Quantitative Trait Loci Analysis and Breeding for Resistance to Common Scab in Potato

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CHAPTER 1

LITERATURE REVIEW

INTRODUCTION TO COMMON SCAB

Potato common scab (CS) is caused by the saprophytic, filamentous, gram-positive, soilborne bacteria *Streptomyces scabies*, *S. acidiscabies*, *S. turgidiscabies*, and other pathogenic *Streptomyces* species (Loria 2001). Symptoms of this disease include scab-like surface, raised, and pitted lesions on the tuber (Loria et al. 1997), which can cause a substantial reduction in marketable yield for potato growers. For seed potato growers in Wisconsin, the seed is considered defective if there are one or more scab lesions (surface, raised, or pitted) that cover more than 5% of the potato tuber surface. The defect is considered serious if one or more scab lesions together cover more than 50% of the tuber surface (Wis. Admin. Code § Certified Seed Potatoes). For CS on potato, there are no above-ground diagnostic symptoms. *Streptomyces scabies* is the potato scab pathogen that was first characterized and is widespread globally, wherever potatoes are grown (Lambert and Loria 1989a).

Thaxter (1892) was the first to describe and isolate the causal agent of CS in North America. The species name was published in 1989 as *Streptomyces scabies* (Lambert and Loria, 1989a). Since then, *S. acidiscabies* (emerging from the United States) (Lambert and Loria 1989b), and *S. turgidiscabies* (emerging from Japan) (Miyajima et al. 1998) have been described. *S. acidiscabies* and *S. turgidiscabies* are thought to have emerged via a horizontal gene transfer event from *S. scabies* (Healy et al. 1999; Bukhalid et al. 1998). A conserved, moble pathogenicity island (325-660 kb) has been identified in *Streptomyces* species (Kers et al. 2005). A pathogenicity island carries virulence genes, is present in pathogenic strains, has different G+C content in comparison to DNA of host bacteria, occupies large chromosomal regions (< 30 kb), represents distinct genetic units, has an association with tRNA genes and/or insertion sequence elements, carries 'mobility' genes, and is unstable (Hacker et al., 1997). Current evidence suggests that *S. scabies* is a heterogeneous species comprised of differing genetic clusters (Goyer and Beaulieu 1997). There is also variation for pathogenicity within *S. scabies* (Goyer

and Beaulieu 1997). Other species of *Streptomyces* that cause CS have been identified (reviewed in Loria 2006).

DIFFERENCES AMONG S. SCABIES, S. ACIDISCABIES, and S. TURGIDISCABIES

Streptomyces scabies is the predominant species that causes CS of potato worldwide (Lambert and Loria 1989a; Lambert and Loria 1989b) and has global distribution (Lambert and Loria 1989a). This species forms grey, smooth spores (0.5 by 0.9 to 1.0 µm) in spiral chains of 20 or more spores. The spores contain melanin (Lambert and Loria 1989a). *S. scabies* strains can be distinguished based on physiological characteristics. Most of the strains evaluated by Lambert and Loria (1989a) do not degrade with xanthine and are susceptible to 10 IU of penicillin G per ml, 20 µg of streptomycin per ml, 10 µg of thallous acetate per ml, 25 µg of oleandomycin per ml, and 0.5 µg of crystal violet per ml. CS caused by *S. scabies* is most severe in the soil pH range of 5.5 to 7.5 (Powelson and Rowe 2008).

S. acidiscabies, also referred to as acid scab, is an acid-tolerant strain that causes scab on potatoes in acidic soils below pH 5.2 (Lambert and Loria 1989b), such as those found in the northeastern United States (Powelson and Rowe 2008). However, it may now be widespread (Loria et al. 2006). Although CS symptoms due to *S. acidiscabies* are indistinguishable from *S. scabies*, the causal agents are distinguishable in culture (Lambert and Loria 1989b). *S. acidiscabies* has flexuous spore chains and produces a red or yellow pH-sensitive diffusible pigment. The spore mass color (white to orange red) is growth-medium dependent. Additionally, it grows on agar media at a pH of 4.0, does not use raffinose as a carbon source, and will tolerate higher concentrations (relative to *S. scabies*) of penicillin G, streptomycin, thallium acetate, crystal violet, and oleandomycin (Lambert and Loria 1989b).

S.turgidiscabies causes erumpent scab lesions on potato in eastern Hokkaido, Japan. Related strains that are pathogenic have been described in Korea (Song et al. 2004) and Finland (Lindholm et al. 1997). This *Streptomyces* species has a grey mass color and flexuous spore chains, with spores that are

smooth and cylindrical. Additionally, it does not produce diffusible pigments (including melanin), and does not grow on agar media at pH 4.0 or 37°C. For a carbon source, it utilizes raffinose and inulin, and is sensitive to streptomycin, penicillin G, polymyxin B, and thallium acetate (Miyajima et al. 1998).

SPECIES CAUSING SCAB IN NORTH AMERICA

In North America, most of the potentially pathogenic species are *S. scabies* and *S. europaeiscabiei*, followed by *S. stelliscabiei*, and species/strain *S. sp*. IdahoX. *S. acidiscabies* and *S. turgidiscabies*; others are less predominant. Species distribution in North America is patchy, with the exception of *S. europaeiscabiei*, which is common in the west (Alaska, Idaho, Ontario) as well as Prince Edward Island, and *S. scabies*, which is predominant in the middle and eastern Midwest (Minnesota, Missouri, Wisconsin, Indiana, and Michigan) as well as Washington, Oregon, and New York. In Maine and Pennsylvania, *S. scabies* and *S. europeiscabiei* are found in approximately equal proportions. Species distribution is variable on a local and regional basis, where a single species will often predominate in a field which may differ from neighboring fields (Wanner 2009). A variety of *S. scabies* strains can be present in a single field within a year. Moreover, the strain profiles within a field may shift from year to year (Wanner 2008).

THAXTOMIN, A VIRULENCE FACTOR FOR PATHOGENICITY

Pathogenic *Streptomyces* species synthesize the phytotoxin thaxtomin (King et al. 1991; King et al. 1992; Bukhalid et al. 1998). Thaxtomin is the primary pathogenicity determinant (Lawrence *et al.* 1990; Healy *et al.* 2000). Thaxtomins were isolated and identified by King et al. (1989), and shown to elicit symptoms (scab lesions) on potato that are the same as those caused by *S. scabies* (King et al. 1989; King et al. 1992; Lawrence et al. 1990). Lesions on potato tubers are typically brown to black and range in morphology. The lesions may appear as isolated or confluent and they may be described as surface, raised, or pitted (Archeleta and Easton 1981; Hooker 1981).

Thaxtomin may be a requirement for the establishment of infection (Loria et al. 1997) because it causes cell collapse (Goyer et al. 2000). It is thought to alter the deposition or composition of cell walls, compromising their integrity (Fry and Loria 2002). Thaxtomin inhibits the biosynthesis of cellulose in expanding and dividing cells walls and induces plant cell hypertrophy in expanding plant tissues (Fry and Loria 2002; Scheible et al. 2003), resulting in a reduction of seedling growth (Scheible et al. 2003; Leiner et al. 1996). Thaxtomin also results in a compromised cell wall by increasing Ca^{2+} which leads to cell wall acidification (Tegg et al. 2005) and activation of enzymes (Cosgrove 1999).

The predominant phytotoxin produced by *S. scabies* is thaxtomin A*,* followed by thaxtomin B (Lawrence et al. 1990; King et al. 1991; King et al. 1992). Other thaxtomins have been identified (King 1992; King and Lawrence 1996). The percentage of potato tuber surface infected was positively correlated with thaxtomin A production in culture (R = 0.60), where an 11% increase in surface area infected corresponded to an increase of 1 µg/mL of thaxtomin A (Kinkel et al. 1998).

Pathogenicity genes for the biosynthesis of thaxtomin (*txtAB* and *txtC*) are on a pathogenicity island (Kers et al. 2005; Loria et al. 1997, 2006). Genes *txtA* and *txtB* (*txtAB*) encode non-ribosomal peptide synthetases (Healy et al. 2000), resulting in a dipeptide that is hydroxylated by an enzyme (cytochrome P450 monooxygenase) that is encoded by *txtC* (Healy et al. 2002). The nitric oxide synthase (NOS) enzyme (TxtD) encoded by *nos*/*txtD* results in a nitrated dipeptide (Kers et al. 2004) along with *txtE*, another P450 monooxygenase gene that encodes TxtE, a unique cytochrome P450 (Barry et al. 2012). Thaxtomin biosynthesis is regulated by the TxtR protein (encoded by *txtR*), a member of the AraC/XylS family of transcriptional regulators and is embedded in the biosynthetic pathway of thaxtomin (Joshi et al. 2007b). The biosynthesis genes just described, along with *txtAB* and *txtC,* are found on the same pathogenicity island (Kers et al. 2005; Loria et al. 1997, 2006).

Nec1 encodes a protein that induces necrosis in plant tissue (Bukhalid and Loria 1997) and contributes to pathogenicity (Bukhalid et al. 1998) and virulence (Joshi et al. 2007a), but is not required for pathogenicity (Wanner 2009). *Nec1* is thought to suppress plant defenses stimulated by thaxtomin (Joshi et al. 2007a). Both the thaxtomin biosynthesis genes and *nec1* are located on a large mobile pathogenicity island in *S. turgisiscabies* (Kers et al. 2005).

Scheible et al. (2003) has identified an *Arabidopsis* mutant resistant to thaxtomin, where resistance is due to a decreased rate of thaxtomin uptake. The cause of resistance is a single, recessive mutation at a nuclear locus. The *TXR1* gene is thought to be a regulator of a thaxtomin transport and plays a required role in normal root growth at increased temperatures and normal shoot growth at all temperatures (Scheible et al. 2003).

