study (Table 1.1). All plant material was grown in the Agricultural Experiment Station Research Greenhouse complex (NDSU, Fargo, ND). Whole leaves were brought to the Electron Microscopy Center (USDA-ARS Northern Crop Science Laboratory, NDSU, Fargo, ND) for processing. The sampling unit consisted of the terminal leaflet and the third primary leaflet. For the identification of the presence of glandular trichomes on the leaflets, both the adaxial and abaxial sides were viewed. The adaxial side of a leaf is the top, upper surface, while the abaxial is the bottom, lower surface of a leaf. According to Tingey and Laubengayer, (1981) fully expanded, mature leaves should be used when screening for glandular trichome density, since glandular trichome density is at its minimum. The third primary leaflet is fully expanded at any growth stage (Dwelle, 2003). Glandular trichome densities will increase proportionately on immature, incompletely expanded leaves (Tingey and Laubengayer, 1981). Pelletier (1990) concluded that Type A glandular trichomes in populations of *S. berthaultii* and *S. tarijense*, which are a general anti-herbivore resistance mechanism, are found in lower altitudes where herbivores are more varied and plentiful. The Type B glandular trichomes, which protect against biotic and abiotic stresses, are found in abundance at higher altitudes since protection against extreme environmental conditions can be priority (Pelletier, 1990). This study supports the hypothesis that plants assign a lower amount of resources for defense at higher altitudes since herbivory is reduced and the need to

tolerate abiotic stresses is more important (Pelletier, 1990). Altitude and age of the plant are two factors that should be taken in consideration when evaluating genotypes for the presence of Type A and Type B glandular trichomes.

Table 1.1. Cultivar and advanced selections, and their parentage, assessed with the Scanning Electron Microscope, Electron Microscopy Center, USDA-ARS Northern Crop Science Laboratory, North Dakota State University, Fargo, ND.

Cultivar/Selection	Female parent	Male parent	Comments
Russet Burbank	Sport of Burbank		Industry standard cultivar
ND2858-1	S. chacoense Bitter	ND1215-1	Breeding selection with glycoalkaloids as CPB defense mechanism
King Harry	N142-72	Pike	Cultivar with Type A glandular trichomes
ND059804C-7	ND2858-1	King Harry	
ND059804C-13	ND2858-1	King Harry	
ND060496C-1	ND2858-1	Norchip	

Lou Sweet, a potato grower in Colorado, found a sport of Burbank in 1914, which Luther Burbank identified, and reported as Russet Burbank (Davis, 1992; Potato Association of America, 2009). Russet Burbank is tolerant to common scab (*Streptomyces* spp.), but susceptible to *Fusarium* and *Verticillium* wilts, leafroll, net necrosis, and Potato Virus Y (Potato Association of America, 2009).

The CPB resistance of ND2858-1 is linked to leptine I and leptine II glycoalkaloids, inherited from the female parent, *S. chacoense* (Lorenzen et al., 2001). Tuber glycoalkaloid content (8.4 mg/100 g fresh weight) is equivalent to commercial cultivars (Lorenzen and Balbyshev, 1997). The ND2858-1 is a good source of host-plant resistance to CPB (Lorenzen and Balbyshev, 1997). It has good vigor and yield, segregates for acceptable glycoalkaloid levels in the tubers, and it crosses well with other tetraploid genotypes (Lorenzen and Balbyshev, 1997).

Plaisted and Tingey (De Jong et al., 2011) at Cornell University first hybridized *S. berthaultii* with a commercial cultivar in 1980. After ten years of selection, and more than five backcrosses, King Harry was released in 2007 (De Jong et al., 2011). King Harry is recognized for its resistance to aphid (*Aphis* spp.), leafhopper (*Empoasca fabae*), flea beetle (*Epitrix* spp.), potato tuber moth (*Phthorimaea operculella*), and CPB (De Jong et al., 2011). De Jong et al. (2011) described King Harry as an early maturing cultivar with high yield. King Harry is known for possessing Type A glandular trichomes, but not Type B glandular trichomes (De Jong et al., 2011).

The ND059804C-7, ND059804C-13, and ND060496-1 lines are three advancing selections from the NDSU potato breeding program. ND059804C-7 and ND059804C-13 are siblings from the cross between ND2858-1 and King Harry. As described above, ND2858-1 is known for its glycoalkaloids based insect resistance and King Harry for glandular trichome based defense. These crosses were made to combine two CPB defense mechanisms, glycoalkaloids and glandular trichomes (Thompson, personal communication).

Jayma Moore at the Electron Microscopy Center (USDA-ARS Northern Crop Science, NDSU, Fargo, ND) facilitated the preparation of samples for evaluation of the presence of glandular trichomes for the cultivars and advanced selections. Leaflets were cut into squares using a new razor blade. The leaf tissue squares were fixed in 2.5% glutaraldehyde in sodium phosphate buffer (Tousimis, Rockville, MD, US) and stored at 4°C overnight. The next day, they were rinsed in buffer and water. Samples were dehydrated utilizing a graded alcohol series from 30% to 100% ethanol. An Autosamdri-810 critical point dryer (Tousimis, Rockville, MD, US), with liquid carbon dioxide as the transitional fluid, was used to dry the samples to the critical point. Leaf tissue samples were attached to aluminum mounts with silver paint (SPI

Supplies, West Chester, PA, US), and spray coated with gold/palladium (Balzers SCD 030, Balzers Union Ltd., Liechtenstein). Images were obtained using a JEOL JSM-6490LV Scanning Electron Microscope (SEM), operating at an accelerating voltage of 15 kV.

Micrographs were printed on a white piece of paper, and identified by cultivar or selection, and the side of the leaflet. All but one of the micrographs were taken with a magnification of X70; one was taken at a magnification of X250. Two squares of 1mm^2 in area were drawn on transparency sheets for the micrographs with a magnification of X70. The first square in the transparency sheet was placed on the top left corner of the micrograph, and the second square on the transparency sheet was placed on the bottom right corner of the micrograph. Type A and Type B glandular trichomes were counted and averaged for each cultivar or selection, and for the abaxial and adaxial side of the leaflet. One square of 0.5 mm^2 in area was drawn on a transparency sheet for the micrographs with a magnification of X250. Type A and B glandular trichomes were counted for that micrograph.

Results and Discussion

The Scanning Electron Micrographs (SEM) of King Harry are presented in Figure 1.1. Glandular trichomes were present on both the adaxial and abaxial sides of the leaflet. Results are indicated with mean values. On the adaxial side (Figure 1.1a), there were 2 Type A and 4 Type B glandular trichomes/mm^2. On the abaxial side (Figure 1.1b), there were 4 Type A and 12 Type B glandular trichomes/mm^2. This indicates that there are more Type B than Type A glandular trichomes on both sides of the leaflets. The micrographs indicate the presence of Type B glandular trichomes. This is contradictory to De Jong et al. (2011) who indicated that King Harry possessed Type A glandular trichomes, but not Type B glandular trichomes. These differences in results could be because the current micrographs were made from leaflets from

plants grown in a greenhouse. Perhaps, De Jong et al. (2011) acquired and made the cultivar release handout utilizing a leaflet from a field grown plant, or maybe they did not use a SEM, but instead a less powerful microscope or means to assess trichome density. The log-like structure on Figure 1.1b is a vascular structure on the abaxial side of the leaflet.

Russet Burbank, the most widely grown cultivar in North America, micrographs are presented in Figure 1.2. Glandular trichomes were present on both the adaxial and abaxial sides of the leaflet. On the adaxial side of the leaf there were 4 Type A and 2 Type B glandular trichomes/mm^2 (Figure 1.2a). On the abaxial side, there were 8 Type A and 29 Type B glandular trichomes/mm^2 (Figure 1.2b). This indicates that there are more Type A than Type B glandular trichomes on the adaxial side, but more Type B than Type A glandular trichomes on the abaxial side. Tingey and Laubengayer (1981) studied the effect of trichome density on green peach

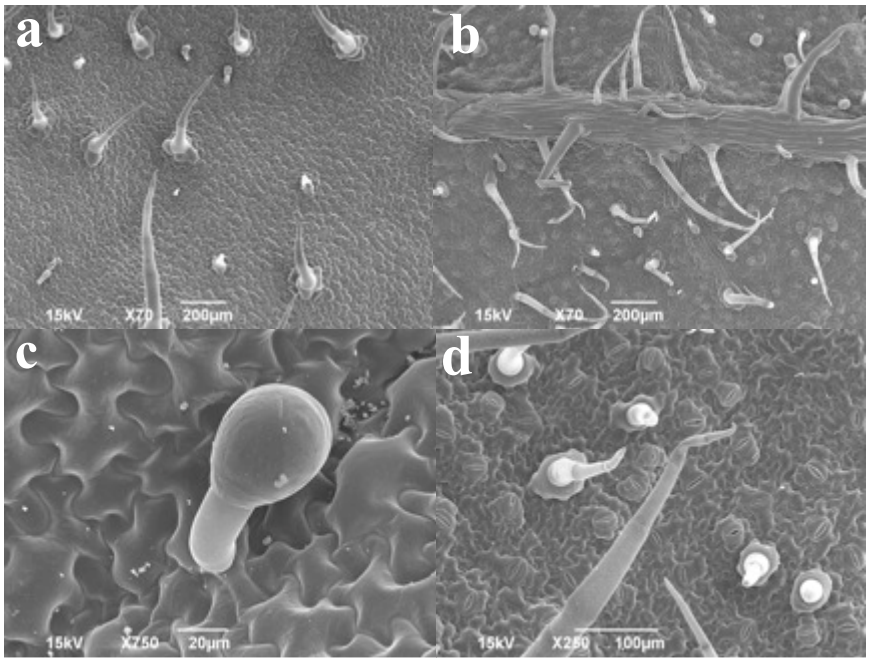

Figure 1.1. Scanning electron micrographs of the leaf surface of King Harry: adaxial side (panel a), abaxial side (panel b), Type A abaxial side (panel c) and Type B abaxial side (panel d). Panels a, and b have a magnification of 70X, the bar representing 200 µm. Panel c has a magnification of 750X, the bar representing 20 µm. Panel d has a magnification of 250X, the bar representing 100 µm. Courtesy of Jayma Moore, Electron Microscopy Center, USDA-ARS Northern Crop Science Laboratory, North Dakota State University, Fargo, ND.

aphid resistance, and found that for a 50% nymphal mortality there was a minimum of 5 Type A and 53 Type B glandular trichomes required. They concluded that clones with a high density of Type A glandular trichomes are a "key parameter" in the selection of parental germplasm (Tingey and Laubengayer, 1981). The interaction between Type A and Type B glandular trichomes significantly increases aphid resistance; therefore, selection of clones bearing both types is crucial (Tingey and Laubengayer, 1981). Neal et al. (1989) confirmed that the presence of Type A glandular trichomes is crucial in CPB resistance and the presence of Type B glandular trichomes increases the expression of resistance. The Type A glandular trichomes four-lobed glands are clearly distinguishable in Figure 1.2c; log-like structures on Figure 1.2b are the vascular structures of the leaf on the abaxial side.

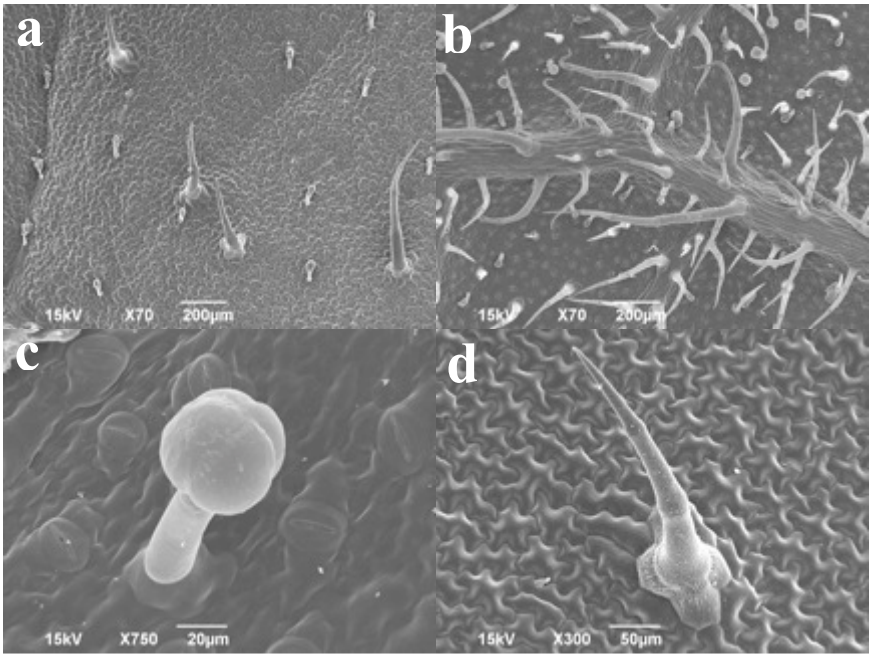

Figure 1.2. Scanning electron micrographs of the leaf surface of Russet Burbank: adaxial side (panel a), abaxial side (panel b), Type A abaxial side (panel c) and Type B adaxial side (panel d). Panels a, and b have a magnification of 70X, the bar representing 200 µm. Panel c has a magnification of 750X, the bar representing 20 µm. Panel d has a magnification of 300X, the bar representing 50 µm. Courtesy of Jayma Moore, Electron Microscopy Center, USDA-ARS Northern Crop Science Laboratory, North Dakota State University, Fargo, ND.

In comparison to King Harry and Russet Burbank, ND2858-1 (Figure 1.3) had the fewest glandular trichomes on the adaxial and abaxial side (Figure 1.3a and 1.3b). On the adaxial side, there were 1 Type A and 1 Type B glandular trichome/mm^2 (Figure 1.3a). On the abaxial side, there were 1 Type A and 4 Type B glandular trichomes/0.5 mm^2 (Figure 1.3b). Thus, the abaxial and adaxial sides have similar populations of Type A glandular trichomes, but more Type B glandular trichomes on the abaxial side. Lorenzen and Balbyshev (1997) reported ND2858-1 as highly resistant to CPB, having good vigor, good tuber yields, progeny would segregate for acceptable levels of tuber glycoalkaloids, and that it crosses well with other tetraploid cultivars. The ND2858-1 has *S. chacoense* as a parent (Lorenzen et al., 2001). Spooner and Clausen (2013) described *S. chacoense* as lacking Type B glandular trichomes.

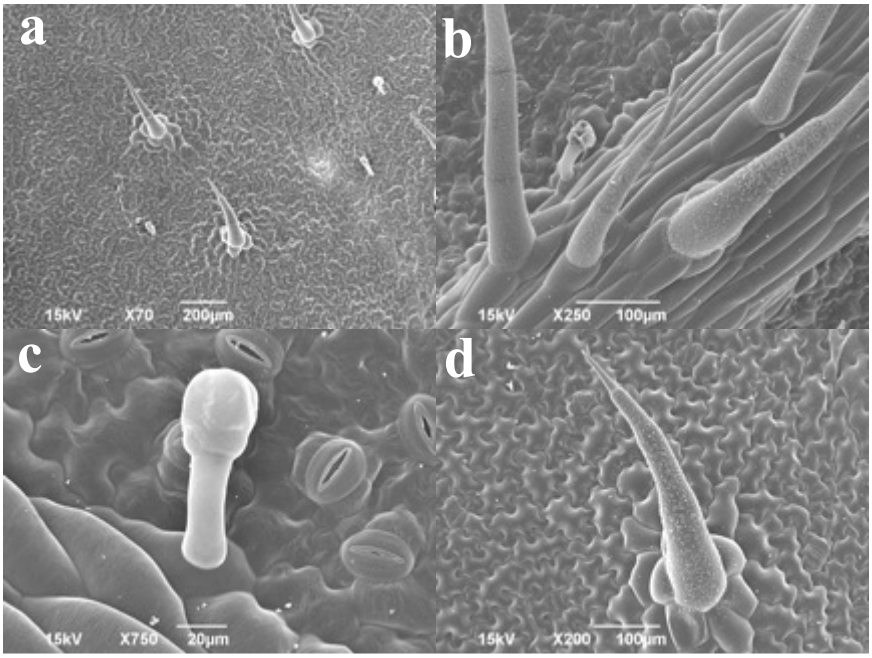

Figure 1.3. Scanning electron micrographs of the leaf surface of ND2858-1: adaxial side (panel a), abaxial side (panel b), Type A abaxial side (panel c) and Type B abaxial side (panel d). Panel a has a magnification of 70X, the bar representing 200 µm. Panel b has a magnification of 250X, the bar representing 100 µm. Panel c has a magnification of 750X, the bar representing 20 µm. Panel d has a magnification of 200X, the bar representing 100 µm. Courtesy of Jayma Moore, Electron Microscopy Center, USDA-ARS Northern Crop Science Laboratory, North Dakota State University, Fargo, ND.