PATHOGENIC STRAIN IDENTIFICATION

Conventional polymerase chain reaction (PCR) assays based on 16S rRNA genes can be used to detect and distinguish among species of *Streptomyces* on scabby tubers (Wanner 2006; Lehtonen et al. 2004). However, the species designations may not indicate pathogenicity because there may be both pathogenic and nonpathogenic strains of a species (Wanner 2006). The pathogenicity of a strain can be confirmed by the presence of thaxtomin (Fry and Loria 2002; Healy et al. 2000; Lawrence et al. 1990; Leiner et al. 1996). The detection of *txtAB* has been perfectly correlated with pathogenicity in more than 100 isolates (Wanner 2006; Wanner 2007). The *txtAB* operon has been used as a marker to determine pathogenic species (Wanner 2006; Wanner 2007; Qu et al. 2008; Qu et al. 2011; Flores-González et al. 2008) to quantify pathogenic species in soil and tubers using a real-time polymerase chain reaction assay (Qu et al. 2008), and to distinguish between symptoms due to pathogenic *Streptomyces* species and those caused by powdery scab in potato (Qu et al. 2011). Powdery scab (PS) is caused by *Spongospora subterranea*, a fungus-like protozoan that produces disease symptoms that may

be confused with CS. PS lesions are tan, blister-like swellings that may rupture and result in outgrowths that look like CS lesions. PS lesions may be distinguished from CS lesions by observing torn edges on the sunken lesions. They may be confirmed by observation with a microscope (Powelson and Rowe 2008).

CS PHENOTYPE SCORING METHODS

Methods for measuring CS disease severity include a variety of categorical and continuous scales that assess surface area affected, lesion type, percent infected tubers for single hills or plots, and pot scores for greenhouse assays and field experiments (Calgari and Wastie 1985; Driscoll et al. 2009; Khatri et al. 2011; Haynes et al. 1997; Leach 1938; Wanner and Haynes 2009). Percent surface area is visually determined on a scale of 0 to 100%. Lesion type may also be scored on a scale of 0 to 5, where 0 = no lesions, 1 = superficial and small, 2 = superficial and coalescing, 3 = raised and small, 4 = raised and coalescing, 5 = pitted lesions of any size or depth (Wanner and Haynes 2009). In general, tubers with smaller surface area infected have a less severe lesion type (Leach 1938; Bjor and Roer 1980). Surface area and lesion type correlation coefficients are positive and range from 0.30 to 0.85 (Bjor and Roer 1980; Lambert et al. 2006). Other methods include determining the percent infected tubers (Caligari and Wastie 1985) and reporting a scab index based on individually scored tubers (Bjor and Roer 1980; Bowers et al. 1996; Liu et al. 1995; Goth et al. 1993; Haynes et al. 1997) or plots (Bradshaw et al. 2008). Assays may be chosen based on the particular objectives of a breeding program and the germplasm utilized. For example, Hiltunen et al. (2011) describe an *in vitro* assay that correlates with field and greenhouse data, and that eliminates only the most susceptible genotypes by screening seedlings with thaxtomin A. In this study, 120 F1 potato genotypes were screened with thaxtomin A in culture medium and assessed for shoot growth. Eighteen were selected based on sensitivity or tolerance and then the selected genotypes were tested in the greenhouse with *S. turgidiscabies* and *S. scabies* and in three fields using a scab index.

Four methods to quantify scab resistance were tested by Leach (1938) using a scale that takes into account the lesion type and percent surface area to obtain a mean scab rating of a seedling family. The most effective methods measured each tuber separately, and then averaged the scores of all tubers in the hill or only tubers that were greater than one inch in diameter. The other two methods detected differences among families, but less significantly so. These assays included a hill rating based on the most susceptible-type lesion or the predominant type of lesion.

Bjor and Roer (1980) concluded that two to three greenhouse pots are needed per genotype to simply identify a resistant genotype. The authors used a scale of one to three for lesion severity and zero to nine for percent surface area to detect differences among genotypes. Goth et al. (1993) reported that a cluster analysis on the combination of percent surface area and lesion type is superior to an overall scab index, such as the one used by Bjor and Roer (1980) in distinguishing potato selections. A cluster index for these two traits provides an efficient method to classify relative resistance of the tested clone to known cultivar standards (Goth et al. 1993).

Caligari and Wastie (1985) reported that 12 plants are needed to detect a difference in mean resistance of 1.3 points on a nine point scale that reflects the percentage of infected tubers. In the same study, a reduction in error variance and wider variation among cultivars was obtained by growing tubers in sandy soil diluted to 50% in the greenhouse. Both Bjor and Roer (1980) and Caligari and Wastie (1985) report a higher disease incidence in greenhouse tests when naturally infested soil was diluted with coarse sand.

However, screens for CS resistance have many sources of variation to consider. Within-greenhouse pot variation may equal the between-pot and between-replicate experiment variation when considering both lesion type and percent surface area, despite efforts to inoculate with a single isolate,

control the inoculum density and maintain a controlled environment in a growth chamber (Wanner and Haynes 2009).

Other assays include a soil-less, hydroponic method utilized by Khatri et al. (2010) based on a nutrient film technique, and a novel pot system described by Khatri et al. (2011). Of these two methods, the novel pot system resulted in more infection than the hydroponic method (Khatri et al. 2011). Yet another method to assess CS resistance in the context of the root systems during growth is computed tomography (CT) scanning (Han et al. 2008).

An assay based on mini tubers obtained by the leaf-bud protocol developed by Lauer (1977), has been used in several studies to assess pathogenicity (Babcock et al. 1993; Kinkel et al. 1998; Lindholm et al. 1997; Lorang et al. 1995). For example, Lindholm et al. (1997) soaked mini-tubers in oatmeal broth mixtures of *S. scabies* strains and incubated them on moist filter paper in the dark. In the greenhouse, Babcock et al. (1993) and Kinkel et al. (1998) made cuttings, placed them into sterilized sand and allowed them to form minitubers, which were then inoculated with a spore suspension and evaluated for CS after three weeks. Lorang et al. (1995) placed cuttings in sterile sand and grew them for two to three weeks in the greenhouse. Then, the minitubers were inoculated with a spore suspension and allowed to develop for seven to ten days until disease symptoms were visible. Similarly, Lawrence et al. (1990) used minitubers formed from dark-grown sprouts to verify pathogenicity of strains.

DISEASE CYCLE AND HOST RANGE OF *STREPTOMYCES SCABIES*

Streptomyces species that cause scab have a wide host range. They result in stunting in both monocots and dicots, including wheat, corn, beet, parsnip, carrot and radish (Goyer and Beaulieu 1997; Hooker 1949; Leiner et al. 1996; Locci 1994; Wanner 2004).

S. scabies is a bacterium that resembles a fungus due to its filamentous morphology. The mycelium is composed of thin (approximately 1 µm), branched hyphae with few or no cross walls. Cylindrical spores (0.6 by 1.5 µm) are produced on the spiral hyphae. Spores are released from the tip of the hyphae by constriction due to cross wall formation. The germ tubes that form develop into a myceloid form (Lambert and Loria 1989a; Agrios 1997). The bacterium is dispersed by spores and survives on seed, in soil, and in soil water (Agrios 1997). The hydrophobic characteristic of the spores allows them to also be transported by arthropods and nematodes (reviewed by Loria 2006). The spores germinate and enter plant tissues through wounds, stomata, and lenticels (Agrios 1997). Other evidence suggests that *Streptomyces* species do not rely on natural openings, and that penetration and growth occurs through the cell walls (Loria et al. 2003). Young tubers can be directly penetrated and are most susceptible in the first two weeks of tuberization (Agrios 1997; Khatri et al. 2011). The tuber is the only known infection site in potato (Powelson and Rowe 2008). However, Han et al. (2008) demonstrated that *S. scabies* affects the emergence and growth of roots in the early stages of development. The pathogen grows through a few cell layers of the tuber, symplastically or apoplastically (Agrios 1997; Loria 2001). As the host cells die, they provide nutrients for the bacterium. Subsequently, the living host cells around the lesion divide and produce layers of cork cells. The pathogen then multiplies, resulting in several cork layers that eventually push outward and form the scab lesion on the tuber (Agrios 1997; Loria 2001). Surface (russet scab), raised (erumpent scab), and pitted lesions (pitted scab) may be observed on the same tuber, and can appear small or coalesced. The

lesion type (severity) and incidence of this disease appears to be dependent on environmental factors and cultivar susceptibility (Locci 1994; Loria et al. 1997).

ABIOTIC FACTORS AND MANAGEMENT PRACTICES

Despite the knowledge that has accumulated since the discovery of the causal agent of CS, there is no single effective method available to control the disease in the field (Lerat et al. 2009; Powelson and Rowe 2008).

CONDITIONS CONDUCIVE TO DISEASE AND MANAGEMENT

Streptomyces species that are pathogenic on potato are capable of causing surface, raised, or pitted lesions on a susceptible genotype given a favorable environment (Archuleta and Easton 1981). Environmental conditions influence the types of lesions observed (shallow, raised, or pitted) (Goyer et al. 1996).

Environmental factors that are conducive to CS caused by *S. scabies* include low soil moisture during tuber initiation, daytime temperatures above 70°F, and a soil pH range of 5.5 to 7.5. The severity of scab lesions increases as the soil pH increases from pH 5.2 to 8.0. Above pH 8.0, scab severity decreases again (Agrios 1997). The recommendation for potato growers is to reduce soil pH to 5.2 or below (reviewed by Locci 1994). Sulfur, acid-forming fertilizers, and gypsum applied before or at planting reduces the soil pH to between 5.0 and 5.2, which helps to suppress CS caused by *S. scabies* (Locci 1994; Powelson and Rowe 2008).

CS is managed on the seed piece by applying the fungicide Mancozeb (Powelson and Rowe 2008) which is typically used to control early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*) in potato (Gullino et al. 2010). Pentachloronitrobenzene (PCNB) (the common name is Quintozene and the trade name is Blocker) is an in-furrow treatment that results in the suppression of CS (Davis et al. 1976; Powelson and Rowe 2008). PCNB is more widely used than formaldehyde, urea

formaldehyde, and manganese sulphate and is typically beneficial in the first year of production (Locci 1994). Chloropicrin, a broad-spectrum soil fumigant, has recently been tested by commercial producers in Wisconsin in an attempt to manage the soilborne diseases CS, potato early dying, and powdery scab (Webster et al. 2013). Although applications with chloropicrin have been variable \ for CS disease control in Wisconsin, there has been a consistent increase in overall yield in fumigated fields (Webster et al. 2013). Ongoing research on the efficacy of chloropicrin on soilborne diseases is being conducted (Webster et al. 2013). Although there are several options to manage CS, there is no one key management tactic for control (Powelson and Rowe 2008).

TIMING OF MANAGEMENT OPTIONS

Timing of management for the suppression of CS includes several factors. In the years prior to planting, crop rotation is effective only when used in combination with other methods. In the year prior to planting, organic amendments may increase disease severity, while non-organic amendments are effective when used with other tactics. At planting, seed treatment is effective only with other strategies as well as water management at tuber initiation (Powelson and Rowe 2008). Common management techniques for soil moisture include maintaining irrigation throughout the growing season at either 45 or 65 kilopascals (kPa) (-0.45 or -0.65 bars) of soil moisture tension (Davis et al. 1976). *S. scabies* will generally cause disease in soils with less than 65 to 70% soil moisture (Gudmestad 2008). As a management technique, growers can maintain soil moisture in the 80 to 85% range from tuber initiation until tubers are 25.4 to 38.1 mm in diameter (Gudmestad 2008; Powelson and Rowe 2008). Irrigation water develops a microfilm around the developing tuber to limit infection (Gudmestad 2008).

GREEN MANURES AND CROP ROTATION

Green manures that inhibit *S. scabies* include, but are not limited to, buckwheat, canola, fallow, oat, rye, and millet (Kinkel 2008; Powelson and Rowe 2008). Plowed red clover and animal manure, such as poultry manure, which increases soil pH, may aggravate CS symptoms. Pulp and municipality wastes have also been shown to reduce the severity of CS (Powelson and Rowe 2008). Increased microbial activity is thought to suppress *S. scabies* in the soil (Kinkel 2008).

A somewhat effective management practice for reducing CS is a disease-suppressive rotation (3-year, mustard/rapeseed-sudangrass/rye-potato). Larkin et al. (2011) found that this rotation, though difficult to carry out in practice due to economic constraints, reduced scab in all three years under irrigated and nonirrigated conditions. In addition, the status quo [barley underseeded with red clover, followed by potato (2-year) and, soil-conserving [additional year of forage grass and reduced tillage (3-year, barley/timothy-timothy-potato)] methods reduced scab in some years. These methods are in contrast to the soil-improving system (added yearly compost amendments to the status quo system), which was not as effective at reducing disease levels.

The disease-suppressive method reduced common scab for all three years in both irrigated and nonirrigated conditions and reduced severity by 25 to 40%, compared to all other cropping systems in the study. However, in contrast to the current thought that an increase in irrigation decreases CS incidence, Larkin et al. (2011) reported a 21% increase in CS incidence over all types of cropping systems, indicating that the relationship between soil moisture and CS is complex. This rotational method is different from the others examined by the observed significant shift in soil microbial community characteristics (Larkin et al. 2011).

Interestingly, soils suppressive to scab are also those that have supported potato monoculture for several years (Lorang et al. 1989; Menzies 1959). Several studies have reported disease suppression

on potato due to pathogenic *Streptomyces* species by the presence of nonpathogenic *Streptomyces* species (Bowers et al. 1996; Hiltunen et al. 2008; Liu et al. 1995; Lorang et al. 1995; Meng et al. 2012; Wanner 2007). Disease suppression also associated with the presence of bacilli and florescent pseudomonads (Meng et al. 2012).

HOST-PATHOGEN INTERACTIONS

The interaction between host and pathogen appears to be complex. Wanner and Haynes (2009) reported specific genotype-pathogen isolate interactions in a study that examined the response of four potato cultivars to different species and molecular types of *Streptomyces* species and isolates that differed in aggressiveness. Factors that may explain some of the complexity are discussed in this section.

RELATIONSHIP BETWEEN DISEASE SEVERITY AND INOCULUM LEVELS

Under greenhouse conditions, CS severity generally increases linearly with a log 10 increase in inoculum concentration. However, the slope of the regression line is affected by cultivar resistance and *S. scabies* strain virulence (Keinath and Loria 1991). An increase in the population of *S. scabies* in the rhizosphere may be dependent on the strains tested and the inoculum density. Keinath and Loria (1991) reported an increase in the rhizosphere population of *S. scabies* with an increase in the initial soil population by assaying four levels of two different pathogenic strains. In contrast, Ryan and Kinkel (1997) found no significant increase in pathogen populations in the rhizosphere by assaying with an inoculum source that contained five different *S. scabies* strains and higher population densities than what Keinath and Loria used. As suggested by Ryan and Kinkel (1997), the higher population densities may have inhibited reproduction of *S. scabies.* Interestingly, the rhizosphere population of *S. scabies* is not consistently related to the severity, incidence, strain virulence, or cultivar resistance (Keinath and Loria 1991).

TUBER PERIDERM AND INFECTION

Penetration of tubers by *S. scabies* is thought to take place through young lenticels, probably because they have not yet formed a layer of suberin (Locci 1994). Lenticels are transformed from stomata that have shed their guard cells (Adams, 1975). Permeability of lenticels to gases and pathogens may be compromised by wet soil, where lenticels proliferate, or dry soil, where suberin deposits form (Adams 1975). Additional possible infection sites are through wounds or larval feeding sites (Locci 1994). Loria et al. (2003) provide evidence that *Streptomyces* species do not rely on natural openings, and that penetration and growth occur through the cell walls.

Infection leading to disease primarily takes place during the first three to four weeks after tuber initiation. Specifically, Khatri et al. (2011) reported a higher percent infected tubers (68%) and percent surface area (11.7%) when inoculum was present at two weeks after tuber initiation compared to a lower percent infection (4%) and area (0.004%) when inoculum was present at eight weeks after tuber initiation. The mean lesion depth was greatest on tubers that were infected from two to three weeks after initiation of tuberization, in contrast to tubers that were inoculated at six to eight weeks, which resulted in mostly superficial lesions. Tuber internodes that are formed first (internodes one through five) are more susceptible to infection compared to internodes that are formed later and are slower to develop (Adams 1975; Khatri et al. 2010; Khatri et al. 2011). The first internodes formed have the greatest amount of time to expand, resulting in more severe lesions when exposed to *S. scabies* (Lapwood et al. 1970).

Exposure to *S. scabies* results in an increased number of cell layers and thickness of the phellem in both tolerant and susceptible genotypes when observed at harvest. In the presence of the pathogen, the thickness of the phellem, number of cell layers and suberin content are increased at 10 days after tuber initiation (Khatri et al. 2011).

A study by Khatri et al. (2011) reports that exposure of a susceptible cultivar (Desiree) to the pathogen results in increased suberin deposition, thickness of phellem, and number of cell layers in the phellem. An increased deposition of suberin can be found in tubers that have been exposed at seven or 21 days after tuber initiation (28% and 38% higher, respectively) when assessed seven days after exposure to *S. scabies.* Desiree also has significantly increased phellem thickness and number of cell layers three weeks after exposure to *S. scabies* when inoculated at seven days after tuber initiation. When inoculated at 21 days after tuberization, there is a significant increase in phellem thickness and number of cell layers at two to three weeks after exposure. Desiree tubers that are exposed to the pathogen seven days after tuber initiation are more susceptible in incidence and severity to CS (27% of tubers with disease) than tubers inoculated 21 days after tuber initiation (<10%).

HOST PLANT RESISTANCE

To date, no cultivars immune to CS are known. There are, however, cultivars that are considered tolerant to CS, including Russet Burbank, Superior, Pike, and Dark Red Norland (Loria 2001; Driscoll et al. 2009). Some examples of susceptible cultivars include Yukon Gold, Shepody, and Kennebec (Lambert et al. 2006). Host plant resistance is considered to be one best options for managing CS (Mishra and Srivastava 2001; Jansky and Rouse 2003; Wanner and Haynes 2009). However, little progress has been made in the introgression of resistance to scab into major cultivars (Douches et al. 1996).

A tolerant host may not show CS lesions yet have large, pathogen-induced increases in periderm thickness (Khatri et al. 2011). The phellem thickness and number of cell layers significantly increases ten days after tuberization when exposed to *S. scabies* for both the tolerant cultivar Russet Burbank and the susceptible cultivar Desiree when compared to the uninoculated controls. It is important to note that

phellem thickness and cell number in the tolerant cultivar was significantly greater than the susceptible cultivar, particularly at 20 to 30 days after tuberization (Khatri et al. 2011).

HERITIBILITY OF RESISTANCE AND QTL IDENTIFICATION

Heritability is a measure of the efficacy of selection made for a particular trait (Fehr 1991). It is dependent on the genetic and nongenetic factors that are involved in the expression of the phenotype. Broad-sense heritability is the ratio of genotypic variance (σ^2_g) to phenotypic variance (σ^2_{ph}). The genotypic variance is comprised of the sum of the dominance (σ^2_D), additive (σ^2_A), and epistatic (σ^2_I) variances. In short, it is the variation due to genetic differences among individuals. The phenotypic variance (σ^2_{ph}) is comprised of the sum of the variance due to the genotype (σ^2_g), experimental error (environmental variance) (σ^2_e), and genotype by environment interaction (σ^2_{gxe}), where genotype performance varies from environment to environment. The environment may be defined by locations and/or years. Narrow-sense heritability is a measure of additive variance, or the genetic variance that is transmitted to the next generation. Heritability measures are valuable because they may direct the breeding methodology to achieve genetic gain. For example, if the heritability is low, more replication may be required to effectively select for a particular trait. Moreover, heritability is not constant because it is dependent on the environment, experimental design and population, among other factors. The variation in heritability values is largely dependent on the experimental parameters of the environment, germplasm evaluated, experimental design, and scale used to quantify the disease. For example, a screening population that has been developed from a cross between a CS-resistant parent and a CS-susceptible parent will have high genetic variation among individuals within the population, which may result in a high heritability value. However, if the trait has a high genotype x environment component, heritability will be lower.

Few studies have reported the heritability of resistance to CS. Haynes et al. (1997) estimated broad-sense heritability (95% confidence intervals) as 0.89 (0.78,0.95) for percent surface area and 0.93 (0.86,0.97) for lesion type. In that study, 23 tetraploid potato genotypes were evaluated in a replicated trial for two years in two locations. Haynes et al. (2009) examined the heritability of CS resistance in a diploid potato population (*Solanum phureja-S. stenotomum*) over three years and assessed for CS resistance using the proportion of tubers with scab lesions. In that study, the broad-sense heritability for resistance was estimated at 0.18 with a 95% confidence interval of 0.15 to 0.35. There was no narrow-sense heritability because there was no variation among families, reflecting a lack of additive variation. Another study by Haynes et al. (2010) estimated broad-sense heritability for CS to range from 0 to 0.78 based on percent surface area, 0.49 to 0.90 based on lesion type, and 0.30 to 0.80 based on proportion of scabby tubers for 17 to 23 advanced genotypes from public potato breeding programs in the United States. The material was evaluated at three locations over five years. As discussed in Haynes et al. (2010), the broad-sense surface area and lesion index values were higher in an earlier study (Haynes et al. 1997). The reported values in the Haynes et al. (2010) study may be lower due to a greater diversity of environments, more diversity in pathogenic *Streptomyces* species (Wanner 2009), or differences among scores by different researchers, despite using the same scale (Haynes et al. 2010). Bradshaw et al. (2008) evaluated the heritability of CS on a tetraploid full-sib family derived from processing clone 12601ab1 and the table cultivar Stirling. The heritability of CS was estimated at 0.660 when quantifying CS on a scale of one to nine. This was one of lowest heritability values in the study, which also examined maturity (0.916), yield (0.896), and other traits (Bradshaw et al. 2008).