The insect resistance of ND2858-1 is attributed to glycoalkaloids and not glandular trichomes, since it contains leptines I and II (Lorenzen et al., 2001). Open stomata can be seen on the abaxial side of the leaf in Figure 1.3c.

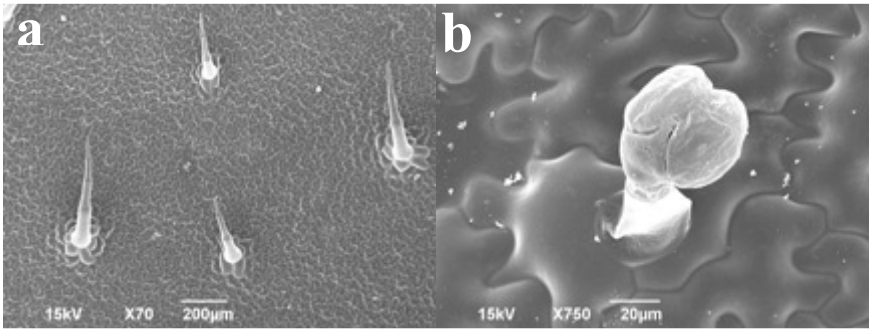

Figure 1.4. Scanning electron micrographs of the leaf surface of ND059804C-7: adaxial side (panel a), and Type A adaxial side (panel b). Panel a has a magnification of 70X, with the bar representing 200 µm. Panel b has a magnification of 750X, the bar representing 20 µm. Courtesy of Jayma Moore, Electron Microscopy Center, USDA-ARS Nothern Crop Science Laboratory, North Dakota State University, Fargo, ND.

Of the six genotypes evaluated by SEM, ND059804C-7 had the fewest number of glandular trichomes per unit area (Figure 1.4). On the adaxial side, there were 1 Type A and 2 Type B glandular trichomes/mm^2 (Figure 1.4a). Glandular trichomes were not observed on the abaxial side of the sample. This indicates that there are more Type B than Type A glandular trichomes on the adaxial side. King Harry, the male parent, had more Type B glandular trichomes mean of 8 than Type A with a mean of 3 on both sides of the leaflets, and ND2858-1, the female parent, had the least glandular trichome density with only a mean of 1 Type A and a mean of 2.5 Type B glandular trichomes per mm^2. The lack of glandular trichomes indicates that ND059804C-7 most likely inherited the trichome density trait from the female parent, ND2858-1. Mehlenbacher et al. (1983) determined that few genes control the density of Type A and Type B glandular trichomes, because the parental phenotypes were recovered in F_2 and backcross generations. The Type A (Figure 1.4b) glandular trichome found appears damaged. The damage

could have been caused in the greenhouse by insect or human activity, by the sampling, or the preparation process.

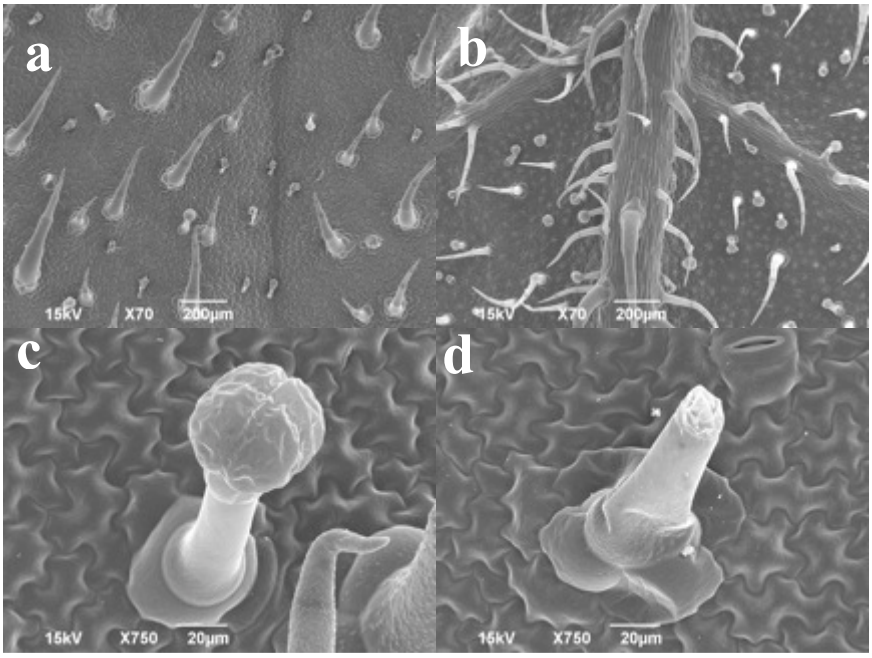

Figure 1.5. Scanning electron micrographs of the leaf surface of ND059804C-13: adaxial side (panel a), abaxial side (panel b), Type A abaxial side (panel c) and Type B abaxial side (panel d). Panels a, and b have a magnification of 70X, with the bar representing 200 µm. Panel c has a magnification of 750X; the bar represents 20 µm. Panel d has a magnification of 750X with the bar representing 20 µm. Courtesy of Jayma Moore, Electron Microscopy Center, USDA-ARS Northern Crop Science Laboratory, North Dakota State University, Fargo, ND.

A sibling of ND059804C-7, ND059804C-13 was the fifth genotype observed. On the adaxial side of ND059804C-13 there were 8 Type A and 10 Type B glandular trichomes/mm^2 (Figure 1.5a). On the abaxial side, there were 10 Type A and 17 Type B glandular trichomes/mm^2 (Figure 1.5b), indicating that there are more Type B than Type A glandular trichomes on both the adaxial and abaxial sides. The ND059804C-13 demonstrates a combination of traits from both parents. ND059804C-13 inherited the glandular trichome quantity trait from King Harry. ND059804C-13 has more Type B glandular trichomes than Type A, which was inherited from ND2858-1. The Type B glandular trichome in Figure 1.5d has a

broken tip. The damage could have been caused in the greenhouse by insect or human activity, by the sampling, or the preparation process.

The ND059804C-7 and ND059804C-13 are two siblings from the cross between ND2858-1 and King Harry. After assessing them with the SEM, there was a distinct difference in the amount of glandular trichomes on both sides of the leaves. The ND059804C-7 had the fewest amount of glandular trichomes whereas the sibling, ND059804C-13 had more. Potatoes are polyploids, which are are "euploids in which the somatic cells possess multiples of complete basic chromosomes sets (x) in excess of the diploid number" (Sleper and Poehlman, 2006). According to Sleper and Poehlman (2006), polyploids are important assets for plant breeders, providing them greater expression of existing genetic diversity. Breeders have the chance to manipulate plant characters by modifying the genome number, and subsequently the quantity of allelic genes that contribute to that particular character (Sleper and Poehlman, 2006). Commercial potatoes are autotetraploid, making the study of inheritance of traits challenging (Sleper and Poehlman, 2006). These siblings demonstrate how segregation of traits varies within a family.

Kalazich and Plaisted (1991) investigated the relationship between trichome characters and agronomic traits in a hybrid cross between *S. tuberosum* and *S. berthaultii*. They concluded that introgression of trichome traits from *S. berthaultii* into *S. tuberosum* is complex. Inheritance of Type B glandular trichomes is more complex than for Type A glandular trichomes (Kalazich and Plaisted, 1991). The backcross generation lacks Type B glandular trichomes, but they can be recovered if the backcross siblings are intermated or selfed (Kalazich and Plaisted, 1991). Kalazich and Plaisted (1991) used simple phenotypic selection for their progeny because of their

strong resemblance to wild species (late maturity, small leaflets, and small tubers) to ensure Type B glandular trichomes were inherited.

Gibson (1979) identified a single dominant gene controlling the presence of Type B glandular trichomes in *S. tarijense* and *S. berthaultii*. However, at least one recessive gene is associated, because F_1 generations of *S. phureja* x *S. berthaultii* and *S. tuberosum* spp. *tuberosum* x *S. berthaultii* hybrid progenies lacked Type B glandular trichomes (Gibson, 1979). F_2 generations had a frequent recurrence of Type B glandular trichomes, indicating that relatively few genes control this trait (Gibson, 1979). Mehlenbacher et al. (1983) determined that few genes control the density of Type A and Type B glandular trichomes because parental phenotypes were recovered in F_2 and backcross generations. Individual plants in a mixed population will demonstrate different phenotypic traits (yield, height, flowering, etc.) due to genetics or environment (Sleper and Poehlman, 2006). Heritability (h^2) is the proportion of the observed phenotypic variation in the progeny that is caused by genetics (Falconer and Mackay, 1996; Sleper and Poehlman, 2006.) Breeders desire high heritability; therefore, phenotypic selection becomes an effective method in identifying the best genotypes from that population. In hybrids of *S. tuberosum* spp. *tuberosum* x *S. berthaultii*, the heritability for the density of Type B glandular trichomes was higher than for the density of Type A glandular trichomes (Mehlenbacher et al., 1984). Type A glandular trichome densities varied for locations (Mehlenbacher et al., 1984). In the current study we did not use multiple counts or locations, since our main purpose was to have a quick assessment of what we had. Multiple counts and locations should be considered for future assessment for glandular trichomes by the NDSU potato breeding program.

The ND060496C-1 selection (Figure 1.6) has a moderate quantity of glandular trichomes compared with the others. On the adaxial side, there were 8 Type A and 2 Type B glandular trichomes/mm^2 (Figure 1.6a). On the abaxial side, there were 7 Type A and 8 Type B glandular trichomes/mm^2 (Figure 1.6b). This indicates that there are more Type A than Type B glandular trichomes on the adaxial side, but more Type B than Type A glandular trichomes on the abaxial side. The ND060496C-1 came from the cross between ND2858-1 and Norchip, which was then crossed with King Harry. The F_1's of the ND2858-1 and King Harry cross had a range of glandular trichomes quantities and now we can see that with a backcross with King Harry we have slowly increased the glandular trichomes quantity. More backcrossing with King Harry might increase the quantity of glandular trichomes on the leaflets. The log-like structure in Figure 1.6b is the vascular structure of the leaf on the abaxial side.

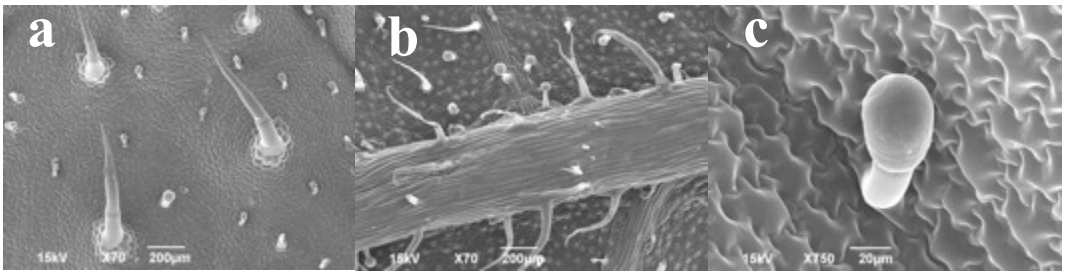

Figure 1.6. Scanning electron micrographs of the leaf surface of ND060496C-1: adaxial side (panel a), abaxial side (panel b), and Type A adaxial side (panel c). Panels a, and b have a magnifications of 70X with the bar representing 200 µm. Panel c has a magnification of 750X; the bar represents 20 µm. Courtesy of Jayma Moore, Electron Microscopy Center, USDA-ARS Northern Crop Science Laboratory, North Dakota State University, Fargo, ND.

The ND059804C-13 (18) had the most Type A glandular trichomes present on the leaflet when assessed using the SEM. King Harry (6), its male parent, was intermediate in ranking of the six cultivars and advanced selections, while ND2858-1 (2), its female parent, had very few. Russet Burbank (12) was intermediate and ND059804C-7 (1) had the fewest number of Type A glandular trichomes present on leaflets when assessed using the SEM. Russet Burbank (31) leaflets had the most Type B glandular trichomes when assessed using the SEM. King Harry

was intermediate in ranking of the six cultivars and advanced selections with 16, while ND059804C-7 had very few Type B glandular trichomes with just 2.

Visual assessment of Type A and Type B glandular trichomes using SEM revealed a range of densities (mean ratio of Type A:Type B). ND059804C-13 (9:13) had the most Type A and Type B glandular trichomes on both adaxial and abaxial side, while its sibling, ND059804C-7 (1:2) had the fewest. The ND2858-1 (1:3) is the female parent, which has few glandular trichomes, and King Harry (3:8), the male parent, is known for its glandular trichomes (De Jong et al., 2011). Russet Burbank (6:15) is an industry standard cultivar. King Harry was expected to have high levels of glandular trichomes based on the literature (De Jong et al., 2011), while Russet Burbank was expected to have the least (Potato Association of America, 2009). The ND059804C-13 and ND060496-1, progeny of King Harry and ND2858-1, exhibit the inheritance of the glandular trichome trait from this parent. The ND059804C-13 qualifies as CPB resistant advanced selection based on Type A and Type B glandular trichomes density (Tingey, and Laubengayer, 1981; Neal et al., 1989).

The benefits of employing SEM include its effectiveness as an accurate way to visually assess the presence of glandular trichomes on potato leaves. Furthermore, once the samples are mounted, they can be stored and reused at any time. A drawback of the SEM is that it takes time between sampling and processing of the samples. Additionally, SEM is quite expensive compared with a compound microscope, making it less appealing for large-scale screening by potato breeding programs.

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CHAPTER 2. SCREENING POTATO GERMPLASM FOR THE PRESENCE OF GLANDULAR TRICHOMES

Abstract

Colorado potato beetle (*Leptinotarsa decemlineata* Say) is one of the most devastating insect pests impacting potato in North America. Glycoalkaloids and glandular trichomes are two approaches in developing insect resistant plants. Potato glandular trichomes can be divided into Type A and Type B glandular trichomes. The research objective was to evaluate North Dakota State University advanced potato breeding selections, progeny families, and cultivars for the presence of Type A glandular trichomes using the Modified Enzymic Browning Assay (MEBA). The experimental approach consisted of screening leaf samples from three field trials in 2011, and two field trials in 2012. MEBA results indicated a high amount of Type A glandular trichomes for Russet Burbank and King Harry. The Ebt 6-21-3 is a promising parental genotype breeding for Colorado potato beetle resistance. Potato breeding programs can utilize the MEBA as early as the F1 generation, saving resources, including time, space, and ultimately funding.

Introduction

Potato production is an important economic resource to the US. In the last five years, more than three billion dollars were introduced to in the US economy, and an average of $192 million to the state economy of North Dakota (USDA-NASS, 2013). Insects, nematodes, bacteria, viruses, and fungi can affect potato; consequently, it needs protection against an assortment of pests. The Colorado potato beetle (CPB) is one of the most devastating insect pests of potato.

The CPB feeds on plant foliage of the *Solanaceae* family, regardless of if they are cultivated or wild species (Ragsdale et al., 2007; Kuhar et al., 2009). The CPB has become resistant to more than 52 different compounds, including all major insecticide classes, since 1950 (Alyokhin et al., 2008). The CPB populations and beetles of varying life stages have different levels of resistance to chemical controls (Alyokhin et al., 2008). Sanitation, cultural practices, physical, biological, microbial, and chemical methods can be applied to manage CPB (Ragsdale et al., 2007). A combination of control methods should be used for management of CPB (Ragsdale et al., 2007). Plant breeders are investigating different combinations and tactics for defense. Glycoalkaloids and glandular trichomes are two plant mechanisms under study (Coombs et al., 2005; Jansky et al., 2009).

The presence of glandular trichomes in wild potato species including *S. berthaultii*, *S. polyadenium,* and *S. tarijense* are recognized as resistant to different insect pests (Tingey and Sinden, 1982; Avé et al., 1986; Dimock and Tingey, 1988; Neal et al., 1989; Kowalski et al., 1992; Hare, 1990; and Pelletier et al., 1990). Potato breeding programs have been using *S. berthaultii*, particularly for introgressing CPB resistance (Hare, 1990).

Glandular trichomes are uni- or multicellular appendages originating from epidermal cells that grow outwards on surfaces of various plant organs (Werker, 2000). They serve several functions in the plant, such as to deter herbivores, guide pollinators, affect photosynthesis, and control leaf temperature and water loss (Rodriguez et al., 1984). Glandular trichomes can also produce pest or pollinator interactive chemicals, which are stored, or volatilized at the plant surface (Wagner, 1991). Glandular trichome secretions have been utilized in a variety of ways, such as in the fragrance, detergent, and medical industries (Tissier, 2012). Pesticide companies have exploited the chemicals found in glandular trichomes for insecticide and behavioral control

options (Peter and Shanower, 1998). Potato glandular trichomes consist of two types: Type A and Type B (Vallejo et al., 1994). Type A glandular trichomes are short trichomes (120-210 μm long) and their apex contains a tetralobulate membrane-bound gland (50-70 μm in diameter) (Vallejo et al., 1994). Type B glandular trichomes are longer trichomes (600-950 μm long), and their tips have an ovoid gland that constantly releases a clear, sticky, viscous exudate (Vallejo et al., 1994). *S. berthaultii* and *S. polyadenium* are known for the presence of Type A glandular trichomes (Avé et al. 1986).

Tingey and Sinden (1982) demonstrated that potato cultivars containing both Type A and B trichomes can provide more resistance against green peach aphid (*Myzus persicae*) and potato leafhopper (*Empoasca fabae*), than cultivars with Type A glandular trichomes alone. Likewise, Neal et al. (1989) concluded that both Type A and Type B glandular trichomes are required for CPB resistance in potato (Neal et al., 1989). Coombs et al. (2005) reported choice and no choice field study evaluations for resistance mechanisms against the CPB. Coombs et al. (2005) concluded that a combination of host plant resistance mechanisms could help breeders achieve their desired goal developing of durable host plant resistance to CPB.

The Enzymic Browning Assay (EBA) and the Modified Enzymic Browning Assay (MEBA) are two tests for polyphenoloxidase (PPO) secreted by Type A glandular trichomes. The EBA and the MEBA have some differences regarding their protocols. For the EBA, leaves are dipped in the reagent solution, whereas in the MEBA, leaves are pressed to a filter paper to allow for rupture of the Type A glandular trichomes (Avé et al., 1986). When samples from the EBA were observed through the microscope, little evidence of trichome rupture was found (Avé et al., 1986). The modification in the MEBA guarantees rupture of Type A glandular trichomes over a specific area (Avé et al., 1986). Avé et al. (1986) determined that MEBA, coupled with

trichome density counts, provide a more precise assessment of enzymic browning by potato glandular trichomes than the EBA. The MEBA depends on the density of Type A glandular trichomes to monitor the presence of PPO; a high optical density score indicates superior Type A glandular trichome quality (Kalazich and Plaistead, 1991).

Vallejo et al. (1994) confirmed that the MEBA is a better protocol for assessing glandular trichomes than a microscope. First, there is a positive correlation between PPO activity and aphid resistance (Ryan et al., 1983). Neal et al. (1989) confirmed that the presence of Type A glandular trichomes is crucial in CPB resistance and the presence of Type B glandular trichomes increases the expression of resistance. Heritability estimates of PPO activity are higher than the estimates for Type A and Type B glandular trichome densities (Vallejo et al. 1994). Lastly, there is a higher uniformity and accuracy in measuring PPO activity, as opposed to counting glandular trichomes (Vallejo et al., 1994), making the MEBA a faster and a less tedious protocol.

An aim of the North Dakota State University (NDSU) potato breeding program is to identify and introgress genetic insect resistance into adapted potato germplasm for adoption by North Dakota producers and growers beyond. The goal of this research was to evaluate NDSU advanced potato breeding selections, progeny families, and cultivars for the presence of glandular trichomes utilizing the MEBA.

Materials and Methods

Three locations and two locations were evaluated with the MEBA (Table 2.1 and Table 2.2), in 2011 and 2012, respectively. In 2011, the locations were Hoople and Absaraka, ND, and Baker, MN. Each plot (genotype) in Hoople, ND (2011, and 2012) consisted of ten hills (plants), with a guard plant (All Blue) at the beginning and end of each plot. In Absaraka, ND and Baker, MN (2011) each plot (genotype) consisted of two four-hill units (plants) for a total of eight hills

in a plot. In 2012, at Grand Forks, ND, the field design was a randomized complete block design (RCBD), with a split-block arrangement, with Factor A as genotypes, and Factor B as treatments (insecticide in-furrow (Imidacloprid 91.79 g/ha) versus no insecticide). There were two replicates, with each plot consisting of five hills, with a guard plant (All Blue) at the beginning and end of each plot.

The sampling unit was the secondary leaflet of the fourth leaf (first fully expanded) from field grown plants. Four replicates, one leaf from four different plants, were analyzed from each plot. The samples were stored in Ziplock ™ storage bags, placed in a cooler and brought to the laboratory where they were kept in the refrigerator at 4°C until analysis was done. Each location took four days to analyze with the MEBA in the laboratory.

The Modified Enzymic Browning Assay protocol by Avé et al. (1986) and Vallejo et al. (1994) was used to screen genotypes for the presence of glandular trichomes. The first step was to prepare small filter paper discs (6 mm) from Whatman filter papers, using a hole puncher. Deionized water was used to wet the filter paper discs, and with the help of tweezers, they were laid onto a green rubber stopper (7 mm). The leaf tissue sample, which was the secondary leaflet of the fourth leaf (one leaflet), was placed on a paper towel, and the green rubber stopper was tapped 15 times on each of the abaxial (low surface of leaf) and adaxial (upper surface) sides. The filter paper disk was dropped into a test tube on ice that contained 3 mLs of 70 mM sodium phosphate buffer (pH 7.0) with 0.075% p-phenylenediamine. All samples were kept in an ice water bath until placed in the shaking water bath. The tubes were placed in a shaking water bath at 37°C for 30 minutes. Samples were placed back in the ice water bath while the optical density (OD) was determined using a Beckman Coulter DU 730 spectrophotometer at 420 nm. A blank

containing the buffer, and the positive control ('King Harry' cultivar) were used to calibrate the spectrophotometer.

Analysis of variance (ANOVA p=0.05) was determined for the MEBA data utilizing the GLM procedure (SAS Institute, 2012). When significance was found, a mean separation test was performed using Tukey analysis (SAS Institute, 2012).

The FREQ procedure (SAS Institute, 2012) was used to group the data into categories from a normally distributed data set. Afterwards, the Chi-square test and the "Good Fit Test" of Kolmogorov-Smirnov were conducted (SAS Institute, 2012). The Chi-square test confirmed that the OD ranges associated with the percentile distributions are correct and the "Good Fit Test" of Kolmogorov-Smirnov confirmed the data was normally distributed. The optical density data was analyzed using Cluster Analysis, and histograms were created with the Univariate Procedure (SAS Institute, 2012).

Table 2.1. Genotypes, with parentage, from field trials at Hoople, and Absaraka, ND, and Baker, MN, in 2011, and Hoople, ND, in 2012.

Genotype	Female parent	Male parent
Atlantic	Wauseon	Lenape
Dakota Crisp	Yankee Chipper	Norchip
Dakota Pearl	ND1118-1	ND944-6
Lenape	47156	B3672-3
NDJL11C-1	ND4708-6PE	N140-201
NDJL11C-3	ND4708-6PE	N140-201
NDJL12C-1	ND4708-6PE	N140-201
NDJL13C-3	ND4710-1	N140-201
NDJL14C-2	ND4710-1	Q115-24
NDJL18C-1	ND4710-1	Q115-24
NDJL19C-1	ND5096-5	Q115-24
NDJL19C-3	ND5096-5	Q115-24
NDJL20C-1	ND5374-9B	N140-201
NDJL20C-4	ND5374-9B	N140-201
NDJL21C-1	ND5374-9B	Q115-24
NDJL21C-3	ND5374-9B	Q115-24
NDJL21C-7	ND5374-9B	Q115-24
NDJL22C-2	ND5374-9B	Q244-6
NDJL22C-3	ND5374-9B	Q244-6
NDJL23C-1	ND5455-3B	N140-201
NDJL23C-2	ND5455-3B	N140-201
NDJL23C-3	ND5455-3B	N140-201
ND049219AB-5	Atlantic	Ebt 6-5-5
ND049305Ab-1	SM8-12	ND7443Ab-72
ND050038-4	ND8305-1	Dakota Crisp
ND059804C-7	ND2858-1	King Harry
ND059804C-13	ND2858-1	King Harry
ND059818C-5	ND4382-17	NY131
ND060496C-1	ND4382-17	King Harry
ND060574B-6	ND6947b-20	ND7192-1
ND060598AB-1	ND7192-1	Ebt 6-5-5
ND060601CAB-2	ND7192-1	ND028804CAb-1
ND060604Ab-24Y	ND7291b-2Y	Ebt 6-5-5
ND060605AB-6	ND7333b-7	Ebt 6-5-5
ND060606CB-2	ND7333b-7	NY131
ND060618CB-2	ND7377Cb-1	EGA970614
ND060618CB-5	ND7377Cb-1	EGA970614
ND060648ABC-1	ND7443AB-76	King Harry

Table 2.1. Genotypes, with parentage, from field trials at Hoople, and Absaraka, ND, and Baker, MN, in 2011, and Hoople, ND, in 2012 (continued).

Genotype	Female parent	Male parent
ND060686C-1	ND7519-1	King Harry
ND060686C-6	ND7519-1	King Harry
ND060695c-6	ND7560C-4	White Pearl
ND060700C-7	ND7560C-4	ND7377Cb-1
ND060705C-8	ND7629C-2	NY131
ND060712C-7	ND7632-3	ND4382-52
ND060837C-7	ND028799C-3	King Harry
ND060838C-14	ND028799C-3	ND860-2
ND071006B-2	SM8-15	ND7495b-6
ND071097C-2	ND7291b-2Y	R91102-2
ND071112-2Y	ND7818-1Y	ND860-2
ND071142C-2	ND8305-1	ND028799C-2
ND071142C-3	ND8305-1	ND028799C-2
ND071146C-1	ND8307-7	ND860-2
ND071154CB-1	ND8477CB-21	ND7799c-1
ND071154CB-2	ND8477CB-21	ND7799c-1
ND071154CB-3	ND8477CB-21	ND7799c-1
ND071155CB-1	ND8477CB-21	ND8304-2
ND071155CB-2	ND8477CB-21	ND8304-2
ND071252B-1	ND028856B-1Russ	ND049423B-3Russ
ND071282CB-1	ND039077B-3	ND028799C-2
ND071282CB-2	ND039077B-3	ND028799C-2
ND071282CB-7	ND039077B-3	ND028799C-2
ND071289CAB-4	ND039104CAB-3	ND028799C-2
ND071334CB-5	ND039209C-3	ND049227B-1
ND071336-1	ND049219-6	ND860-2
ND071378B-63	ND049497B-4	ND049227B-1
ND071396C-1	ND049321C-4	ND860-2
ND071401CB-1	ND049321C-4	ND049285CB-4
ND071402C-1	ND049323C-8	ND860-2
ND081450CB-1	Dakota Diamond	Allegria
ND081452CB-1	Dakota Diamond	ND8277B-5
ND081456B-1Y	Gala	ND860-2
ND081456B-3Y	Gala	ND860-2
ND7379B-6	EB8109-1	Jacqueline Lee
ND7379b-7	EB8109-1	Jacqueline Lee
ND7381B-17	EB8109-1	ND860-2

Table 2.1. Genotypes, with parentage, from field trials at Hoople, and Absaraka, ND, and Baker, MN, in 2011, and Hoople, ND, in 2012 (continued).

Genotype	Female parent	Male parent
ND7403B-5	JND88166-1	EB8109-1
ND7550C-1	ND4382-51	ND4778-2
ND7601-9	ND5250-8	S440
ND7601-10	ND5250-8	S440
ND8316-1	ND3828-15	White Pearl
ND860-2	ND78-3	ND9583-1
NDJL24C-2	ND5455-3B	N140-201
NDJL25C-1	ND5812-1	ND5873-15
NDJL27C-1	ND5812-1	Q115-24
NDJL30C-2	ND5873-57	Q115-24
NDJL7C-2	ND4382-51	N140-201
NDJL7C-3	ND4382-51	N140-201
NDJL7C-4	ND4382-51	N140-201
NDJL8C-1	ND4382-15	Q115-24
NDJL8C-3	ND4382-51	Q115-24
Snowden	Lenape	Wischip

Table 2.2. Genotypes with parentage evaluated from the field trial at Grand Forks in 2012.

Genotype	Female parent	Male parent	Reference
Ebt 5-31-2	P2-3	Katahdin	Gillen and Novy, 2007
Ebt 5-31-3	P2-3	Katahdin	Gillen and Novy, 2007
Ebt 5-31-4	P2-3	Katahdin	Gillen and Novy, 2007
Ebt 5-31-5	P2-3	Katahdin	Gillen and Novy, 2007
Ebt 6-21-12	P2-3	Katahdin	Gillen and Novy, 2007
Ebt 6-21-2	P2-3	Katahdin	Gillen and Novy, 2007
Ebt 6-21-3	P2-3	Katahdin	Gillen and Novy, 2007
Ebt 6-21-4	P2-3	Katahdin	Gillen and Novy, 2007
Ebt 6-21-5	P2-3	Katahdin	Gillen and Novy, 2007
Ebt 6-5-2	P2-3	Katahdin	Gillen and Novy, 2007
Ebt 6-5-5	P2-3	Katahdin	Gillen and Novy, 2007
J101-K6	*S. bulbocastanum*	*S. tuberosum* (J101)	James et al., 1997
J101-K6-A22	*S. bulbocastanum*	*S. tuberosum* (J101)	James et al., 1997
J103K-7	*S. bulbocastanum*	*S. tuberosum* (J103)	James et al., 1997
J138-A12	*S. bulbocastanum*	*S. tuberosum* (J138)	James et al., 1997
ND060836ABc-15	ND028799C-3	Ebt 6-5-5	Thompson, personal communication
ND060898AB-1	ND039986AB-3	EB8109-1	Thompson, personal communication
ND071289CAB-3	ND039104CAB-3	ND028799C-2	Thompson, personal communication
ND2858-1	*S. chacoense*	ND1215-1	Lorenzen and Balbyshev, 1997
ND7443Ab-72Russ	S440	Ebt 5-31-5	Thompson, personal communication
P2-4	2-7-4D	Katahdin	Gillen and Novy, 2007
King Harry	N142-72	Pike	De Jong et al., 2011
Red Norland	Sport of Norland		Thompson, personal communication
Russet Burbank	Sport of Burbank		Davis, 1992

Results and Discussion

The combined analysis of variance for the OD from the MEBA from Absaraka and Hoople, ND, and Baker, MN, in 2011, demonstrated that main effects (location and genotype) were significantly different, but the genotype by location interaction was not significantly different (Table 2.3).