As discussed by Haynes et al. (2009), the disparity between the reported levels of heritability between the Haynes et al. (2009) and Haynes et al. (1997) studies may be due to several factors which also may explain the differences among all studies discussed in this chapter. One explanation is the

difference in the methodologies used to quantify CS – Haynes et al. (1997) quantified percent surface area and lesion type, whereas Haynes et al. (2009) quantified CS as proportion of scabby tubers. Other factors to consider include inherent genetic differences between the populations, differences in breeding strategies that resulted in the tested populations, and differences due to ploidy levels, where more possibilities for multi-allelic interactions at a locus and inter-locus complementary gene action are present at the tetraploid level.

To date, there has been only one report of quantitative trait loci (QTL) for scab resistance in the literature. Bradshaw et al. (2008) reported two QTLs for CS (chromosomes two and six), but no single QTL explained more than 8.2% phenotypic variability in the tetraploid *S. tuberosum* population. Chapter three of this dissertation describes a QTL for resistance to CS on chromosome eleven in an F2 population with an *S. tuberosum* female grandparent (US-W4) and *S. chacoense* male grandparent (524-8, source of resistance). This QTL explained approximately 24% phenotypic variability for percent surface area and 17% for lesion type in this population.

BREEDING FOR RESISTANCE TO COMMON SCAB

Introgression of new sources of germplasm resistant to CS is one potential solution to obtaining genotypes with high levels of resistance across environments (Haynes et al. 1997). CS is a disease that has been studied for several years in potato. The causal agent was described as *Oospora scabies* by Thaxter in 1890, then renamed *Actinomyces scabies* by Gussow in 1914, then *Streptomyces scabies* by Waksman and Henrici in 1948. In 1926, Fellows reported the relationship between CS and growth of the potato tuber and speculated on the involvement of phytotoxins. Barker and Lawrence (1963) then found that scab-like lesions developed on immature potato tubers from sterilized extracts of scab lesions from field infected tubers. In 1989, King et al. isolated and characterized two phytotoxins directly associated with CS. Several more recent studies have been committed to developing germplasm

with enhanced resistance to CS and determining the genetic basis for resistance to CS (Haynes et al. 2009; Bjor and Roer 1980; Bradshaw et al. 2008; Caligari and Wastie 1985). However, the range in broad-sense heritability values reported in the literature is very wide, from 0.18 to 0.93 (Haynes et al. 1997; Haynes et al. 2009; Haynes et al. 2010; Bradshaw et al. 2008). These data indicate that screening for resistance to CS leads to variable results, depending on the methodology and populations used. For example, Haynes et al. (2010) reports significant genotype x location interactions in all six years of evaluation for percent surface area and in five years for lesion type. This indicates the need for replication to categorize genotypes for CS. Replication across environments may be important as a breeding objective to identify a durable and stable source of resistance, while replication within an environment may be important if a distinction among genotypes is crucial under a specific set of agronomic conditions. Secondly, increasing replications would likely increase heritability values in a particular population. By increasing the number of replications, the heritability ratio increases. For example, if the heritability is calculated on an entry-mean basis, the equation is as follows:

$$h^2 = \frac{\sigma_g^2}{\sigma_e^2/rt + \sigma_{ge}^2/t + \sigma_g^2}$$

Where h^2 is the broad sense heritability, σ_g^2 is the genetic variance, σ_e^2 is the experimental error, σ_{ge}^2 is the genotype by environment interaction, *r* is the number of replications, and *t* is the number of test environments (Fehr 1991).

The population assessed by Haynes et al. (2009) had dominance variation (non-additive for resistance) as revealed by significant variation among families, and no additive variation (narrow-sense heritability that reflects the degree to which selection for CS on parents will be transmitted to the next generation). Because of this, the authors suggest that it may be feasible to transfer the high levels of resistance in the population to the tetraploid level using 4x-2x crosses, a breeding method also

suggested by Alam (1972). It has been demonstrated that a portion of non-additive genetic variation can be transmitted via inter-tetraploid crosses (4x-4x) (Wricke and Weber 1986), as well as unilateral sexual polyploidization (4x-2x), and bilateral sexual polyploidization (2x-2x) (Haynes 1990, 1992). The method of transferring resistance to CS via unilateral sexual polyploidization (4x-2x) has been demonstrated by Murphy et al. (1995). Five of the eight families developed from scab-resistant diploids of *S. phureja*, *S. stenotomum*, and haploid *S. tuberosum* crossed to a susceptible tetraploid variety (Shepody) had a scab index lower than the mid-parent value. The study by Murphy et al. (1995) supports the hypothesis developed by Alam (1972) that two independent loci are required for resistance to CS. At one locus, one or more dominant alleles confer resistance and homozygous recessive alleles confer resistance at a second locus. Cipar and Lawrence (1972) also reported resistance from haploids extracted from tetraploid cultivars Avon and Hindenburg. The authors suggested that scab resistance is controlled by more than one locus (probably two or three loci) with a simple mode of inheritance. In contrast to the one or few gene model observed in haploid or diploid populations, tetraploid populations tend to have more complex gene action (Dees and Wanner 2012; Driscoll et al. 2009; Dionne and Lawrence 1961; Goth et al. 1993; Haynes et al. 2010). Nearly continuous variation in severity at the tetraploid level suggests that multiple genes are involved (Dees and Wanner 2012; Driscoll et al. 2009).

Despite a century of breeding, relatively few CS resistant cultivars are available. This is due, at least in part, to the selection process based on phenotype and lack of genotype information. To date, the selection process based on phenotype for CS requires an inoculated field, greenhouse or growth chamber that is managed for high disease pressure. In the field, the environmental conditions can be controlled only to a limited extent (such as water availability), and may result in a trial with little differentiation among potato clones due to lack of disease pressure. Even in an environment that is

controlled, such as the greenhouse and growth chamber, rating the symptoms is difficult, because the range in symptoms of the tubers within an experimental unit (such as a pot or hill) may vary from none to severe (Dees and Wanner 2012). The consideration for added replication (discussed above) decreases the number of potato clones that a breeder is able to test, given that the resource allocation is the same, therefore potentially decreasing breeding progress. The number of replications and scoring methods are discussed in chapter two of this dissertation. A potential high-throughput phenotypic assay may be to select with Thaxtomin A (Wilson et al. 2010; Hiltunen et al. 2011). Wilson et al. (2010) isolated somatic cell variants of Russet Burbank with strong to extreme resistance to CS with thaxtomin A as a positive selective agent. Hiltunen et al. (2011) also used Thaxtomin A as a selection agent to eliminate the most susceptible progeny from a population by measuring the shoot growth of the seedlings. These assays may help to eliminate the need for field screening and increase the amount of germplasm available with resistance to CS in breeding programs (Dees and Wanner 2012).

Recently, the potato genome has been published and the single nucleotide polymorphism (SNP) Infinium 8303 Potato Array has been released (Hamilton et al. 2011). The high-throughput method of collecting and analysis of genotypic information will potentially allow breeders to select using molecular markers. QTL analysis, the preliminary work toward the development of a molecular marker for resistance to CS, was conducted and is reported in chapter three of this dissertation. Selection with a molecular marker would reduce or eliminate the need for early generation screening in the field, greenhouse, or growth chamber.

Germplasm that demonstrates greater resistance than historic cultivars (such as Russet Burbank and Atlantic) has been identified in the last decade (Dees and Wanner 2012; Douches et al. 2001; Haynes et al. 2010; Tarn et al. 2003), of which some clones are from a successful recurrent selection

program (Tarn et al. 2003). The release of this material contributes toward a broader germplasm base that may be used to develop cultivars with greater and more stable resistance across environments.

One potential source of novel for germplasm for resistance for CS is wild relatives of potato. Wild *Solanum* species have made important contributions to cultivar development (Maxted et al. 2012). Moreover, most wild species can hybridize with the cultivated potato (Hanneman Jr. 1989; Camadro, 2010). These species have made important contributions for disease resistance and improved quality (Jansky 1990; Hawkes 1945, 1958; Rieman et al. 1954; Rudorf 1958; Ross 1966, 1979; Plaisted and Hoopes 1989; Bradshaw and Ramsay 2005; Bradshaw 2009).

Hosaka et al. (2000) screened one hundred accessions of 18 wild diploid potato species through three cycles of selection and selected several (322) resistant genotypes. Wild species *S. bukasovii*, *S. canasense*, and *S. multidissectum* produced the most resistant clones. Resistant accessions of the cultivated diploid species *S. phureja* (De Maine et al. 1993) and wild species *S. chacoense* (Dionne and Lawrence 1961) have also been identified.

Due to the intensity of labor required for screening for CS resistance, the use of marker-assisted selection (MAS) would likely lead to significant gains in selection for resistance. One method to initiate marker development is QTL analysis. Bradshaw et al. (2008) identified two QTLs for CS in a *S. tuberosum* tetraploid population. Chapter three of this dissertation discusses the analysis and results of a QTL study for resistance to CS in a diploid, cultivated x wild species (*S. chacoense*) hybrid population. Using MAS, coupled with a strong source of resistance from a novel, diploid source that produces 2n pollen will allow breeders to develop potato cultivars with resistance to CS. It is notable to mention that cultivars derived from 4x-2x crosses, where the tetraploid is a highly-selected cultivar and the diploid male is a haploid x wild species hybrid or cultivated diploid x haploid hybrid, results in heterosis for yield (Jansky 2009; De Jong et al. 1981; Bani-Aameur et al. 1991; Tai and De Jong 1991; Buso et al. 1999, 2000,

2003; Alberino et al. 2004) and provides greater yield stability and appearance across environments than 4x-4x crosses or 2x-4x crosses (Jansky 2009; Darmo and Peloquin 1990; Ortiz et al. 1991).