Table 2.3. Mean squares for optical densities from the Modified Enzymic Browning Assay, a glandular trichome assessment tool in potato, from Absaraka and Hoople, ND, and Baker, MN, in 2011.

Sources of Variation	df	Mean squares	F value
Location	2	3.21	***
Genotype	97	0.09	***
Location x Genotype	152	0.05	
Error	755	0.06	
CV% 37			

*** Significant at 0.001 probability level.

Location was significantly different suggesting that it had an effect on the OD's from the MEBA (Table 2.3). According to the North Dakota Agricultural Weather Network, 2012 had slightly higher air temperature, higher average bare soil temperature, and higher solar radiation, but less rainfall than 2011 (NDAWN, 2014). We did not run statistics for the weather data therefore, it cannot be said with certainty that the weather conditions were a factor affecting the OD's from each location. The literature lacks information regarding impact of location on glandular trichomes, other than altitude (Horgan et al., 2009). Perhaps it could be due to subtle differences in lighting or changes in temperature that influenced the beetle/larvae feeding behavior. The CPB life cycle depends on temperature for many functions including emergence, laying of eggs, egg hatching, and soil burrowing of adult beetles (Anonymous, 1981). For future research, air temperature, rainfall, radiation, and bare soil temperature should be taken into consideration along with data collection.

Genotype was significantly different, indicating that it had an effect on the OD's from the MEBA (Table 2.3). The parents of the genotypes used in this trial are NDSU lines, commercial lines, and Ebt lines (Table 2.1 and Table 2.2). This array of genetics provides a wide range of OD readings (Table 2.5). The NDSU lines have inherited glandular trichomes, glycoalkaloids, or both (Thompson, personal communication). The Ebt lines were created when Gillen and Novy (2007) reported that somatic hybridization was the solution to the obstacle for sexual

hybridization between *S. tuberosum* and *S. etuberosum*, allowing for introgression of the glandular trichome traits. *Solanum etuberosum* Lindl., a wild potato species native to Chile, is resistant to Potato Virus Y, Potato Leaf Roll Virus, Potato Virus X, and green peach aphid (*Myzus persicae* S.) (Gillen and Novy, 2007; Spooner, 2013).

The analysis of variance for the OD from the MEBA from Hoople, ND, in 2012, did not detect significant differences for the main effect (genotype) (Table 2.4). The analysis of variance for the OD from the MEBA from Grand Forks, ND, in 2012, detected that the main effect (genotypes) was significantly different (Table 2.4). Genotypes were expected to be different due to the parentage of the genotypes and their differences in the inheritance of glandular trichomes (Table 2.1 and Table 2.2). The parents of the genotypes used in this trial are NDSU lines, commercial lines, Ebt lines and other wild species (Table 2.1 and Table 2.2). The NDSU selections contain glycoalkaloids, glandular trichomes, or both (Thompson, personal communication), whereas the Ebt lines have inherited the glandular trichome trait (Gillen and Novy, 2007) from *S. berthaultii*, *S. etuberosum* or both.

Table 2.4. Mean squares and F values for optical density from the Modified Enzymic Browning Assay, a glandular trichome assessment tool in potato, from potato genotypes grown at Hoople and Grand Forks, ND, in 2012.

	Hoople, ND			Grand Forks, ND		
Sources of Variation	df	Mean squares	F values	df	Mean squares	F values
Genotype	81	0.03		23	0.10	***
Error	238	0.04		152	0.05	
CV%		66			42	

*** Significant at 0.001 probability level.

The means and standard deviations for the OD's from the MEBA for the Absaraka and Hoople, ND, and Baker, MN, 2011, and Hoople and Grand Forks, ND, 2012, samples are provided in Table 2.5. The means and standard deviations for the OD range from 0.07±0.07 (NDJL25C-1) to 0.93±0.30 (NDJL8C-1).

Vallejo et al. (1994) reported that high OD values indicate high enzymic activity. The goal is to acquire genotypes with a high OD, indicating high Type A glandular trichome density. Greater Type A glandular trichome density indicates more PPO activity; PPO inhibits CPB feeding (Vallejo et al., 1994). As seen on Table 2.5, a change in OD's from year to year can be detected; the 2011 data demonstrates higher OD's than the 2012. The 2011 trials were planted later in the calendar year than 2012, therefore there was a difference in calendar sampling dates. The weather conditions for the 2011 field trials were rainy and colder than the 2012 at the time of planting; therefore, the 2011 trials were planted later in the calendar year (NDAWN, 2014).

Table 2.5. Means and standard deviations for optical densities from the Modified Enzymic Browning Assay, a glandular trichome assessment tool, used to assess genotypes from Absaraka, Hoople, and Grand Forks, ND, and Baker, MN, in 2011 and 2012.

	Combined	Hoople	Grand Forks		Combined	Hoople	Grand Forks
Genotype	---2011----	----------2012----------		Genotype	-----2011-----	----------2012----------	
Atlantic	0.58±0.25	0.21±0.15	-	ND060606CB-2	0.54±0.21	0.24±0.18	-
Dakota Crisp	0.60±0.20	0.15±0.05	-	ND060618-5	0.84±0.21	0.11±0.04	-
Dakota Pearl	0.69±0.28	0.28±0.22	-	ND060618CB-4	0.63±0.29	-	-
Ebt 5-31-2	-	-	0.55±0.21	ND060618CB-5	0.60±0.13	0.25±0.20	-
Ebt 5-31-3	-	-	0.45±0.12	ND060648ABC-1	0.72±0.27	0.27±0.24	-
Ebt 5-31-4	-	-	0.51±0.12	ND060686C-1	0.61±0.31	-	-
Ebt 5-31-5	-	-	0.57±0.16	ND060686C-6	0.42±0.24	0.25±0.14	-
Ebt 6-21-12	-	-	0.64±0.27	ND060695c-6	0.75±0.22	-	-
Ebt 6-21-2	-	-	0.56±0.21	ND060700C-7	0.59±0.17	-	-
Ebt 6-21-3	-	-	0.76±0.32	ND060705C-8	0.78±0.28	0.44±0.34	-
Ebt 6-21-4	-	-	0.35±0.19	ND060712C-7	0.62±0.28	0.37±0.35	-
Ebt 6-21-5	-	-	0.37±0.07	ND060836ABc-15	-	-	0.42±0.13
Ebt 6-5-2	-	-	0.67±0.13	ND060837C-7	0.63±0.21	0.18±0.15	-
Ebt 6-5-5	-	-	0.45±0.22	ND060838C-14	0.59±0.27	0.22±0.16	-
J101-K6	-	-	0.47±0.21	ND060898AB-1	-	0.71±0.30	-
J101-K6-A22	-	-	0.63±0.51	ND071006B-2	0.67±0.18	-	-
J103K-7	-	-	0.39±0.14	ND071097C-2	0.65±0.29	0.21±0.18	-
J138-A12	-	-	0.51±0.22	ND071112-2	0.49±0.27	0.43±0.24	-
King Harry	-	-	0.59±0.18	ND071289CAB-3		-	0.45±0.09
Lenape	0.67±0.22	0.28±0.14	-	ND071142-3	0.68±0.15	-	-
ND049219AB-5	0.70±0.29	0.32±0.15	-	ND071142C-2	0.59±0.39	0.41±0.11	-
ND049305Ab-1	0.79±0.29	0.28±0.22	-	ND071142C-3	0.70±0.32	0.35±0.16	-
ND050038-4	0.72±0.29	0.33±0.20	-	ND071146C-1	0.51±0.26	0.28±0.15	-
ND059804C-13	0.56±0.19	0.29±0.15	-	ND071154CB-1	0.56±0.25	0.24±0.21	-
ND059804C-7	0.72±0.28	0.23±0.17	-	ND071154CB-2	0.56±0.21	0.23±0.16	-

Table 2.5. Means and standard deviations for optical densities from the Modified Enzymic Browning Assay, a glandular trichome assessment tool, used to assess genotypes from Absaraka, Hoople, and Grand Forks, ND, and Baker, MN, in 2011 and 2012 (continued).

	Combined	Hoople	Grand Forks		Combined	Hoople	
Genotype	----2011---	----------2012----------		Genotype	-----2011-----	----------2012----------	
ND059818C-5	0.68±0.29	0.32±0.18	-	ND071154CB-3	0.56±0.25	0.20±0.06	-
ND060496C-1	0.59±0.15	0.30±0.16	-	ND071155CB-1	0.55±0.30	0.22±0.17	-
ND060574B-6	0.63±0.26	0.28±0.08	-	ND071155CB-2	0.54±0.18	0.44±0.25	-
ND060598AB-1	0.68±0.36	-	-	ND071252B-1	0.62±0.22	-	-
ND060601CAB-2	0.70±0.33	0.37±0.23	-	ND071282CB-1	0.55±0.15	0.21±0.09	-
ND060604Ab-24Y	0.46±0.24	0.15±0.10	-	ND071282CB-2	0.55±0.17	0.15±0.08	-
ND060605AB-6	0.47±0.24	0.33±0.26	-	ND071282CB-7	0.53±0.25	0.22±0.10	-
ND071289CAB-4	0.67±0.31	-	-	NDJL14C-2	0.66±0.25	0.38±0.21	-
ND07128CB-1	0.86±0.15	-	-	NDJL19C-1	0.75±0.16	0.27±0.18	-
ND071334CB-5	0.70±0.31	0.20±0.14	-	NDJL19C-3	0.79±0.24	0.23±0.07	-
ND071336-1	0.62±0.27	0.34±0.13	-	NDJL20C-1	0.62±0.21	0.29±0.10	-
ND071378B-63	0.57±0.22	0.12±0.16	-	NDJL21C-1	0.71±0.35	0.41±0.30	-
ND071396C-1	0.65±0.17	0.26±0.20	-	NDJL21C-3	0.79±0.30	0.51±0.34	-
ND071401CB-1	0.57±0.34	0.27±0.21	-	NDJL21C-7	0.69±0.20	0.36±0.19	-
ND071402C-1	0.66±0.25	0.35±0.27	-	NDJL22C-2	0.58±0.20	0.23±0.15	-
ND081450CB-1	0.75±0.19	0.29±0.17	-	NDJL22C-3	0.57±0.24	0.24±0.16	-
ND081452CB-1	0.56±0.34	0.21±0.14	-	NDJL23C-1	0.68±0.26	0.23±0.20	-
ND081456B-1	0.68±0.18	0.29±0.17	-	NDJL23C-3	0.71±0.24	0.25±0.14	-
ND081456B-3	0.62±0.37	0.17±0.17	-	NDJL23C-4	0.56±0.18	0.35±0.13	-
ND2858-1	-	-	0.33±0.14	NDJL24C-2	0.61±0.26	0.26±0.23	-
ND73796-7	0.78±0.19	-	-	NDJL25C-1	0.59±0.34	0.07±0.07	
ND7379B-6	0.62±0.29	-	-	NDJL27C-1	0.75±0.18	0.33±0.16	
ND7379b-7	0.66±0.23	-		NDJL2BC-2	0.41±0.11	-	
ND7381B-17	0.55±0.26	0.39±0.09	-	NDJL30C-2	0.62±0.21	0.28±0.19	

Table 2.5. Means and standard deviations for optical densities from the Modified Enzymic Browning Assay, a glandular trichome assessment tool, used to assess genotypes from Absaraka, Hoople, and Grand Forks, ND, and Baker, MN, in 2011 and 2012 (continued).

	Combined	Hoople	Grand Forks		Combined	Hoople	Grand Forks
Genotype	----2011---	----------2012----------		Genotype	-----2011-----	----------2012----------	
ND7403B-5	0.50±0.26	0.29±0.24	-	NDJL310C-2	0.90±0.30	-	
ND7443Ab-72Russ	-	-	0.44±0.16	NDJL7C-2	0.61±0.22	0.45±0.30	
ND7550C-1	0.56±0.20	0.25±0.26	-	NDJL7C-3	0.65±0.26	0.56±0.33	
ND7601-10	0.68±0.26	0.46±0.10	-	NDJL7C-4	0.58±0.22	0.41±0.28	
ND7601-9	0.55±0.19	-	-	NDJL8C-1	0.93±0.30	0.27±0.25	
ND8316-1	0.73±0.24	0.28±0.14	-	P2-4	-	-	0.44±0.20
ND860-2	0.45±0.26	0.27±0.17	-	Red Norland	-	-	0.52±0.19
NDJL13C-3	0.68±0.20	0.49±0.16	-	Russet Burbank	-	-	0.54±0.11
NDJL18C-1	0.57±0.17	0.28±0.19	-	Snowden	0.55±0.20	0.30±0.22	-
NDJL20C-4	0.74±0.27	0.33±0.24	-				
NDJL23C-2	0.59±0.25	0.13±0.04	-				
NDJL8C-3	0.72±0.22	0.31±0.18	-				
NDJL11C-1	0.68±0.20	0.46±0.0	-				
NDJL11C-3	0.88±0.24	0.33±0.23	-				
NDJL12C-1	0.60±0.21	0.29±0.18	-				

Vallejo et al. (1994) utilized *S. berthaultii* (Ber), a hybrid between *S. phureja* and *S. stenotomum* (Phu-Stn), hybrid between Phu-Stn and Ber (F_1), backcross between Phu-Stn and F_1 bulk (BC) and reciprocal backcross between F_1 and Phu-Stn bulk (BC_R). Ber had the highest PPO activity recorded with an OD reading of 0.42±0.23, followed by BC_R with 0.42±0.06, F_1 progeny with 0.39±0.17, Phu-Stn genotypes with 0.26±0.08, and lastly, BC progeny with average reading of 0.16±0.03 at 470 nm (Vallejo et al., 1994). In comparison, our findings had OD's lower and higher than Vallejo et al. (1994). The parents of the genotypes used in this trial are NDSU lines, commercial lines, and Ebt lines (Table 2.1 and Table 2.2). Ebt lines were created when Gillen and Novy (2007) reported that somatic hybridization was the solution to the obstacle for sexual hybridization between *S. tuberosum* and *S. etuberosum*, allowing for introgression of the glandular trichome traits. The OD's of the Ebt lines revealed that the introgression of the glandular trichome trait was successful since the MEBA results categorized them as high for Type A glandular trichome quantity.

The Chi-square and Kolmogorov-Smirnov tests were conducted, and were significantly different (Table 2.6) (SAS Institute, 2012). The Chi-square test confirmed that OD ranges with the percentile distributions are correct (Figure 2.1). The "Good Fit Test" of Kolmogorov-Smirnov confirmed that the data were normally distributed.

OD ranges with the percentile distribution of the data were used to create OD categories (Table 2.6) to make the data easier to read and classify before conducting cluster analysis. The OD ranges and data were divided into a total of five categories: very low, low, medium, high and very high (Table 2.6) Type A glandular trichome quantity. The higher the Type A glandular trichome density is, the higher the PPO activity, translating to inhibition of CPB feeding (Vallejo et al., 1994), the goal of this study.

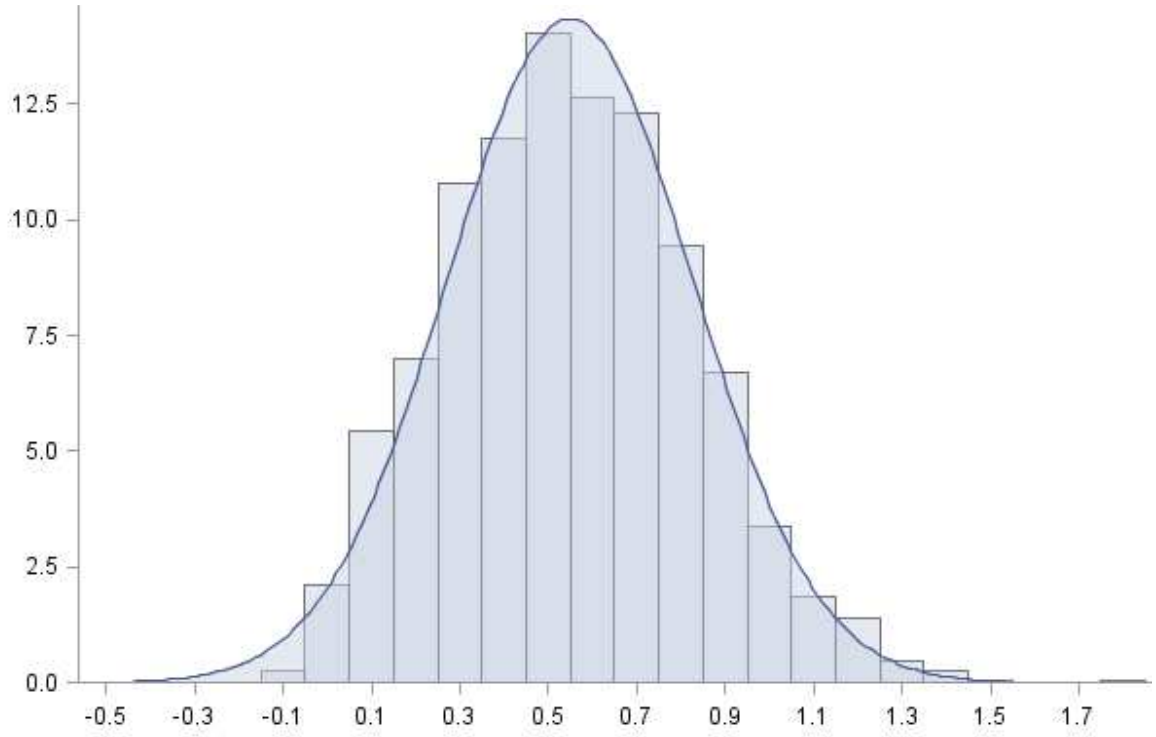

Figure 2.1. Bell curve, indicating the distribution of optical densities for genotypes grown at Absaraka and Hoople, ND, and Baker, MN, in 2011, and Hoople and Grand Forks, ND, in 2012.

Table 2.6. Categories, OD range, and percentile distribution of the MEBA data across genotypes for trials grown in 2011 and 2012, across all locations.

Category	OD range	Percentile distribution
Very Low	Less than 0.179	10%
Low	0.179-0.345	25%
Medium	0.345-0.540	50%
High	0.540-0.736	75%
Very High	More than 0.736	More than 75%
Chi-square (0.05) 149.7***		
Kolmogorov-Smirnov 0.048***		

Cluster analyses were made for the OD measurements from 2011 across three locations, and from 2012 for two locations (Figure 2.1). The first cluster analysis results from the Absaraka and Hoople, ND, and Baker, MN in 2011, which includes 98 genotypes and found in

Table 2.7. The 98 genotypes fell under the Very High (more than 0.736 OD range) to Medium (0.345-0.540 OD range) in the OD ranges for Type A glandular trichomes utilizing Table 2.6. The majority of the genotypes in 2011 across three locations indicate high levels of Type A glandular trichome activity, which can be translated to insect resistance due to glandular trichome quantity on foliage (Vallejo et al., 1994). More replicates and locations should be evaluated with these genotypes to retest with the MEBA, and to statistically confirm the results. The NDSU potato breeding program should then test the genotypes with high OD values in a field trial to assess defoliation and to confirm CPB resistance due to the presence of glandular trichomes. Subsets of these genotypes (24) were tested at the Northern Plains Potato Growers Association (NPPGA) Research Farm at Grand Forks, ND, in 2012 and 2013. The results of that field study will be discussed in Chapter 3: Field evaluation of potato genotypes to determine defoliation and oviposition by Colorado potato beetle (Thesis: Rodríguez-García, 2015).

For this set of data (Absaraka and Hoople, ND, and Baker, MN, in 2011), the top five genotypes according to the MEBA, having a high OD (OD and category range) are NDJL11C-3 (0.8799, Very High), ND07128CB-1 (0.8570, Very High), ND060618CB-5 (0.8438,Very High), NDJL21C-3 (0.7914, Very High) and NDJL19C-3 (0.7888, Very High). The parentage of ND07128CB-1, and ND060618CB-5 are from crosses between NDSU lines from the potato breeding program. The pedigrees of NDJL11C-3, NDJL21C-3, and NDJL19C-3 have parents with *Solanum berthaultii* in their pedigree from the Cornell University potato breeding program. *Solanum berthaultii* is classified as an insect resistant species due to the presence of Type A glandular trichomes on the foliage (Avé et al., 1986, Gibson, 1971; Gibson, 1974; Tingey and Laubengayer, 1981; Gregory et al., 1986; Dimock and Tingey, 1988). *Solanum berthaultii* has demonstrated resistance to aphids, leafhoppers, flea beetles, spider mites, and CPB (Gibson,

1971; Gibson, 1974; Tingey and Laubengayer, 1981). Hybrids between *Solanum berthaultii* (wild parent) and *Solanum tuberosum* L. (cultivated potato) have shown resistance to CPB due to the high density of glandular trichomes (Neal et al., 1989; Kowalski et al., 1992).

Table 2.7. Genotype, cluster number, and optical density mean for the 98 potato genotypes analyzed using optical densities obtained from the Modified Enzymic Browning Assay assessment tool for leaflet samples from Absaraka and Hoople, ND, and Baker, MN, in 2011.

Genotype	Cluster number	OD Mean	Range[†]	Genotype	Cluster number	OD Mean	Range
NDJL11C-3	5	0.8799	VH	NDJL30C-2	1	0.6189	H
ND07128CB-1	5	0.8570	VH	ND060712C-7	1	0.6188	H
ND060618CB-5	5	0.8438	VH	ND7379B-6	1	0.6188	H
NDJL21C-3	3	0.7914	VH	NDJL20C-1	1	0.6183	H
NDJL19C-3	3	0.7888	VH	NDJL7C-2	1	0.6136	H
ND73796-7	3	0.7840	VH	ND060686C-1	1	0.6094	H
ND060705C-8	3	0.7788	VH	NDJL24C-2	1	0.6075	H
ND060695c-6	3	0.7501	VH	ND060618CB-5	1	0.6040	H
NDJL19C-1	3	0.7484	VH	Dakota Crisp	1	0.6018	H
NDJL27C-1	3	0.7459	VH	NDJL12C-1	1	0.6013	H
ND081450CB-1	3	0.7446	VH	NDJL23C-2	1	0.5913	H
NDJL20C-4	3	0.7366	VH	ND060838C-14	1	0.5908	H
ND049305Ab-1	3	0.7860	H	NDJL25C-1	1	0.5900	H
ND060648ABC-1	3	0.7275	H	ND071142C-2	1	0.5879	H
ND8316-1	3	0.7268	H	ND060496C-1	1	0.5871	H
NDJL8C-3	2	0.7186	H	ND060700C-7	1	0.5868	H
ND059804C-7	2	0.7157	H	NDJL22C-2	1	0.5830	H
ND050038-4	2	0.7150	H	NDJL7C-4	1	0.5756	H
NDJL23C-3	2	0.7085	H	Atlantic	1	0.5748	H
ND071334CB-5	2	0.7008	H	NDJL22C-3	1	0.5698	H
ND049219AB-5	2	0.6958	H	ND071378B-63	1	0.5671	H
ND060601CAB-2	2	0.6958	H	ND071401CB-1	1	0.5648	H
ND071142C-3	2	0.6945	H	ND7550C-1	1	0.5644	H
NDJL21C-7	2	0.6912	H	ND071154CB-2	1	0.5638	H
Dakota Pearl	2	0.6868	H	ND059804C-13	1	0.5633	H
ND060598AB-1	2	0.6830	H	ND081452CB-1	1	0.5602	H
NDJL13C-3	2	0.6810	H	ND071154CB-3	1	0.5592	H
NDJL11C-1	2	0.6806	H	NDJL23C-4	1	0.5560	H
ND071142-3	2	0.6800	H	ND071154CB-1	1	0.5545	H
NDJL23C-1	2	0.6794	H	Snowden	1	0.5535	H
ND059818C-5	2	0.6793	H	ND071282CB-2	1	0.5523	H
ND081456B-1	2	0.6778	H	ND7381B-17	1	0.5522	H
ND7601-10	2	0.6762	H	ND7601-9	1	0.5457	H
Lenape	2	0.6702	H	ND071155CB-1	1	0.5454	H
ND071289CAB-4	2	0.6677	H	ND071282CB-1	1	0.5446	H

Table 2.7. Genotype, cluster number, and optical density mean for the 98 potato genotypes analyzed using optical densities obtained from the Modified Enzymic Browning Assay assessment tool for leaflet samples from Absaraka and Hoople, ND, and Baker, MN, in 2011 (continued).

Genotype	Cluster number	OD Mean	Range†	Genotype	Cluster number	OD Mean	Range
ND071006B-2	2	0.6656	H	ND060606CB-2	1	0.5434	H
ND071402C-1	2	0.6604	H	ND071155CB-2	1	0.5413	H
ND7379b-7	2	0.6603	H	ND071282CB-7	1	0.5291	M
NDJL14C-2	2	0.6573	H	ND071146C-1	4	0.5048	M
ND071097C-2	2	0.6458	H	ND7403B-5	4	0.4977	M
ND060618CB-4	1	0.6337	H	ND071112-2Y	4	0.4883	M
ND060574B-6	1	0.6323	H	ND060605AB-6	4	0.4715	M
ND060837C-7	1	0.6304	H	ND060604Ab-24Y	4	0.4603	M
ND071336-1	1	0.6237	H	ND860-2	4	0.4500	M
ND071252B-1	1	0.6208	H	ND060686C-6	4	0.4233	M
ND081456B-3Y	1	0.6206	H	NDJL2BC-2	4	0.4050	M

† Range of OD for glandular trichomes: M= medium, H= high, VH= Very high.

A second cluster analysis was conducted for the 82 genotypes from Hoople, ND, in 2012 and can be found in Table 2.8. The 82 genotypes were categorized, based on the ranges in Table 2.6, from Very Low (less than 0.179 OD range) to High (0.540-0.736 OD range), based on optical density (OD), an indicant measure of Type A glandular trichome quantity. The majority of the genotypes for Hoople, ND, 2012, fell into the categories of Low (0.179-0.345 OD range) and Medium (0.345-0.540), indicating a lack of, or relatively few, Type A glandular trichomes. According to this set of data alone, these genotypes should not be considered CPB-resistant due to the lack of Type A glandular trichomes quantity. However, the data from 2011 had the same cultivars and they were categorized as Very High (more than 0.736 OD range) and High (0.540-0.736 OD ranges) based the OD readings (Table 2.6). The difference in OD's from 2011 to 2012 may be due to the sampling time, and preparation of the samples. The data from 2011 and 2012 had a difference in the calendar year for the sampling dates. The 2011 data was sampled a month later in the calendar year than the 2012.

Table 2.8 Genotype, cluster number, and optical density mean for the 82 potato genotypes analyzed using optical densities obtained from the Modified Enzymic Browning Assay assessment tool for leaflet samples from field trial at Hoople, ND, in 2012.

Genotype	Cluster number	OD Mean	Range†	Genotype	Cluster number	OD Mean	Range
NDJL7C-3	2	0.5633	H	NDJL30C-2	1	0.2780	L
NDJL21C-3	2	0.5133	M	Dakota Pearl	1	0.2775	L
NDJL13C-3	2	0.4890	M	ND8316-1	1	0.2768	L
NDJL11C-1	1	0.4640	M	ND071146C-1	1	0.2758	L
ND7601-10	2	0.4608	M	ND060574B-6	1	0.2745	L
NDJL7C-2	4	0.4445	M	ND071401CB-1	1	0.2740	L
ND071155CB-2	4	0.4408	M	NDJL19C-1	1	0.2732	L
ND060705C-8	3	0.4345	M	ND860-2	3	0.2728	L
ND071112-2Y	4	0.4308	M	ND060648ABC-1	4	0.2725	L
ND071142C-2	2	0.4130	M	NDJL8C-1	1	0.2685	L
NDJLC-4	1	0.4128	M	ND071396C-1	1	0.2580	L
NDJL21C-1	4	0.4048	M	NDJL24C-2	1	0.2568	L
ND7381B-17	1	0.3930	M	ND060618CB-5	1	0.2540	L
NDJL14C-2	1	0.3773	M	ND7550C-1	1	0.2503	L
ND060601CAB-2	1	0.3733	M	NDJL23C-3	1	0.2445	L
ND060712C-7	1	0.3695	M	ND060606CB-2	1	0.2383	L
NDJL21C-7	1	0.3603	M	NDJL22C-3	1	0.2380	L
NDJL23C-4	1	0.3483	M	ND071154CB-1	1	0.2368	L
ND071402C-1	1	0.3477	M	NDJL23C-1	3	0.2338	L
ND071142C-3	1	0.3475	M	NDJL21C-7	1	0.2333	L
NDJL27C-1	2	0.3265	M	ND071154CB-2	1	0.2280	L
NDJL20C-4	2	0.3258	M	ND059804C-7	1	0.2258	L
ND060686C-6	2	0.2520	M	NDJL19C-3	1	0.2245	L
ND071336-1	1	0.3420	L	ND060838C-14	1	0.2230	L
ND050038-4	1	0.3313	L	ND071282CB-7	1	0.2218	L
NDJL11C-3	1	0.3283	L	ND071155CB-1	1	0.2155	L
ND060605AB-6	3	0.3268	L	ND071097C-2	1	0.2125	L
ND059818C-5	1	0.3185	L	Atlantic	1	0.2120	L
ND049219AB-5	1	0.3185	L	ND071282CB-1	1	0.2110	L
NDJL8C-3	4	0.3110	L	ND081452CB-1	1	0.2080	L
Snowden	6	0.3010	L	ND071334CB-5	1	0.2010	L
ND060496C-1	1	0.3000	L	ND060837C-7	3	0.1768	L
NDJL12C-1	4	0.2938	L	ND081456B-3Y	3	0.1685	VL
ND059804C-7	1	0.2915	L	ND060604Ab-24Y	1	0.1530	VL
ND7403B-5	4	0.2910	L	Dakota Crisp	1	0.1508	VL
NDJL20C-1	1	0.2905	L	ND071282CB-2	3	0.1480	VL
ND081456B-1	1	0.2853	L	NDJL23C-2	1	0.1313	VL
ND081450CB-1	1	0.2845	L	ND071378B-63	3	0.1190	VL
NDJL18C-1	1	0.2810	L	ND060618CB-2	1	0.1103	VL
Lenape	5	0.2810	L	NDJL25C-1	1	0.0700	VL
ND049305Ab-1	1	0.2803	L	ND071334CB-5	1	0.201	L

† Range of OD for glandular trichomes: VL= very low, L= low, M= medium, and H= high.

The top five genotypes for Hoople, ND, in 2012, based on their MEBA OD and their category are NDJL7C-3 (0.5633, High), NDJL21C-3 (0.5133, Medium), NDJL13C-3 (0.4890, Medium), ND7601-10 (0.4608, Medium), and NDJL11C-1 (0.4640, Medium). The NDJL7C-3, NDJL21C-3, NDJL13C-3, and NDJL11C-1 have one parent that is a *Solanum berthaultii* line from Cornell University. *Solanum berthaultii* is known to be insect resistant due to Type A glandular trichomes in their vegetation (Avé et al., 1986, Gibson, 1971; Gibson, 1974; Tingey and Laubengayer, 1981; Gregory et al., 1986; Dimock and Tingey, 1988).