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REVIEW OF VERTICILLIUM WILT

Verticillium wilt (VW) is caused by the soilborne fungi *Verticillium albo-atrum* (Reinke and Barth) and *Verticillium dahliae* (Kleb) (Martin et al. 1982; Nicot and Rouse 1987; Weingartner et al. 1974). The disease is a persistent problem (Jansky 2009), is distributed throughout growing regions of the world, and can cause yield reductions up to 50% (Powelson and Rowe 1993). *V. dahliae* is widespread in the United States, particularly in areas where the average daily summer temperatures exceed 25°C (Johnson and Dung 2010; Powelson and Rowe 2008), including Wisconsin. *V. albo-atrum* causes disease in production areas that are cooler and do not normally exceed temperatures of 21°C such as Maine, the Red River Valley, and southern California (Powelson and Rowe 2008). Root-lesion nematodes (*P. penetrans*) can interact with *V. dahliae* and result in potato early dying disease (MacGuidwin 2008). In some cultivars, co-infection will result in symptoms greater than if the organisms were acting separately (MacGuidwin 2008).

The symptoms of VW in potato include wilting, vascular discoloration, premature senescence, chlorosis, necrosis, and yield reduction (Johnson et al. 2010; Rowe et al. 1987). *Verticillium* spp. may be present in the soil and germinate in response to plant root exudates (Mol 1995) or in the vascular system of infected tuber seed pieces (Dung et al. 2013; Easton et al. 1972, Omer et al. 2000, Tsror et al. 1999). Infection and disease are favored by moist soils and high rates of evapotranspiration (Powelson

and Rowe 2008). Microsclerotia of *Verticillium* can survive in the soil for up to fifteen years (Pataky 1997).

One of the most effective current techniques for managing VW is to fumigate the soil (Powelson and Rowe 2008). Fumigation can reduce soil populations of *V. dahliae* by 85 to 95% depending on the chemical, application rate, and soil environmental conditions at the time of application (Powelson and Rowe 2008). However, fumigants are costly (Rowe et al. 1987; Young 1956), can destroy beneficial microorganisms in the soil (Pegg 1974), and can have negative human health and environmental effects (Noling 1997). Soil solarization has been found to reduce levels of *V. dahliae*, nematodes, and other soilborne pathogens (Hopkins and Hirnyck 2008; Jimenez-Diaz et al. 2012; MacGuidwin 2008). Green manure crops have been found to suppress potato early dying in some locations (Kinkel 2008; Powelson and Rowe 2008; Davis et al. 1996).

Host resistance offers promise as a favorable alternative to minimizing VW in potato (Corsini et al. 1988; Mohan et al. 1990). Of 15 leading cultivars in the United States and Canada, Norland, Kennebec, Russet Norkotah, Superior, and Yukon Gold are considered susceptible or very susceptible. A resistant clone is Dakota Pearl, and mildly resistant clones include Goldrush, Ranger Russet, and Umatilla (Whitworth and Davidson 2008). However, potato cultivars with the greatest resistance are not widely used in commercial production (Powelson et al. 1993). Resistance to VW has been demonstrated to be stable (Corsini et al. 1985) and heritable (Corsini et al. 1990). In potato, the genetic mechanism for resistance to VW was reported to be polygenic (Hunter et al. 1968). In two cultivated, diploid mapping populations of potato, three chromosomes were linked with resistance to VW (Simko et al. 2004b).

Genes for resistance to nearly all potato diseases have been identified in wild *Solanum* species (Bamberg et al. 1994, Jansky 2000, Ortiz 1998). Resistance genes from the wild species have been integrated by breeding programs by crossing them to *S. tuberosum* haploids and evaluating the

segregating progeny (Iwanaga et al. 1989; Ortiz et al. 1990; Swiezynski et al. 1989, Swiezynski et al. 1991). Resistance to VW has been identified in several wild potato species (Concibido et al. 1994; Lynch et al. 1997; Simko et al. 2004b). A single dominant gene in *S. chacoense* was reported to be responsible for resistance to infection and colonization *V. albo-atrum* (Lynch et al. 1997). Resistance to VW was identified in diploid interspecific clones (Jansky and Rouse 2000), with complimentary gene action of two dominant alleles controlling resistance (Jansky et al. 2004).

Functional resistance genes to VW in potato have not yet been characterized. However, Schaible et al. (1951) demonstrated that a single dominant gene (*Ve*) was responsible for resistance to *V. albo-atrum* in tomato, and is located on chromosome nine (Diwan et al. 1999). Genes within the tomato *Ve* locus have been isolated and confer resistance to *V. dahliae* in transformed potato clones (Kawchuk et al. 2001). In tomato, the *Ve* locus is comprised of two closely linked genes, *Ve1* and *Ve2* (Kawchuk et al. 2001). The functional expression of Ve1 and Ve2 genes results in resistance in potato (Kawchuk et al. 2001. However, the mechanism behind resistance due to *Ve* is not clear (Halterman et al. 2009). For example, Chen et al. (2004) suggest that *Ve* prevents the pathogen from spreading in the xylem tissue, while Williams et al. (2002) suggest a more active immune response to VW in tomatoes with *Ve* (Halterman et al. 2009). The salicyclic acid pathway appears to be involved as a defense mechanism against *V. dahliae* in potato (Derksen et al. 2013). Derksen et al. (2013) tested five defense genes associated with the salicylic acid defense signaling pathway, and found that four of them (*PAL1*, *PAL2*, *PR-1*, and *PR-2*) are regulated at the transcriptional level in response to *V. dahliae*.

The *Ve1* gene in tomato was used to detect *Ve* homologs (*StVe1*) in diploid and tetraploid potato (Simko et al. 2004a). In this candidate gene approach, the authors tested for a candidate gene marker and resistance to *V. dahliae* and found that the QTL comprised an eleven member family and encoded leucine-rich repeats similar to the tomato *Ve* genes. However, the homologs do not appear to confer

resistance (Fei et al. 2004; Simko et al. 2004a). Liu et al. (2012) overexpressed the *StVe1* gene in transgenic potato clones, resulting in enhanced resistance to *V. dahliae*. A molecular marker was developed by Bae et al. (2008a) using the sequence information from *Ve* orthologs and was found to be effective for selection of the gene interspecific wild *Solanum* species breeding populations.

Breeding programs face many challenges when selecting for resistance to VW, most likely due to the inability to effectively identify resistant clones (Halterman et al. 2009). The methods for quantifying the disease vary, are difficult to perform as high-throughput screens, and may vary due to the environment (Halterman et al. 2008). Breeding progress has been made using unilateral sexual polyploidization (Frost et al. 2006). Similarly, a high proportion of high-yielding, VW-resistant clones are expected if diploids (2x) with high yields and VW resistance are used in 4x-2x crosses (Bae et al. 2008b).

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REVIEW OF COLD INDUCED SWEETENING

Low temperature storage of potato tubers is advantageous in that it retards growth and spread of pathogens and delays sprouting. When tubers are stored at temperatures less than 10°C, however, they accumulate sucrose and reducing sugars (glucose and fructose) - a process known as cold-induced sweetening (CIS) (Dale and Bradshaw 2003; Sowokinos 2001a). CIS is a serious problem of the processing industry because it results in an unacceptable, dark, and bitter-tasting product when fried at high temperatures (Dale and Bradshaw 2003). The unacceptable dark color is dependent on the amount of reducing sugars, which undergo a non-enzymatic Maillard reaction at high temperatures (Talburt et al. 1975). In the food processing industry, the culinary quality of chips and French fries is determined by the amount of reducing sugars in the tuber (Brown et al. 1990; Kirkman 2007; Li et al. 2013; Mackay et al. 1990; Xiong et al. 2002).

The biochemical pathway of starch breakdown to sucrose and into reducing sugars is complex (Sowokinos 2001a; Blenskinsop et al. 2004), and many enzymes have been identified that are associated with this process (Richardson et al. 1990; Zrenner et al. 1993; Borovkov et al. 1996; Hill et al. 1996; Zrenner et al. 1996; Nielsen et al. 1997; Reimholz et al. 1997; Lorberth et al. 1998; Sowokinos 2001b; Kumar et al. 2004; McKenzie et al. 2005). During CIS, there is a net accumulation of sucrose, and some sucrose is transported to the vacuole where it is hydrolyzed to glucose and fructose (Isla et al. 1998) primarily by vacuolar acid invertase (Matsuura-Endo et al. 2004). There is a strong association between vacuolar acid invertase activity and the accumulation of reducing sugars in cold storage (Matsuura-Endo et al. 2004).

QTL have been identified for CIS resistance (Menéndez et al. 2002). Using a candidate gene approach, Menéndez et al. (2002) identified QTL for reducing sugars on five linkage groups. Douches and Freyre (1994) identified six QTL for chip color across four chromosomes (two, four, five, and ten).

Bradshaw et al. (2008) identified four QTL for chip color variation on four linkage groups. Chapter three of this dissertation identifies two QTL for chip color on two chromosomes (four and six). Li et al. (2005a) identified an association between chip color and DNA variation on one chromosome (nine) at the invertase locus *invGE/GF*. The locus consists of invertase genes *invGE* and *invGF* and colocalizes with cold-sweetening QTL *Sug9* (Li et al. 2005). However, the *invGE*/*GF* locus explains only one of about twenty cold-sweetening QTL (Li et al. 2005; Menendez et al. 2002).

Resistance to CIS is at least partially attributed to the vacuolar invertase inhibitor (*INH2*) gene, which has post-translation regulation of acid invertase (Mackenzie et al. 2013). Overexpression of *INH2* during cold storage in CIS-susceptible potato clones reduced acid invertase activity (Mackenzie et al. 2013). The *Vlnv* gene plays a key role in CIS, and can be controlled effectively by suppressing the gene through targeted breeding or biotechnology (Bhaskar et al. 2010). Li et al. (2013) demonstrated that combinations of two or three marker alleles significantly improved average chip quality after cold storage. The most reproducible markers for improving average chip quality after cold storage are Pain1-8c or $Pain1_{prom}$-d/e (Li et al. 2013).