The third cluster analysis was conducted for the 24 genotypes from Grand Forks, ND, in 2012, and can be found in Table 2.9. The 24 genotypes were categorized using Table 2.6 as Low (0.179-0.345 OD ranges) to Very High (more than 0.736 OD ranges) based on OD, a measure of Type A glandular trichome population. According to this data set alone, these genotypes are in the Medium range an indirect measure of Type A glandular trichome quantity. The top five genotypes for Grand Forks, ND, in 2012, based on their MEBA OD and category are Ebt 6-21-3 (0.7626, Very High), ND060898AB-1 (0.7065, High), Ebt 6-21-12 (0.6413, High), J101-K6-A22 (0.6308, High) and Ebt 6-5-2 (0.6732, High), and thus can be classified as CPB-resistant due to glandular trichome density. More replicates and locations should be utilized to test these genotypes in field trials for CPB defoliation and results correlate with OD readings. In Chapter 3 of (Thesis: Rodriguez-Garcia, 2015) we found out the top five yielding genotypes to be ND071289CAB-3, ND060857CAB-3, Ebt 6-21-3, Ebt 5-31-3 and Ebt 6-21-4. The Ebt 6-21-3 looks like a promising genotype for CPB resistance and also combines good yield potential. It has a high OD, which helps in the CPB resistance, and is a high yielder under the circumstances of an insect attack.

Table 2.9. Genotype, cluster number, and optical density mean for the 24 potato genotypes analyzed using optical densities obtained from the Modified Enzymic Browning Assay assessment tool for leaflet samples obtained from field trial at Grand Forks, ND, in 2012.

Genotype	Cluster number	OD Mean	Range†
Ebt 6-21-3	5	0.7626	VH
ND060898AB-1	3	0.7065	H
Ebt 6-5-2	3	0.6732	H
Ebt 6-21-12	3	0.6413	H
J101-K6-A22	3	0.6308	H
King Harry	2	0.5858	H
Ebt 5-31-5	2	0.5687	H
Ebt 6-21-2	2	0.5554	H
Russet Burbank	2	0.5415	H
Ebt 5-31-2	2	0.5519	M
Red Norland	2	0.5229	M
Ebt 5-31-4	2	0.5104	M
J138-A12	2	0.5088	M
J101-K6	1	0.4674	M
Ebt 6-5-5	1	0.4508	M
ND071289CAB-3	1	0.4473	M
Ebt 5-31-3	1	0.4466	M
ND7443Ab-72Russ	1	0.4428	M
P2-4	1	0.4379	M
ND060836ABc-15	1	0.4199	M
J103K-7	4	0.3851	M
Ebt 6-21-5	4	0.3733	M
Ebt 6-21-4	4	0.3479	M
ND2858-1	4	0.3313	L

† Range of OD for glandular trichome quantity: L= low, M= medium, H= high, VH= Very high.

The Scanning Electron Micrographs from Chapter 1 Assessment of potato germplasm for the presence of glandular trichomes using the Scanning Electron Microscope (Thesis: Rodríguez-García, 2015) reconfirm the results from the MEBA. Russet Burbank (Figure 1.2) had an OD of 0.5415 (Table 2.9) classifying it as having High Type A glandular trichome quantity. King Harry (Figure 1.1) had an OD of 0.5858 (Table 2.9), also classifying it as High Type A glandular trichome quantity. ND2858-1 (Figure 1.3) had an OD of 0.3313 (Table 2.9) classifying it as having Low Type A glandular trichome quantity. ND2858-1 has *S. chacoense* as a parent; its

insect resistance relies on glycoalkaloids, rather than glandular trichomes (Lorenzen and Balbyshev, 1997).

The MEBA is a screening tool, and the goal is to acquire genotypes with high OD's that translate to high densities of Type A glandular trichomes (Avé et al., 1986). High densities and volumes of Type A glandular trichomes are needed for CPB resistance (Tingey and Laubengayer, 1981; Neal et al., 1989). Avé et al. (1986) determined that MEBA, combined with trichome density counts, provide a more precise assessment of enzymic browning by potato glandular trichomes than EBA alone. In this research, trichome counts were not made and correlated with the MEBA. For future research and validation of the MEBA results, trichome counts should be made, in combination with the MEBA. A weakness of the MEBA is the sampling time and the ice protocol. For the sampling time, leaves need to be fully matured and expanded (Tingey and Laugenbayer, 1981). For the ice protocol, if not handled correctly, high OD will occur due to the p-phenylenediamine reacting with the air.

The NDSU germplasm has the potential to exploit the Type A glandular trichome trait. More research needs to be done with the NDSU lines to confirm the CPB resistance due to glandular trichomes. If utilizing the MEBA, sampling time and preparation will be key for the best results. More locations and years are needed to determine the inheritance of the glandular trichome trait in NDSU lines.

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CHAPTER 3. FIELD EVALUATION OF POTATO GENOTYPES TO DETERMINE DEFOLIATION AND OVIPOSITION BY COLORADO POTATO BEETLE

Abstract

Colorado potato beetle (*Leptinotarsa decemlineata* Say.) (CPB) is a destructive insect pest affecting potato (*Solanum tuberosum* L.) in North America. Glycoalkaloids and glandular trichomes are being studied to produce insect resistant potato plants. The research objective was to discover potato genotypes that experienced little to no defoliation by CPB, due to the presence of glandular trichomes. A two-year field trial in Grand Forks, ND, was conducted, with weekly evaluations including defoliation ratings, number of larvae and adult beetles, number of egg masses, and number of eggs in two egg masses. The top four CPB resistant clones based on defoliation ratings were Ebt 6-21-2, Ebt 6-21-3, J101-K6 and P2-5. The top four yielding clones were ND071289CAB-3, ND060857CAB-3, Ebt 6-21-3, and Ebt 5-31-3. The Ebt 6-21-3 is a promising genotype for parental use in cultivar development due to the presence of Type A glandular trichomes and high yield potential.

Introduction

Worldwide, potato ranks fifth among crops for total production, after sugar cane (*Saccharum officinarum*), corn (*Zea mays L.*), rice (*Orzya sativa*), and wheat (*Triticum aestivum*) (FAO, 2012). Potato production was valued at more than three billion dollars in the US (2009-2013), while the value of North Dakota's production, was more than $192 million (USDA-NASS, 2013).

The Colorado potato beetle (Order: Coleoptera and Family: Chrysomelidae) is a foliage consumer (Abdelhaq, 2006). Cultivated and wild plants of the *Solanaceae* family are susceptible

to CPB feeding (Ragsdale et al., 2007; Kuhar et al., 2009). Larvae and adults can eat entire leaves without discerning leaf tissues, consuming around 40 and 10 cm^2/day of foliage, respectively (Alyokhin et al., 2008; Alyokhin, 2009). Potato plants in the early tuber-bulking phase can only tolerate about 10% defoliation (Ragsdale et al., 2007; Alyokhin, 2009). It was thought that defoliation by herbivores decreases plant productivity based on the hypothesis that less photosynthetic area would reduce plant growth (Hare, 1990). However, the relationship between defoliation and plant growth is not lineal and simple, as it might seem (Hare, 1990). Annual crops must exceed a threshold between 5 and 30% of defoliation before primary productivity is reduced (Mattson and Addy, 1975). Plant species sensitivity depends on plant development (Hare, 1990). The economic threshold for adult beetles is an average of 25 adults per 50 plants surveyed, for small larvae 200 per 50 plants, and for large larvae, around 75 per 50 plants (Cooperative Extension: Potatoes, 2014).

Sanitation, cultural practices, physical, biological, microbial, and chemical controls are a few of the control methods used to manage CPB (Ragsdale et al., 2007). Nonetheless, CPB has developed resistance to more than 52 different compounds belonging to all major insecticide classes, since 1950 (Alyokhin et al., 2008)

The CPB life cycle begins in early spring or early summer, depending on temperature and the physiological state of the beetle, with the emergence of overwintering adult beetles from the ground (Anonymous, 1981). After emergence, beetles walk or do short flights to the closest potato field for feeding (Anonymous, 1981). Mating starts after feeding, between 10°C (no feeding) to a maximum at 25°C (Anonymous, 1981). A day or two after mating, females oviposit 10 to 30 eggs at a time (15 to 30°C) on the abaxial (lower) side of the leaves in orderly rows (Anonymous, 1981). Females can oviposit up to 2000 eggs until midsummer (Anonymous,

1981). Eggs will hatch 4 to 12 days after being laid if temperatures are above 12°C; larvae begin to feed immediately after emerging (Anonymous, 1981). Larvae shed their skins four times during the course of a two to three week period, which are the only times they will stop feeding (Anonymous, 1981). Larvae hatched from the same egg batch stay together through their first molt on the abaxial leaf surface; then they will move to the terminal buds (Anonymous, 1981). Depending on soil conditions, matured larvae fall to the ground and burrow in to the soil at different depths (Anonymous, 1981). The CPB cycle could start again if the weather conditions are warm enough for beetles to reemerge; if not, hibernation will occur until the following spring (Anonymous, 1981).

Several wild potato species are referred as resistant to CPB, including *S. chacoense* Bitter, *S. berthaultii* Hawkes, *S. tarijense* Hawkes, *S. demisssum*, *S. vernei* Bitter et Wittm., *S. acaule* Bitter, *S. hjertingi* Hawkes, *S. jamesii* Torr., *S. polyadenium* Greenm, *S. demissum*, and *S. neocardenasii* Hawkes and Hjert (Gibson, 1976; Sanford et al., 1984; Dimock et al. 1986; Sinden et al., 1986; Dimock and Tingey, 1988). Insect resistance relies on glandular trichomes (*S. berthaultii*, *S. tarijense*, *S. polyadenium*) and glycoalkaloids (*S. chacoense*, and *S. neocardenasi*) (Gibson, 1976; Dimock et al., 1986; Sinden et al., 1986; Dimock and Tingey, 1988).

Glycoalkaloids fluctuate quantitatively and qualitatively, depending on photoperiod, intensity of light, soil moisture, stage of plant growth, tuber storage conditions, and tuber tissue wounding (Tingey, 1984). All potato cultivars contain solanine and chaconine, and are considered unsafe when total tuber glycoalkaloid levels exceed 20 mg/100 g fresh weight (Sinden and Sanford, 1981). Human poisoning due to potato glycoalkaloids can cause mild gastrointestinal effects that can begin within 8 to 12 hours of ingestion and are resolved after a day or two (Lawley, 2013). Other reported symptoms include stomach cramps and headaches

(Lawley, 2013). Symptoms of more serious cases have included neurological problems such as hallucinations, paralysis, and death (Lawley, 2013).

Another approach breeders are investigating for CPB control is the use of glandular trichomes for insect resistance. Trichomes are uni- or multicellular appendages originating from epidermal cells (Werker, 2000). They can grow outward on the surface of various plant organs (Werker, 2000). There are two main types of trichomes: glandular and non-glandular trichomes (Vallejo et al., 1994). Glandular trichomes come in a variety of shapes and structures, having in common, metabolically active cells, and the ability to store or secrete specialized metabolites (Tissier, 2012). These insecticidal mixtures of chemicals are often used in the pesticide industry (Peter and Shanower, 1998). Potato glandular trichomes are divided into two types: Type A and Type B (Vallejo et al., 1994). Type A glandular trichomes are short trichomes (around 120 to 210 μm long), and their apex contains a tetralobulate membrane-bound gland (50 to 70 μm in diameter) (Vallejo et al., 1994). Type B glandular trichomes are long trichomes around 600 to 950 μm, and their tips have an ovoid gland that constantly releases a clear, sticky, viscous exudate (Vallejo et al., 1994). An advantage of the presence of glandular trichomes over glycoalkaloids is that often genotypes with high foliar glycoalkaloid levels also have unacceptable levels in tubers.

A target of the North Dakota State University (NDSU) Potato Breeding Program is to identify, and introgress genetic insect resistance into adapted potato germplasm for adoption by Northern Plains potato producers. The goal of this research was to investigate CPB feeding, and oviposition habits, on potato genotypes (selections and cultivars), and the effect on tuber yield in treated (CPB insecticide in-furrow) versus untreated production scenarios.

Materials and Methods

Twenty-four potato genotypes were used for this study at the Northern Plains Potato Growers Association (NPPGA) Research Farm at Grand Forks, ND, in 2012 (Table 3.1) and 2013 (Table 3.2). Potato seed tubers were acquired from the North Dakota State University (NDSU) Potato Breeding Program. In 2013, there was a lack of seed for four genotypes (Ebt 6-21-2, Ebt 6-5-2, J101-K6-A22, and ND060898AB-1); they were replaced by (ND2861-1, ND2861-2, ND060857CAB-3, and P2-5). The field trial objective was to evaluate defoliation, beetle/larval population in plots, and oviposition.

Seed was prepared for planting on 23 May 2012, and 14 June 2013, and the planting dates were 23 May 2012, and 14 June 2013, respectively. Seed was treated with Maxim MZ after cutting. The within-row spacing was 30.48cm and the row spacing was 91.44cm. The field design was a randomized complete block design (RCBD), with a split-block arrangement, with two replicates. Factor A was genotypes (24) and factor B was treatments (2).

Table 3.1. Genotypes and their parentage for the CPB defoliation field trial at Grand Forks, ND, in 2012.

Entry number	Genotype	Female parent	Male parent	Reference
1	Ebt 5-31-2	P2-3	Katahdin	Gillen and Novy, 2007
2	Ebt 5-31-3	P2-3	Katahdin	Gillen and Novy, 2007
3	Ebt 5-31-4	P2-3	Katahdin	Gillen and Novy, 2007
4	Ebt 5-31-5	P2-3	Katahdin	Gillen and Novy, 2007
5	Ebt 6-5-2	P2-3	Katahdin	Gillen and Novy, 2007
6	Ebt 6-5-5	P2-3	Katahdin	Gillen and Novy, 2007
7	Ebt 6-21-2	P2-3	Katahdin	Gillen and Novy, 2007
8	Ebt 6-21-3	P2-3	Katahdin	Gillen and Novy, 2007
9	Ebt 6-21-4	P2-3	Katahdin	Gillen and Novy, 2007
10	Ebt 6-21-5	P2-3	Katahdin	Gillen and Novy, 2007
11	ND071289CAB-3	ND039104CAB-3	ND028799C-2	Thompson, personal communication
12	Ebt 6-21-12	P2-3	Katahdin	Gillen and Novy, 2007
13	J101-K6	*S. bulbocastanum*	*S. tuberosum* (J101)	James et al., 1997
14	J103K-7	*S. bulbocastanum*	*S. tuberosum* (J103)	James et al., 1997
15	J101-K6-A22	*S. bulbocastanum*	*S. tuberosum* (J101)	James et al., 1997
16	J138-A12	*S. bulbocastanum*	*S. tuberosum* (J138)	James et al., 1997
17	ND060836ABc-15	ND028799C-3	Ebt 6-5-5	Thompson, personal communication
18	P2-4	2-7-4D	Katahdin	Gillen and Novy, 2007
19	ND060898AB-1	ND039986AB-3	EB8109-1	Thompson, personal communication
20	ND7443Ab-72Russ	S440	Ebt 5-31-5	Thompson, personal communication
21	ND2858-1	*S. chacoense*	ND1215-1	Lorenzen and Balbyshev, 1997
22	King Harry	N142-72	Pike	De Jong et al., 2011
23	Red Norland	Sport of Norland	-	Thompson, personal communication
24	Russet Burbank	Sport of Burbank	-	Davis, 1992

Table 3.2. Genotypes and their parentage for the CPB defoliation field trial at Grand Forks, ND, in 2013.