Breeding progress has been made toward developing clones that accumulate low amounts of reducing sugars in cold storage (Love et al. 1998; Hamernik et al. 2009). However, no widely used cultivars fail to undergo CIS when stored at 0 to 4°C. Accessions of the wild potato species (*S. raphanifolium* and *S. chacoense*) show promise as a source of resistance to CIS (Hamernik 1998; McCann et al. 2010). Exploring the natural variation in wild species for *Vlnv* expression and introgression into cultivars (Bhaskar et al. 2010) may be one way to accelerate breeding progress in the next century.

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CHAPTER 2

SAMPLING STRATEGIES FOR ASSESSING COMMON SCAB (*STREPTOMYCES SCABIES*) AND VERTICILLIUM WILT (*VERTICILLIUM DAHLIAE*) IN POTATO

ABSTRACT

Effective and efficient methods to assay for disease resistance in potato are crucial for breeding progress. Assays for Verticillium wilt (VW) and common scab (CS) are time-consuming processes. Thus, sampling strategies to reduce standard deviation of the mean of a clone while minimizing sample number were examined. To evaluate VW, seven standard cultivars were planted at Hancock, Wisconsin, in a field high in VW disease pressure. To evaluate CS, three standard cultivars and four breeding lines were planted at Antigo, WI in a field high in CS disease pressure. Both VW and CS were planted in replicated trials and assessed for two years. Stem-to-stem variability for VW was higher than other tested factors in most clones for both years. A collection of nine to eleven stems from seven to eight plants reduces the standard deviation of the estimated CFU mean of a clone by 75%. This finding is further supported by resource allocation calculations, which determined that a total of twelve stems should be collected, with one stem per plant sampled from four plants in each of three blocks. The use of all stems in the analysis resulted in a higher coefficient of variance (CV) and percent variability not explained by the model than the use of the four most diseased stems per block. Thus, a sampling strategy that considers the four most diseased stems may be desirable for a lower CV and percent variability explained by the model in order to have a higher probability of detecting differences among clones. However, the ranking of clones may be different from the ranking of clones from a model that considers all stems. Moreover, diseased stems can only be observed in hindsight. For CS, tuber-to-tuber variation within a plot was higher than other parameters. However, unlike VW, significant differences were detected among clones. Analysis of seven to eight plots per clone using the average of the four most diseased tubers per plot reduces the standard deviation for the estimated mean of disease response (PSA or LT) of a clone by 75%. The CV and percent variability not explained by the model were lowest when the four most diseased tubers per plot were considered. The four most

diseased tubers are easily observed and require less time to score than all tubers in a plot. This strategy will likely result in clonal rankings similar to those based on all tubers in the plot.

INTRODUCTION

Breeders strive to conserve resources by identifying efficient methods to effectively screen for resistance to plant diseases. In addition, they must distinguish among test individuals for disease resistance responses. Individuals are typically identified by measuring the phenotype, which is the product of environmental and genetic effects (Bernardo 2010). In order to carry out genetic studies on traits such as disease resistance or improved quality, large and segregating populations are needed to characterize genetic ratios and identify favorable clones (Little 1945; Jansky 2009a). In tetraploids, segregation ratios for qualitative traits cannot be precisely predicted because they depend on the crossover frequency between the gene and the centromere, which may be environmentally variable (Jansky 2009a). In addition, genetic studies are difficult to carry out on quantitative traits that are difficult to precisely measure, such as resistance to scab in potato (Krantz and Eide 1941; Little 1945). Variable data can also result from environmental dynamics (Frost 2005; Frost et al. 2007). A phenotyping protocol that provides an accurate reflection of the genotype is desired. This information allows breeders to classify or rank genotypes for the measured response variable for the trait of interest.

Potato breeding programs focus on developing cultivars with disease resistance (Jansky, 2009a). Breeding programs evaluate tens of thousands of clones per year, and then select only a small proportion of clones for evaluation in replicated trials in subsequent years (Jansky 2009a; Louwes and Neele 1987). The potato diseases common scab (CS) and Verticillium wilt (VW) both result in marketable yield loss in potato (*Solanum tuberosum*). They are caused mainly by the soilborne

bacterium *Streptomyces scabies* and the soilborne fungus *Verticillium dahliae*, respectively (Agrios 1997). Methods for measuring CS include categorical and continuous scales that assess tuber surface area affected, lesion type, and percent infected tubers in greenhouse assays or field trials (Calgari and Wastie 1985; Driscoll et al. 2009; Jansky and Rouse 2003; Khatri et al. 2011; Haynes et al. 1997; Wanner and Haynes 2009).

VW may be quantified based on symptom expression or measurement of pathogen populations in host tissue (Akeley et al. 1956; Davis et al. 1983; Hoyos et al. 1991; Hunter et al. 1968; Frost et al. 2007; Jansky and Rouse 2003). The latter method more accurately explains yield loss due to VW (Frost et al. 2007). When measuring pathogen levels in host stems, it is common to find variability among plants and stems of the same clone (Frost 2004; Jansky and Rouse 2000; Slattery 1981). Previous studies have identified a large range in the mean levels of conidia in sap of susceptible clones (Dan et al. 2001; Frost et al. 2007; Jansky and Rouse 2000; Jansky 2009b). Therefore, mean colony counts may not effectively distinguish differences among susceptible clones (Jansky 2009b).

Despite the abundant literature for CS and VW resistance evaluations, the recommended number of experimental units has not yet been reported. The objective of this research was to determine the most effective method to score an individual genotype for CS and VW. Methods of data collection for VW include analysis with all stems, the four most diseased stems, the mean of all stems per block, the median of all stems per block, and the mean of the four most diseased stems per block. Methods of data collection for CS include all tubers per plot, the four most diseased tubers per plot, the mean of all tubers per plot, the median of all tubers per plot, and the mean of the four most diseased tubers per plot per trait [percent surface area (PSA) and lesion type (LT)].

For each of these methods, the following are considered: the relative amount of variation due random effects, including the variables block, block by clone, and error (represented by the covariance

parameter estimates); the standard deviation of the estimated sample mean for disease severity (using iterations based on data), coefficient of variance; percent variability not explained by the model; and resource allocation calculations (only for VW). Differences in clonal ranking with all samples will also be considered. The purpose of this research is to guide future field experiments. Recommendations are based on methods that make efficient use of resources in terms of sampling effort.

MATERIALS AND METHODS

CS Trials. Clones were evaluated in a randomized complete block design near Antigo, Wisconsin, in a field that provides high CS disease pressure. Eight clones varying in levels of susceptibility [524-8 (most resistant), Superior, Atlantic, Megachip, Scab1, Scab14, US-W4, and W2324-1 (most susceptible)] were placed in seven plots in each of the two blocks. Each plot consisted of two plants. The tubers from the plants within a plot were bulked together. The 2011 field was planted on May 25 and harvested on October 3. The 2012 field was planted on May 11 and harvested on October 2. All tubers from each plot were harvested by hand and transported to Madison, Wisconsin. The ten largest tubers from each plot were washed and scored for percent surface area (PSA) (on a scale of 0 to 100%, increments of 5%) and lesion type (LT) (0 = no lesions, 1 = superficial and small, 2 = superficial and coalescing, 3 = raised and small, 4 = raised and coalescing, 5 = pitted and small, 6 = pitted and coalescing). If a plot contained fewer than 10 tubers, then all tubers were washed and scored. Further details about the clones used for this trial and the VW trial are found in Table 1.

A diploid F2 breeding population was planted in the same field and evaluated in the same manner. The F2 population was developed from a cross between two randomly chosen F1s from a cross between diploids *S. tuberosum* US-W4 (susceptible to CS) and *S. chacoense* 524-8 (resistant to CS). This population was evaluated in another study which examined the genetic basis for resistance to CS

through quantitative trait loci (QTL) analysis. In the current study, this population was used to compare clonal rankings based on an average of all tubers from a plot with that from the average of the four most diseased tubers per plot. In 2011, two plots per F2 clone were planted with one in each of two blocks. In this trial, 51 clones were evaluated. In 2012, a total of six plots per F2 clone were planted in two blocks (totaling three plots per block). In this trial, the remaining 44 clones of the population, plus the 51 tested in 2011 were evaluated.

VW Trials. Clones were evaluated in a randomized, replicated trial near Hancock, Wisconsin, in a field that is managed for high VW disease pressure. Seven clones varying in levels of susceptibility [Ranger Russet (most resistant), Atlantic, Russet Burbank, Red Norland, White Pearl, Superior, and Russet Norkotah (highly susceptible)] were planted in four-hill units in three blocks. In 2011, the field was planted on May 4, and destructively sampled for stem sap collection on August 2. In 2012, the field was planted on May 8, and destructively sampled on August 7. The destructive stem sampling method provides one measure of resistance by determining the number of *Verticillium* colonies within stem sap. Each basal stem piece (10 cm in length) was surface disinfested, and squeezed to collect 100 µl sap, which was plated on a selective medium, incubated in the dark for two weeks, and evaluated for number of Verticillium colonies. Up to four stems per plant were evaluated.

Data Analysis. Analyses of variance (ANOVAs) were generated in SAS (ver. 9.2) using the mixed model procedure (SAS Institute Inc., Cary, NC), including the use of the pdmix800 package (Saxton, 1998). Restricted maximum likelihood (REML) was used to determine covariance parameter estimates, p-values for genotypes and LS means for the ANOVA. General linear models (GLM) determined type I sums of squares, which were used to calculate the percent variability not explained by the model and the coefficient of variance. Models for VW were run with all stems, the four most diseased stems per block, the average of all stems per block, the median of all stems per block, and the average of the four most

diseased stems per block per genotype. Models for CS were run with all tubers from a plot, the four most diseased tubers in a plot, the average of all tubers from a plot, the median of all tubers from a plot, and the average of the four most diseased tubers in a plot. Analysis for each CS phenotype (PSA and LT) was conducted separately. Data quality checks, including the distribution of residuals, were also performed in SAS. All other analyses, including iterations, Spearman rank correlations, and Pearson correlations were generated in R (ver. 2.15.2). Correlation values were calculated with the transformed LS means from the ANOVA. Genotype was fixed for the mixed procedure. Block, block by genotype, and plant nested within block by genotype were considered to be random variables. The numbers of *Verticillium* colonies in sap and the PSA for CS were square root transformed to meet normality assumptions. In the tables depicting LS means of the colony counts (VW) and PSA (CS), the LS means of untransformed data are presented.