Entry number	Genotype	Female parent	Male parent	Reference
1	Ebt 5-31-2	P2-3	Katahdin	Gillen and Novy, 2007
2	Ebt 5-31-3	P2-3	Katahdin	Gillen and Novy, 2007
3	Ebt 5-31-4	P2-3	Katahdin	Gillen and Novy, 2007
4	Ebt 5-31-5	P2-3	Katahdin	Gillen and Novy, 2007
5	Ebt 6-5-5	P2-3	Katahdin	Gillen and Novy, 2007
6	Ebt 6-21-3	P2-3	Katahdin	Gillen and Novy, 2007
7	Ebt 6-21-4	P2-3	Katahdin	Gillen and Novy, 2007
8	Ebt 6-21-5	P2-3	Katahdin	Gillen and Novy, 2007
9	Ebt 6-21-12	P2-3	Katahdin	Gillen and Novy, 2007
10	J101-K6	*S. bulbocastanum*	*S. tuberosum* (J101)	James et al., 1997
11	J103-K7	*S. bulbocastanum*	*S. tuberosum* (J103)	James et al., 1997
12	J138-A12	*S. bulbocastanum*	*S. tuberosum* (J138)	James et al., 1997
13	ND2858-1	*S. chacoense*	ND1215-2	Lorenzen and Balbyshev, 1997
14	ND2861-1	*S.polytrichom*	ND1215-1	Thompson, personal communication
15	ND2861-2	*S.polytrichom*	ND1215-1	Thompson, personal communication
16	ND7443Ab-72Russ	S440	Ebt 5-31-5	Thompson, personal communication
17	ND060836ABc-15	ND028799C-3	Ebt 6-5-5	Thompson, personal communication
18	ND060857CAB-3	ND028804CAB-4	N4131	Thompson, personal communication
19	ND071289CAB-3	ND039104CAB-3	ND028799C-2	Thompson, personal communication
20	P2-4	S440	Ebt 5-31-5	Gillen and Novy, 2007
21	P2-5	S440	Ebt 5-31-5	Gillen and Novy, 2007
22	King Harry	N142-72	Pike	De Jong et al., 2011
23	Red Norland	Sport of Norland	-	Thompson, personal communication
24	Russet Burbank	Sport of Burbank	-	Davis, 1992

Treatment one had insecticide applied in-furrow (Imidacloprid 91.79 g/ha) at planting, and treatment two had no insecticide (control) applied. Each plot consisted of five hills (plants), with a guard plant (All Blue) at the beginning and end.

This germplasm was chosen for its variety in host-plant resistance towards CPB, such as glandular trichomes, glycoalkaloids, or both. Ebt lines were selected for their glandular trichome trait and resistance to several other pest and diseases (Gillen and Novy, 2007). Ebt lines were created through somatic hybridization between *S. tuberosum* and *S. etuberosum*, introgressing the glandular trichome trait (Gillen and Novy, 2007). ND2858-1 is a good source of host-plant resistance to CPB due to glycoalkaloids (Lorenzen and Balbyshev, 1997). King Harry was chosen for the glandular trichomes, and its known resistance towards aphids (De Jong et al., 2011). The NDSU lines were nominated because the genotypes had a combination of: a.) glandular trichomes, b.) glycoalkaloids, and c.) both (glandular trichome and glycoalkaloids) (Thompson, personal communication). Red Norland and Russet Burbank are two commercial cultivars used as controls (Davis, 1992).

The data was collected twice a week, beginning approximately a month after planting, through until harvest. In 2012, the data was collected on the following dates: 14 July, 17 July, 20 July, 24 July, 27 July, 31 July, 3 August, 7 August, 10 August, and 14 August, for a total of 10 data collection days. In 2013, data was collected: 31 July, 5 August, 9 August, 12 August, 16 August, 19 August, 23 August, 26 August, 30 August, 3 September, and 5 September, for a total of 11 data collection days. Defoliation was rated on a 0 to 5 scale (0: no defoliation and 5: 80-100% defoliation) (Table 3.3). The number of egg masses per plot and the number of eggs in two random egg masses per plot were counted.

Table 3.3. Defoliation rating used to assess damage caused by larvae and adult beetles of *Leptinotarsa decemlineata* Say. on the potato field trials at Grand Forks, ND, in 2012 and 2013.

Defoliation rating	Description
0	No visible damage
1	20% damage or less on the plant's foliage
2	From 20 to 40% damage on the plant's foliage
3	From 40 to 60% damage on the plant's foliage
4	From 60 to 80% damage on the plant's foliage
5	From 80 to 100% damage on the plant's foliage

Analysis of variance (p= 0.001) was determined for stand, number of egg masses, number of eggs in two egg masses, number of adults, number of larvae and yield for 2012 and 2013 evaluation dates (10 and 11, respectively) utilizing the GLM procedure (SAS Institute, 2012). When the F-test was significant, a mean separation test was performed using the LSD (p=0.05) (SAS Institute, 2012). Defoliation rating was analyzed using the MIXED procedure with least significant (LS) means (SAS Institute, 2012).

Results and Discussion

The analysis of variance for stand, number of egg masses, number of eggs in two egg masses, number of adults, number of larvae and yield, from Grand Forks, ND, in 2012, detected significance for a main effect (genotype) for stand, for beetle adults (17 July), and larvae count (17 July) (Table 3.4 to Table 3.6). There was also a significant difference for the main effect

(insecticide in-furrow treatment) for egg count (27 July), and larvae count (10 August) (Table 3.4 to Table 3.6). Stand count was significantly different for the genotypes; therefore, each genotype emerged differently. The majority of the genotypes had nearly 100% emergence. Ebt 5-31-3, Ebt 5-31-4 and ND2858-1 were the only three genotypes with poor emergence (Table 3.7). This could be due to strong dormancy, poor seed quality, or environmental conditions (Dwelle, 2003).

The means, and Least Significance Difference (LSD) of the genotypes for stand, number of adults, and number of larvae from the Grand Forks, ND, in 17 July 2012, trials can be found in Table 3.7. The number of adult beetles on 17 July was significantly different for genotypes (Table 3.7). Ebt 6-5-5 and King Harry were the only two genotypes with a higher number of adult CPB on 17 July, in comparison to the other genotypes (Table 3.7). This could be due to the fact that the CPB life cycle is greatly influenced by temperature and physiological state of the adult beetles (Anonymous, 1981). Emergence of overwintering adult beetles depends greatly on temperature at this time of the year (17 July), therefore there were still adult beetles emerging. The number of larvae on 17 July was significantly different for genotypes. Most of the genotypes had larvae infestation that day (Table 3.7). Based on the CPB life cycle, date, and stage of crop growth, most of the larvae have or should have hatched and start their feeding (Anonymous, 1981).

Table 3.4. Mean squares for yield, stand, egg masses, eggs, adults and larvae for biweekly evaluations from 14 July to 20 July 2012, at Grand Forks, ND.

			-------14 July---		----------17 July----------				-----20 July-----		
Sources of Variations	df	Stand	Egg masses†	Eggs‡	Egg masses	Eggs	Adults	Larvae	Adults	Larvae	Yield
		%	----------no.----------								
Rep §	1	41	0.01	7.7	0.08	40.2	0.73	0.00	0.08	0.73	37128
Genotype	23	100***	0.03	21.5	0.05	25.2	0.11*	0.19**	0.14	0.10	8566
Error (a)	23	81	0.03	24.3	0.05	25.2	0.05	0.07	0.18	0.07	6083
Treatment	1	28	0.01	12.1	0.01	6.0	1.68	11.28	7.00	2.07	29151
Error (b)	1	40	0.01	3.7	0.01	6.0	0.73	0.18	0.01	0.73	5654
Trt ¶ x Genotype	23	37	0.03	24.2	0.05	27.0	0.11	0.15	0.10	0.10	4298
Error	21	108	0.04	26.9	0.06	29.6	0.06	0.06	0.15	0.08	4343

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

† Egg masses=Number of egg masses on five potato plants.

‡ Eggs found in two random egg masses per plot of five potato plants.

§ Rep=Replicate

¶ Trt=Treatment

Table 3.5. Mean squares for egg masses, eggs, adults and larvae for biweekly evaluations from 24 July to 31 July 2012, at Grand Forks, ND Colorado potato beetle defoliation study.

		24 July				27 July				31 July			
Sources of Variation	df	Egg masses †	Eggs ‡	Adults	Larvae	Egg masses	Eggs	Adults	Larvae	Egg masses	Eggs	Adults	Larvae
		no.											
Rep §	1	0.27	138	0.17	0.01	0.32	123	0.41	0.00	0.99	336	0.17	0.00
Genotype	23	0.18	101	0.29	0.13	0.27	202	0.12	0.04	1.26	507	0.20	0.02
Error (a)	23	0.22	137	0.29	0.05	0.40	230	0.13	0.04	1.11	277	0.18	0.03
Treatment	1	0.10	188	0.36	0.48	7.39	4537*	1.25	0.05	52.03	20197	3.03	0.00
Error (b)	1	0.10	24	0.05	0.01	0.05	4	0.41	0.05	0.92	284	0.05	0.01
Trt ¶ x Genotype	23	0.10	47	0.18	0.12	0.27	202	0.09	0.04	1.30	469	0.10	0.03
Error	21	0.08	25	0.16	0.11	0.40	225	0.15	0.05	1.25	344	0.19	0.04

* Significant at 0.05 probability level.

† Egg masses=Number of egg masses on five potato plants.

‡ Eggs found in two random egg masses per plot of five potato plants.

§ Rep=Replicate

¶ Trt=Treatment

Table 3.6. Mean squares for egg masses, eggs, adults and larvae for biweekly evaluations from 3 August to 10 August 2012, at Grand Forks, ND Colorado potato beetle defoliation study.

		3 August				7 August				10 August			
Sources of Variation	df	Egg masses †	Eggs ‡	Adults	Larvae	Egg masses	Eggs	Adults	Larvae	Egg masses	Eggs	Adults	Larvae
Rep §	1	0.53	499	0.09	0.01	1.49	768.18	0.23	0.05	0.5	365	0.17	0.00
Genotype	23	1.30	283	0.15	0.01	1.78	441.55	0.18	0.02	0.1	286	0.17	0.17
Error (a)	23	1.76	238	0.14	0.01	0.94	208.43	0.19	0.04	0.7	294	0.12	0.15
Treatment	1	66.50	17159	3.41	0.01	17.14	5874.56	2.79	0.05	35.6	15441	4.55	0.95***
Error (b)	1	0.28	16	0.01	0.01	0.56	29.56	1.14	0.05	1.1	284	0.05	0.00
Trt ¶ x Genotype	23	1.23	271	0.15	0.01	1.34	315.16	0.12	0.04	0.1	273	0.21	0.18
Error	21	1.36	181	0.10	0.01	1.49	493.44	0.16	0.02	0.8	329	0.19	0.12

*** Significant at 0.001 probability level.

† Egg masses=Number of egg masses on five potato plants.

‡ Eggs found in two random egg masses per plot of five potato plants.

§ Rep=Replicate

¶ Trt=Treatment

Table 3.7. Mean and least significant difference (LSD) across genotype for stand, number of adults, and larvae for the Colorado potato beetle defoliation study at Grand Forks, ND, on 17 July 2012.

Genotype	Stand†	Adults‡	Larvae §
Ebt 5-31-2	95.0¶	0.0	0.0
Ebt 5-31-3	20.0	0.0	0.5
Ebt 5-31-4	66.7	0.3	0.3
Ebt 5-31-5	100.0	0.0	0.5
Ebt 6-5-2	100.0	0.0	0.5
Ebt 6-5-5	100.0	0.5	0.0
Ebt 6-21-2	100.0	0.0	0.3
Ebt 6-21-3	100.0	0.3	0.5
Ebt 6-21-4	100.0	0.0	0.5
Ebt 6-21-5	100.0	0.0	0.5
ND071289CAB-3	100.0	0.3	0.5
Ebt 6-21-12	100.0	0.0	0.0
J101-K6	95.0	0.0	0.5
J103-K7	95.0	0.0	0.3
J101-K6-A22	100.0	0.3	0.8
J138-A12	100.0	0.0	0.8
ND060836ABc-15	100.0	0.0	0.3
P2-4	100.0	0.3	0.3
ND060898AB-1	90.0	0.0	0.5
ND7443Ab-72Russ	100.0	0.3	0.5
ND2858-1	50.0	0.3	0.0
King Harry	100.0	0.5	0.5
Red Norland	100.0	0.3	0.3
Russet Burbank	100.0	0.3	0.5
LSD (0.05)	13.3	0.4	0.4

† % of plants emerged out of five potato plants.
‡ Number of adults (Colorado potato beetle) found in a five-plant plot.
§ Number of larvae (Colorado potato beetle) found in a five-plant plot.
Values are the mean.

Table 3.8. Mean number of eggs (27 July 2012) and larvae (10 August 2012) for treatments from Colorado potato beetle defoliation study at Grand Forks, ND.

Treatment	Eggs†	Larvae‡
Insecticide in-furrow§	13.40	0.29
No insecticide	0.00	0.09
LSD (0.05)	5.31	0.01

† Eggs found in two random egg masses per plot.
‡ Number of larvae found in a five-plant plot.
§ Imidacloprid at 91.79 g/ha

The insecticide in-furrow treatment had an effect on the number of eggs on 27 July and larvae on 10 Aug at Grand Forks, ND (Table 3.8). The insecticide in-furrow treatment was significantly different for number of eggs (27 July), compared to no insecticide treatment (control). Similarly, the insecticide in-furrow treatment was significantly different for larvae count (10 August), compared to the no insecticide treatment (control). Some potential reasons for this could be that the first recorded resistance of imidacloprid in the Midwestern United States was in 2004 (Szendrei et al., 2012). Imidacloprid was registered to manage CPB in potatoes in 1995 (Szendrei et al., 2012). The CPB resistance to imidacloprid, in combination with how beetles move within a field, maybe a cause for this difference in numbers of eggs and larvae in the treated versus untreated plots (Table 3.8). The CPB movement across the field may be a cause for this difference in significance. When overwintering beetles emerge in the spring, they scatter by walking and flying to their host (Hare, 1990). Potentially, these adult beetles may have emerged near a tree line, or an adjacent section of the field, and therefore, came in contact first with the in-furrow treated plots. The data collected for 14 August is not presented because there were no significant differences for any of the data points.

The defoliation rating for the damage caused by the larvae and adult beetles of CPB can be found on Table 3.3. The relative effect of defoliation for the treatments on the 24 genotypes were made for the 10 dates of the 2012 growing season at Grand Forks, ND. We will only present the first, and last data collection days (14 July 2012 and 14 August 2012), which can be found in Figures 3.1 and 3.2. Relative effect measurements are made to "express the outcome in one group relative to that in the other" (Higgins and Green, 2011). Relative effect is a non-parametric measurement determined via SAS, using the Kruskal Wallis Test (SAS Institute, 2012). According to McDonald (2014), the Kruskal-Wallis test is "a non-parametric test, which

means that it does not assume that the data come from a distribution that can be completely described by two parameters, mean and standard deviation (the way a normal distribution can)." In this study we wanted to compare treatment one (insecticide in-furrow) versus treatment two (no insecticide or the control).

The relative effect graphs present on the y-axis the entry numbers and on the x-axis the relative effect (RE). In Table 3.1, you can find the entry number with its corresponding genotype. The relative effect (RE) in this study is correlated to the defoliation rating. The lower the RE is, the lower the defoliation rating. The insecticide in-furrow treatment stayed consistent overall. Therefore, any genotype with no insecticide that appears below the insecticide in-furrow treatments are our least defoliated genotypes, and therefore presumably our most resistant genotypes.

The following genotypes were rated as resistant to CPB defoliation on 14 July 2012 (Figure 3.1): Ebt 5-31-3 (Entry # 2), Ebt 5-31-4 (Entry # 3), Ebt 6-21-2 (Entry # 7), Ebt 6-21-3 (Entry # 8), Ebt 6-21-12 (Entry # 12), J101-K6 (Entry # 13), ND060898AB-1 (Entry #19), and ND7443Ab-72 Russ (Entry #20). The Ebt lines have the glandular trichome trait introgressed thru somatic hybridization of *S. tuberosum* and *S. etuberosum* (Gillen and Novy, 2007). ND7443Ab-72 Russ parents are S440 and Ebt 5-31-5 from which it may have inherited the glandular trichome trait (Thompson, personal communication).