In the VW data set, 24 randomly selected stems within a clone and year were run with 1000 iterations to determine the number of stems to sample to reduce the standard deviation of the estimated colony forming unit (CFU) count mean of a clone by 75% clone within a year. The iterations were performed by taking a subsample of the 24 stems for one through 24 stems and obtaining an average number of CFU per iteration. Mean CFU and standard deviation of the estimated means were obtained from the 1000 iterations for each number of stems tested. In 2012, White Pearl was excluded from analysis because it did not produce 24 stems. Similarly, the number of plants required to reduce the standard deviation of a clone within a year was determined by averaging the colony counts of stems collected from a plant, and then performing iterations for the twelve plants of each genotype and year. In 2012, Atlantic, Russet Norkotah, Red Norland, and White Pearl had less than twelve plants and were not used in this analysis.

The CS data set was run with 500 iterations within a trait and year to determine the number of plots required to reduce the standard deviation of the estimated mean of PSA or LT of a clone by 75%. The iterations were run using the plot mean of the four most diseased tubers from one to thirteen plots. A mean of a subsample of randomly selected plots per iteration was obtained. A mean (and standard deviation) was then obtained for each number of tested plots for each clone within a year. In 2011 and 2012, genotype 524-8 had less than thirteen plots of data and was excluded from this analysis.

Resource allocation calculations for VW were used to determine the number of plants and blocks from which the stems should be sampled that result in the lowest variance. Variance components from the ANOVA were used as the numerator for the calculations. The denominator values are the number of blocks, stems, and plants to test. The equation is as follows:

$$var = \frac{\sigma^2\ of\ error}{stems * plants * blocks} + \frac{\sigma^2\ of\ plants(block * clone)}{plants * blocks} + \frac{\sigma^2\ of\ block * clone}{blocks}$$

Combinations of stems per plant, plants per block, and number of blocks were tested such as that the total number stems ranged between five and twelve. This parameter was set based on the results of number of stems to sample in the iterations described previously.

RESULTS

***2011 and 2012 Verticllium*.**

Overall Analyses. *Verticillium* colony sap means were higher in 2011 than 2012, whether all stems or the four most diseased stems were used in the analysis (Tables 2 to 3). No significant differences ($p < 0.05$) were detected among clones for 2011 or 2012. In all models for 2011 except for the model that considers the average of all stems per block, White Pearl had the highest average number of colonies. In 2012, White Pearl was the highest for the models that consider all stems, average of all stems per block, and the median of all stems per block. Superior had the highest average number of colonies for all

models considering the four most diseased stems. The lowest colony counts for 2011 were observed in Ranger Russet for all models. In 2012, the lowest colony counts were in Atlantic for the models that consider all stems, the average of all stems per block, and the median of all stems per block. In models with the four most diseased stems, Red Norland had the lowest colony counts. The most diseased stems did not tend to come from a particular plant of a clone in either 2011 or 2012 (data not shown).

Ranking of clones among models. The ranking of clones in 2011 was similar ($p < 0.05$; rho = 0.82 to 0.96) among models that considered all stems, the four most diseased stems, the median of all stems per block, and the average of all stems per block (Tables 2 to 4). Clone ranking based on a model that considered the average of all stems per block was different than rankings based on other models. In 2012, clone rankings were the same ($p < 0.05$, rho = 0.96 to 1.00) among models based on individual stems, the average of all stems per block, and the median of all stems per block. Clone rankings were the same ($p < 0.05$, rho = 0.76 to 0.96) among models that considered the four most diseased stems and average of the four most diseased stems per block. The clone ranking was not the same ($p > 0.05$) between years.

Sources of variation. Mean and median scores based on all stems differed from those based on the four most diseased stems per block for relative amounts of variation, as reflected in the covariance parameter estimates. The relative amounts of variation also differed from year to year (Tables 2 and 3). The stem-to-stem variation (error term) was higher for most clones than for any other source of variation in both 2011 and 2012. The next highest source of variation was from plant to plant when considering all stems in the model. Conversely, when the four most diseased stems per block per genotype were used in the model, the block by genotype interaction was the next highest source of variation.

The error variation due to genotype differed between years and whether all or the four most diseased stems were considered (Tables 2 and 3). The extreme clones maintained similar rankings across years and sampling strategies (all stems versus the four most diseased stems). Considering all stems in the model, White Pearl had the highest and Ranger Russet the lowest amount of stem-to-stem plus unknown variation in 2011. In 2011, considering either all stems or the four most diseased stems per block per clone, the clone with the most stem-to-stem variation was White Pearl and the least was Ranger Russet. In 2012, considering the four most diseased stems, Russet Burbank had the most stem-to-stem variation, while Russet Norkotah had the lowest. Considering all stems, Superior had the highest stem-to-stem variation and White Pearl had the lowest.

Coefficient of variance (CV) and percent variability. Coefficient of variance (CV) values and percent variability not explained by the model were highest for the sampling strategy using all stems in the analysis for both years as compared to the analysis with the four most diseased stems (Tables 4 and 5). The highest CV among the mean of all stems, the mean of the four most diseased stems, and the median per block was the median of all stems per block for both years (Tables 4 and 5). The lowest CV was observed when the mean of the four most diseased tubers per block was used. The highest percent variability not explained by the model was the analysis with the mean of all stems per block in 2011 and the mean of the four most diseased stems per block in 2012. The lowest percent variability not explained by the model was the analysis with the mean of the four most diseased stems per block in 2011 and the median of all stems per block in 2012.

Minimization of standard deviation around the mean for stems. As the number of stems assayed increased, the estimated standard deviation around the mean of the iterations decreased for all clones (Figures 1 and 2). Overall, the standard deviation when nine to eleven stems were sampled was reduced by 75% compared to that when only one stem was sampled (Table 4). In 2011, ten stems from Atlantic,

Superior, Russet Norkotah, Ranger Russet, White Pearl, and Red Norland, and eleven from Russet Burbank reduced the standard deviation by 75%. In 2012, nine stems from Russet Burbank, ten stems from Atlantic, Ranger Russet, Red Norland, and Russet Norkotah, and eleven stems from Superior reduced the standard deviation by 75%.

Minimization of standard deviation around the mean for plants. As the number of plants assayed increased, the standard deviation around the mean of the iterations decreased for all clones (Figure 3 and 4). Seven or eight plants (all assayed stems were averaged by plant) reduced the standard deviation by 75%. Seven plants of Red Norland and White Pearl, and eight plants of Atlantic, Ranger Russet, Russet Burbank, Russet Norkotah, and Superior reduced the standard deviation by 75% in 2011. In 2012, eight plants of Ranger Russet, Russet Burbank, and Superior reduced the standard deviation by 75%.

Resource allocation. The variance for 2011 and 2012 was lowest when one stem per plant was collected from four plants in each of three blocks (Table 6).

***2011 and 2012 Scab*.**

Overall Analyses. Significant differences were detected among genotypes under all tested models ($p < 0.05$) (Tables 7 to 9). In both years, LS means were higher in models where the four most diseased tubers per clone per plot were used than in those using all tubers. Within a trait and year, the LS means for clones were similar whether all tubers, the mean of all tubers or the median of all tubers were used in the model. PSA was higher in 2012 than 2011. LT score was higher in 2012 for models that included the four most diseased tubers, the median of all tubers, and the mean of the four most diseased tubers. LT was higher in 2011 for models with all tubers and the mean of all tubers. In 2011, W2324-1 had the highest PSA and LT LS mean value among the tested genotypes. In 2012, Scab1 had the highest PSA LS

mean value, and W2324-1 had the highest LT value. However, Scab1 and W2324-1 were not significantly different from each other for all models in either year for PSA. Genotype 524-8 had the lowest PSA and LT LS mean value in both years for both traits.

Ranking of clones among models. Ranking of the LS means of clones for PSA and LT was similar in 2011 for all combinations of PSA and LT (analysis with all tubers, the four most diseased tubers, the mean of all tubers per plot, the median of all tubers per plot, and the mean of the four most diseased tubers per plot) (rho = 0.79 to 0.81, $p < 0.05$) (Tables 7 to 9). However, rankings of clones for PSA were different than those for LT in 2012 (rho = 0.24 to 0.40, $p > 0.05$). Within a trait (PSA or LT) and year, the rank was similar (rho = 0.83 to 1.00, $p < 0.05$) for all combinations. Ranking of clones for PSA in 2011 was similar to that in 2012 for all combinations (rho = 0.83 to 0.90, $p < 0.05$). For LT, ranking between 2011 and 2012 was significant for all combinations of PSA and LT (rho = 0.76 to 0.86, $p < 0.05$). The only exception was the analysis of LT with all tubers; the clones were not ranked the same as in 2011 (rho = 0.62 to 0.64, $p > 0.05$). The Pearson correlation coefficient between PSA and LT of a particular model and year was 0.75 to 0.82 ($p = 0.01$ to 0.03).

The similarity of clonal ranking between the average of all tubers from a plot and the four most diseased tubers from a plot was tested in two different years using an F2 breeding population segregating for resistance to common scab. For each trait (PSA and LT) and year, the rankings of the genotypes were similar when the four most diseased tubers were averaged per plot or the average of all tubers per plot was used (rho = 0.94 to 0.98, $p < 0.0001$).

Sources of variation - PSA. Variance due to block and block by clone was negligible compared to that due to a plot (Table 7). When all tubers were considered in the model, there was more tuber-to-tuber variation than plot-to-plot variation for five (2011) and six (2012) of the tested clones. When the four most diseased tubers were considered in the model, there was only one clone (Scab14) in one year

(2012) that had higher tuber-to-tuber variation than plot-to-plot variation. The remainder of the clones in 2011 and 2012 had a residual estimate less than the plot covariance parameter estimate, indicating higher plot-to-plot variation.

Some clones had higher levels of residual variation (tuber-to-tuber variation within a plot) than others (Table 7). For both 2011 and 2012, the susceptible clone US-W4 had the highest tuber-to-tuber variation and the resistant clone 524-8 the lowest when all tubers were considered. When the four most diseased tubers per plot were considered, the genotype with the highest residual variation was Scab14 in both years. In 2011 and 2012, Megachip and 524-8, respectively, had the lowest residual variation.

The models that examined the mean of all tubers, the median of all tubers, or the mean of the four most diseased tubers per plot had residual variation that represents the plot-to-plot variation (Table 7). No clone had consistently high or low plot-to-plot variation.