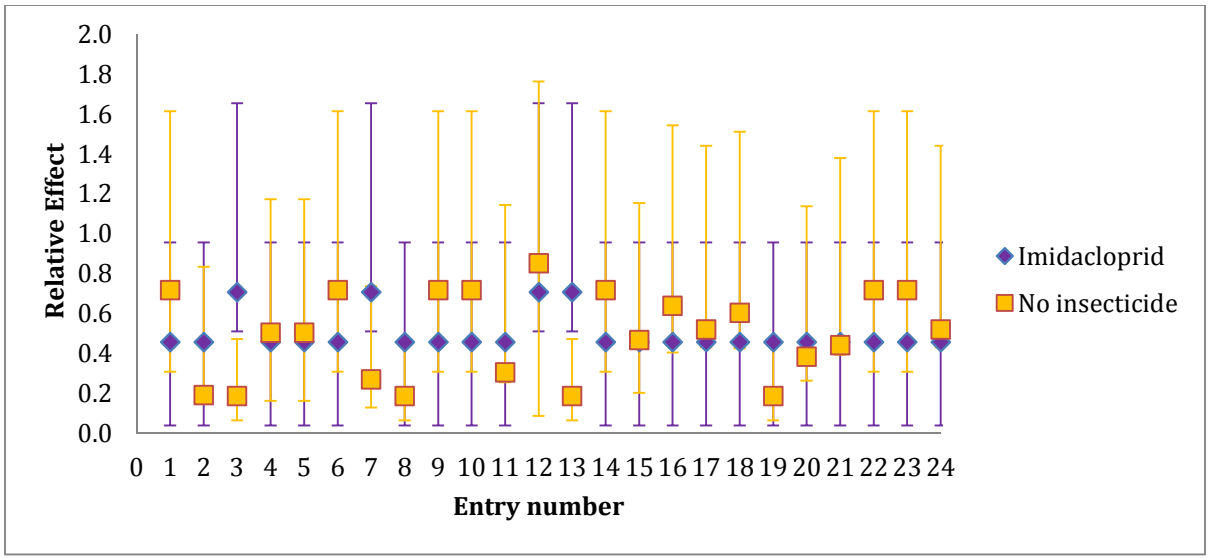

Figure 3.1. Relative effect of defoliation for treatments with and without insecticide (Imidacloprid 91.79 g/ha) for each of 24 genotypes on 14 July 2012, at Grand Forks, ND. Bars indicate the upper and lower confidence limit at 95%.

On 14 August 2012 the genotypes related as resistant to CPB defoliation (Figure 3.2) were: Ebt 5-31-4 (Entry #3), Ebt 6-5-2 (Entry #5), Ebt 6-21-2 (Entry #7), Ebt 6-21-3 (Entry #8), Ebt 6-21-4 (Entry #9), ND060836ABc-15 (Entry #17), P2-4 (Entry #18), and King Harry (Entry #22). The Ebt lines contain the glandular trichome trait thanks to somatic hybridization between *S. tuberosum* and *S. etuberosum* (Gillen and Novy, 2007). King Harry is known to be resistant to several insect pests and for having Type A glandular trichomes (De Jong et al., 2011).

There was a trend where CPB defoliation increased during the middle of the growing season and later on diminished. This trend agrees with the CPB annual life cycle (Anonymous, 1981). At the beginning of the growing season, defoliation occurs by the overwintering beetles that emerge and are reproducing (Anonymous, 1981). At the peak of the growing season, newly emerged larvae will take over the defoliation (Anonymous, 1981). At the end of the season adult beetles begin to burrow into the soil, therefore, defoliation declined as it comes to a stop (Anonymous, 1981). Potato tuber production is not affected by single defoliation attacks at the beginning or ending of the growing season (Hare, 1990). Tubers are near full size at the end of

the season, thus defoliation has little or no effect on them (Sparks et al., 1959). Most of the damage to yield is caused near the middle of the growing season during rapid tuber bulking, when tuber production can be reduced from 13% to 100%, depending upon level of defoliation (Hare, 1990).

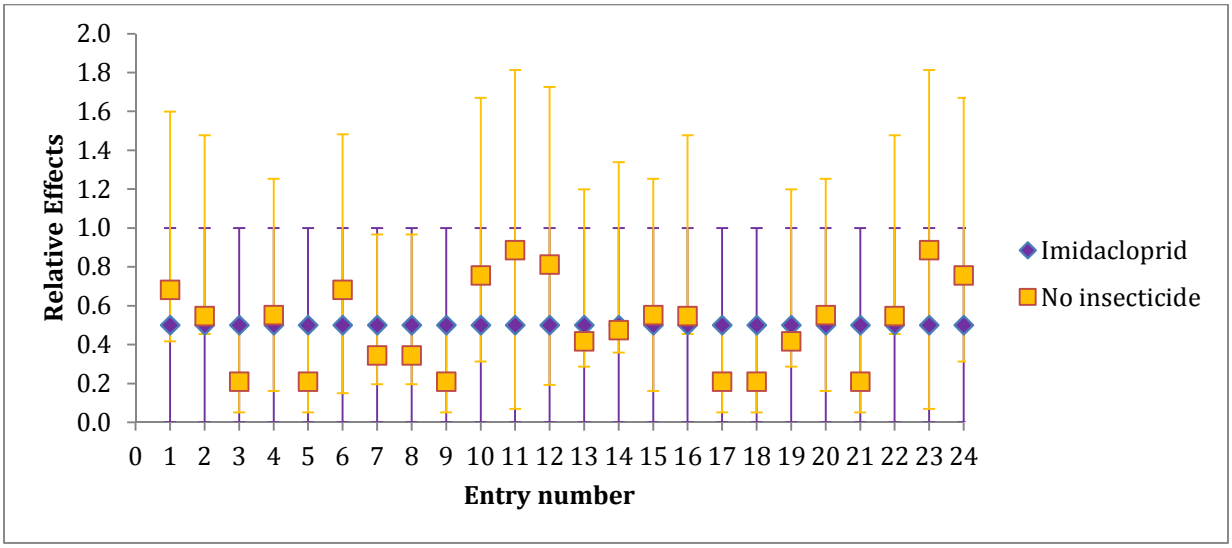

Figure 3.2. Relative effect of defoliation for treatments with and without insecticide (Imidacloprid 91.79 g/ha) for each of 24 genotypes on 14 August 2012, at Grand Forks, ND. Bars indicate the upper and lower confidence limit at 95%.

From the 10 data collection dates in 2012, a total of 18 genotypes demonstrated resistance, but only four were consistent: Ebt 6-21-2 (Entry # 7), Ebt 6-21-3 (Entry # 8), J101-K6 (Entry # 13), and ND060898AB-1 (Entry # 19). Ebt lines have the glandular trichome trait thanks to the wild species used in the somatic hybridization (Gillen and Novy, 2007). ND060898AB-1 parentage is *S. bulbocastanum* and *S.etuberosum* (Thompson, personal communication). *S. bulbocastanum* is known for its late blight resistance genes and not for the glandular trichome trait (Lokossou et al., 2010).

The analysis of variance for stand, number of egg masses, number of eggs in two egg masses, number of adults, number of larvae, and yield at Grand Forks, ND, in 2013, detected significances for the main effect (genotypes) for yield (Table 3.9) and number of adults beetles

(16 August 2013), and the main effect (treatment) for the number of egg masses (16 August 2013) (Table 3.10). Yield was significantly different for genotype, therefore each genotype was affected differently, which was expected (Table 3.9). The top five yielding genotypes were ND071289CAB-3, ND060857CAB-3, Ebt 6-21-3, Ebt 5-31-3, and Ebt 6-21-4. Ebt lines have the glandular trichome trait thru the somatic hybridization of *S. tuberosum* and *S. etuberosum* (Gillen and Novy, 2007). The NDSU lines parentage are found in Table 3.1 and Table 3.2. In Chapter 2: Screening potato germplasm for the presence of glandular trichome (Rodríguez-García, 2015) we utilized the Modified Enzymic Browning Assy (MEBA) to determine an estimate of the presence of Type A glandular trichomes the genotypes had. The results were categorized as Very Low, Low, Medium, High and Very High for the quantity of Type A glandular trichomes (Table 2.6). ND071289CAB-3 was categorized as a Medium, Ebt 6-21-3 as Very high, Ebt 5-31-3 as Medium, and Ebt 6-21-4 Medium for their quantity of Type A glandular trichomes.

Table 3.9. Mean squares for yield, stand, egg masses, number of eggs, adults and larvae for biweekly evaluations from 9 and 12 August 2013, at Grand Forks, ND.

				----------9 August----------				------12 August-----		
Sources of Variation	df	Stand	Egg masses†	Eggs‡	Adults	Larvae	Egg masses	Eggs	Larvae	Yield
		%		----------no.----------						g
Rep §	1	41	0.04	63	0.01	0.04	0.26	257.0	0.01	4061
Genotypes	23	21	0.04	63	0.01	0.02	0.09	94.0	0.03	17915***
Error (a)	23	42	0.04	63	0.01	0.02	0.09	94.0	0.03	1220
Treatment	1	60	0.04	63	0.01	0.04	0.01	0.84	0.01	34756
Error (b)	1	70	0.04	63	0.01	0.04	0.01	0.84	0.09	148
Trt ¶ x Genotypes	23	20	0.04	63	0.01	0.02	0.10	105.0	0.03	1268
Error	23	109	0.04	63	0.01	0.02	0.10	105.0	0.29	2012

*** Significant at 0.001 probability levels.

† Egg masses=Number of egg masses on five potato plants.

‡ Eggs found in two random egg masses per plot in five potato plants.

§ Rep=Replicate

¶ Trt=Treatment

Table 3.10. Mean squares for egg masses, eggs, adults and larvae for biweekly evaluations conducted 16 Aug to 5 September 2013, at Grand Forks, ND.

		----------16 Aug----------				----------19 Aug----------				23 Aug	26 Aug	30 Aug	5 Sept
Sources of Variation	df	Egg masses †	Eggs ‡	Adults	Larvae	Egg masses	Eggs	Adults	Larvae	Adults	Larvae	Larvae	Adults
		----------no----------											
Rep §	1	0.38	106	0.09**	0.00	0.04	18.0	0.01	0.04	0.04	0.04	0.01	0.01
Genotypes	23	0.14	45	0.11	0.04	0.04	18.0	0.01	0.02	1.0	0.02	0.01	0.01
Error (a)	23	0.16	50	0.03	0.04	0.04	20.0	0.01	0.02	0.02	0.02	0.01	0.01
Treatment	1	0.04***	41	0.01	0.04	2.70	0.10	0.01	0.0	1.0	0.04	0.01	0.01
Error (b)	1	0.00	2	0.09	0.04	0.04	16.0	0.01	0.0	0.04	0.04	0.01	0.01
Trt ¶ x Genotypes	23	0.17	53	0.03	0.04	0.04	21.0	0.01	0.02	0.025	0.46	0.01	0.01
Error	21	0.17	55	0.03	0.04	0.04	20.0	0.01	0.02	0.20	0.02	0.01	0.01

, * Significant at 0.01, and 0.001 probability levels, respectively.

† Egg masses=Number of egg masses on five potato plants.

‡ Eggs found in two random egg masses per plot in five potato plants.

§ Rep=Replicate

¶ Trt=Treatment

ND060857CAB-3 was not an entry in the trials evaluated with the MEBA. Therefore, we do not know a few of the genotypes evaluated with both the MEBA and CPB defoliation field trials are showing some additional promising qualities, may or may not be due to the presence of glandular trichomes or not including high yield and CPB resistance.

The 2013 defoliation rating data is not presented because there were no significant differences for genotypes, treatments, number of adult beetles, number of larvae, number of egg masses, number of eggs in two egg masses and yield. This was due to little defoliation because of a lack of CPB activity during the 2013 growing season. The only CPB activity recorded for the 2013 trial was the presence of adult beetles on Ebt 6-21-3, Ebt 6-21-12, J101-K6, ND2861-1 and P2-4 in the month of August (Rodríguez-García, 2015). Some potential reasons for a lack of the insect pest include that the trial was moved year to year to other areas of the farm due to the need for crop rotation and perhaps the field might have been less infected with CPB due to lack of winter survival, fewer acres of potatoes in the immediate area the previous year, or other. Overwintering beetles emerge and feed on the first potato plants they encounter (Anonymous, 1981). They may feed and become ill or die, or maybe unable to lay eggs which hatch into surviving larvae. Initial feeding is often a chance or opportunity for the beetles, which could may mean that they could have died from glycoalkaloids toxicity (Hare, 1990).

It was very important that the same researcher did the defoliation rating and the egg mass, and egg number counts in the field, in order to minimize the error due to researcher rating. Different researchers might equate to variable results, and the data would not be uniform for statistical analyses.

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SUMMARY

The objective of this thesis was to study and analyze the presence of glandular trichomes on potato genotypes, and their impact on defoliation by the Colorado potato beetle potato. The experimental approach consisted of using the Scanning Electron Microscope (SEM) to assess the presence of glandular trichomes (Chapter 1), the use of the Modified Enzymic Browning Assay (MEBA) to screen leaves for the quantity of glandular trichomes (Chapter 2), and a two-year field trial to evaluate CPB defoliation and oviposition on potato genotypes (Chapter 3).

The SEM provided a closer look at the morphology and quantity of glandular trichomes on potato leaves. The SEM photographs provided a range of glandular trichome densities, on the six genotypes evaluated. Densities and ratio information will be valuable to the NDSU potato breeding program and others in developing breeding strategies employing multiple defense mechanisms such as the presence of glandular trichomes or foliar glycoalkaloids against insect pests, including the CPB. Identification of CPB resistance will require multiple years and locations to statistically confirm the effectiveness of the protocol.

To enhance and aid in the selection of CPB resistant cultivars, the MEBA was done to confirm its efficiency as a rapid screening and selection tool. An advantage of the assay is that it can be implemented as soon as the F_1 generation is available. Rapid identification at this stage would reduce field space and time because selection for specialized genotypes with appropriate density of Type A glandular trichomes can be conducted and the population narrowed before a selection reaches more advanced stages of cultivar development. The MEBA protocol may need further refinement prior to adoption by the NDSU potato breeding program. This information can assist breeders in making more informed decisions in utilizing an advanced selection or a

released cultivar for CPB resistance breeding, as well as, a quick identification of progeny genotypes with potential resistance to CPB.

The SEM and MEBA results corroborate those of the other. Visual assessment of Type A and Type B glandular trichomes using SEM revealed a range of densities (mean ratio of Type A: Type B). Russet Burbank (6:15) had an OD of 0.5415 classifying it as having High Type A glandular trichome quantity. King Harry (3:8) had an OD of 0.5858, also classifying it as High Type A glandular trichome quantity. ND2858-1 (1:3) had an OD of 0.3 classifying it as having Low Type A glandular trichome quantity.

More research needs to be done with the NDSU lines to confirm CPB resistance due to glandular trichomes. If utilizing the MEBA, sampling time and preparation will be key for the best results. More locations and years are needed to determine the inheritance of the glandular trichome trait.

The MEBA results substantiate the results from the field trials. In the field trials ND071289CAB-3, Ebt 6-21-3, Ebt 5-31-3, and Ebt 6-21-4 were CPB resistant to defoliation. The MEBA results were categorized as Very Low, Low, Medium, High and Very High for their quantity of Type A glandular trichomes. According to the MEBA, ND071289CAB-3 was categorized as a Medium, Ebt 6-21-3 as Very high, Ebt 5-31-3 as Medium, and Ebt 6-21-4 Medium for their quantity of Type A glandular trichomes. More CPB resistance research should be done with these four clones to verify the resistance. Additionally, they should be evaluate for foliar glycoalkaloid levels in order to verify the mechanism or combination of mechanisms providing resistance.

Finally, potato breeding programs should use a variety of methods to evaluate glandular trichome mediated resistance. Some methods like SEM might be a bit expensive but MEBA and

CPB field trials are achievable. The key component to evaluate true CPB resistance lies in having as many locations and years, as possible, with several methods to confirm the data.