Sources of variation – LT. LT variance due to block and block by clone was negligible compared to variance due to plot (Table 8). Tuber-to-tuber variation (residual variation) for all clones was higher than plot-to-plot variation in both 2011 and 2012 when all tubers were considered in the model. In both years, when the four most diseased tubers were considered in the model, five clones for 2011 and 2012 had more tuber-to-tuber variation than plot-to-plot variation.

For the models that examined the mean of all tubers, the median of all tubers, or the mean of the four most diseased tubers per plot, US-W4 had the highest residual variation. This clone contributed more plot-to-plot variation than all other tested clones for this trait. There was no single clone that consistently had the lowest residual variation.

Coefficient of variance (CV) and percent variability. The lowest coefficient of variance for both years and traits was obtained from the model that considered the four most diseased tubers, followed by the

one based on the average of the four most diseased tubers per plot (Tables 9 and 10). The highest values were in the models based on the mean or median of all tubers.

For both years and traits, the lowest percent variability not explained by the model occurred when scores for the four most diseased tubers were averaged. Depending on the year and trait, the poorest fit to the model occurred in analyses based on the mean or median of all tubers in a plot.

Minimization of standard deviation around the mean for plots. For all tested clones, the standard deviation of the mean of the iterations decreased as the number of plots increased (Figures 5 to 8). Seven to eight plots were needed to reduce the single plot standard deviation by 75% (Table 10). For 2011 PSA, seven plots were needed for Atlantic, and eight plots were needed for Megachip, Scab1, Scab14, Superior, US-W4, and W2324-1. In 2012, eight plots were needed for all clones to effectively reduce the standard deviation.

In 2011 LT, seven plots were needed for Scab14 and W2324-1, and eight plots were needed for Atlantic, Megachip, Scab1, Superior, and US-W4 reduce the standard deviation by 75% from the highest standard deviation value of a clone. In 2012, seven plots from Superior and eight plots from Atlantic, Megachip, Scab1, Scab14, Superior, US-W4, and W2324-1 are needed to reduce the standard deviation by 75%.

DISCUSSION

Effective resource allocation in screening for VW and CS allows breeding programs to make progress toward developing resistant germplasm and varieties. Identifying assay methods that reduce the standard deviation of mean of a clone yet minimize the number of samples to collect contributes to this goal. The most effective ways to score for VW and CS were evaluated in this study by taking into

consideration the sources of variation, coefficient of variance (CV) values, and percent variability not explained by the model.

***Using the most diseased samples compared to all samples*.** Not surprisingly, using data from only the most diseased samples results in higher LS means for both VW and CS (Tables 2 and 3; Tables 8 and 9). The results for clonal ranking among models were not consistent for VW. In 2011, the clonal ranking was interchangeable for analyses with all stems, the four most diseased stems per block, the mean of the four most diseased stems per block, and the median of all stems per block. The only exception was using the mean of all stems per block (Table 4). In 2012, the ranking of the genotypes was similar when considering all stems, the mean of all stems per block, and the median of all stems per block. The same ranking was not obtained by taking the four most diseased stems per block or the mean of the four most diseased stems per block (Table 4). This discrepancy may be explained in part by the observation that between-year repeatability declines for late season samples (Frost 2004). However, for VW, collecting only the most diseased stems may not be practical to implement, since the most diseased are only recognized in hindsight, after the sap has been extracted and culture plates read.

In contrast, the rankings of clones for a trait within a year were not significantly different from each other for CS in both 2011 and 2012 (Table 10). Therefore, one could analyze the data with the four most diseased tubers, an average of the four most diseased tubers, all tubers, an average of the four most diseased tubers, or the median of all tubers per plot and obtain a similar clonal ranking. As a high throughput method, the four most diseased tubers per plot for each trait may be scored. Moreover, in the F2 breeding population there was also a similarity in clonal ranking when using either the average score of all tubers from a plot or the four most diseased tubers per plot in each of two years. The Pearson correlation between PSA and LT is strong and significant for both years ($r = 0.8$). Other studies have reported similar correlations (Bjor and Roer 1980; Lambert et al. 2006; Loria 1981). Leach et al.

(1938) also noted the trend that the lower the LT, the smaller the PSA, yet acknowledged that exceptions occur. This is similar to the observed differences between PSA and LT in clonal ranking for a particular year (Table 8 and 9). Despite the high correlation between PSA and LT, Jansky and Rouse (2003) reported a low, yet significant correlation between PSA of scab and percent of tubers with scab lesions and suggest that the traits should be scored separately. Goth et al. (1993) suggest the use of cluster analysis that combines PSA and LT to compare advanced breeding lines to standard cultivars.

***Sources of variation and detection of significant differences among clones*.** As illustrated by the covariance parameter estimates (Tables 2 and 3), the highest source of variation for VW was generally stem-to-stem (residual values). The residual variation values were generally substantially higher than the other sources of variation. This concurs with other studies that have reported variability among plants and stems of a clone (Frost 2004; Jansky 2009b; Jansky and Rouse 2000; Slattery 1981). Due to this variability, Jansky (2009b) suggested that clones cannot be distinguished using mean colony counts. In the present study, significant differences were not detected among clones. This is likely because the stem-to-stem variation was high enough to mask any significant differences among clones. Significant differences were detected among clones in other studies, but may be due to the germplasm being tested (Jansky and Rouse 2000, 2003). Some clones have higher variation than others (see residuals of the covariance parameter estimates), although there was no single clone that was the most or least variable from year to year, nor does variation appear to be consistently related to the susceptibility of a clone (Tables 2 and 3).

For CS, the largest source of variation was tuber-to-tuber when including all tubers in the analysis, and plot-to-plot for all other methods (Tables 8 and 9). Unlike VW, the presence of tuber-to-tuber or plot-to-plot variation still allowed for the detection of significant differences among clones, regardless of the method of analysis.

***Consideration of the coefficient of variance (CV) and percent variability not explained by the model*.**

For VW, the CV values and percent variability not explained by the model were lower for the analysis with the four most diseased stems than with all stems (Tables 4 and 5). Given the stem-to-stem variation observed, it is not surprising that selecting the four most diseased stems results in a reduction of the ratio of standard deviation (σ) to the mean (μ). The advantage of using the sampling strategy based on the four most diseased stems (lower CV and percent variability not explained by the model) is the higher likelihood of detecting significant differences among clones. Despite the relatively lower CV and percent variability not explained by the model values using the four most diseased stems per block, researchers still need to go through the process of collecting and processing samples to determine those that are most diseased. A future experiment that associates a relationship between the appearance of the stem in the field and the number of colonies extracted from it may allow for an efficient assay using the four most diseased stems per block. However, analyses with the four most diseased stems per block may result in differences in clonal ranking compared to those based on all stems (Tables 2 to 4). Although analysis with the four most diseased stems may be a method for breeders to consider when eliminating the most susceptible test clones, it may not provide insight on the interaction between the host and the pathogen. There are environmental dynamics not controlled by the genetic makeup of the potato clone that result in variable data (Frost 2005; Frost et al. 2007).

The CV and percent variability not explained by the model were lowest when using the four most diseased tubers per plot for both scab phenotypes, PSA and LT (Tables 10 and 11). Therefore, this is the most favorable method to quantify scab. Analysis of the four most diseased tubers per plot can be based on either individual tubers in the data set (totaling four data points per plot), or an average of the four most diseased as the data point (totaling one data point per plot).

***Balancing standard deviation with number of experimental units*.** Researchers have an interest in minimizing the number of samples collected in order to maximize the output from resource expenditures. Therefore, an iteration based on true data was conducted. Based on this work for VW, it is recommended that nine to eleven stems be collected to reduce the standard deviation. Based on iterations with an average of all stems of a plant, seven to eight plants should be assayed. This is supported by the resource allocation calculations (Table 6). The recommended design, based on both 2011 and 2012 data is to collect one stem per plant from four plants in three blocks. A high-throughput way to screen for scab is to consider the four most diseased tubers per plot for each scab trait (PSA and LT). In this study, it was not difficult to accurately distinguish the most diseased for each trait at first glance when scoring. Based on the simulation from data in this study, it is recommended that seven to eight plots (with an average of the four most diseased tubers per plot) be assayed to reduce the standard deviation by 75%.

McCann et al. (2012) performed similar analyses to determine resource allocation for chip color, tuber sugar concentrations, and tuber dry matter. For these traits, the authors reported that variance of the clone genotypic means was reduced by limiting replications at a location and increasing the number of locations or years. Unreplicated trials are used for traits that are sensitive to genotype x environment interactions (Moreau et al. 2000; Brown and Glaz 2001).

CONCLUSIONS

Researchers strive to balance resources with data quality. This study provides insight on efficient methods to collect data for VW and CS. The recommendation to screen for VW is to collect one stem per plant from four plants in three blocks, totaling twelve stems. This method allows for reduced variation and the need to track only block and genotype when collecting in the field. Plant does not need

to be tracked because only one stem is gathered per plant. All stems should be used in the analysis, despite having an unfavorable goodness-of-fit (higher CV value and percent variability not explained by the model). It is favored over taking the four most diseased stems because diseased stems are only recognized in hindsight (after all stems have been assayed). However, selection may be most efficient for a breeder if only the most diseased stems are assayed. Future work to pursue increased efficiency may be to track the appearance of the disease when collecting the stem and identify a possible relationship between the appearance of the stem and the amount of *Verticillium* colonies extracted from the sap. It is interesting to note that relatively high or low stem-to-stem variability is not a consistent characteristic of one particular clone.

The recommendation to screen for CS is to evaluate five to eight plots using an average of the four most diseased tubers per plot per CS phenotype (PSA and LT). The CV and the percent variability not explained by the model were lowest when the four most diseased tubers per plot were considered, indicating a favorable goodness-of-fit.

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TABLES AND FIGURES

Table 1. *Description of clones used for the Verticillium wilt (VW) and Common Scab (CS) evaluation*.

Clones for Common Scab (CS) Evaluation						
Clone	**Ploidy**	**Species composition**	**Primary use**	**Maturity**	**Common Scab**	**Verticillium wilt**
Atlantic	4x	*S. tuberosum*	[2]fresh market; chipping	[3]mid-season	[3]tolerant	[3]tolerant
Megachip	4x	*S. tuberosum*	[4]chipping after storage	[4]medium-late	[4]greater resistance than Snowden	.
Superior	4x	*S. tuberosum*	[3]early fresh market	[3]medium-early	[3]resistance	[3]highly susceptible
W2324-1	4x	*S. tuberosum*	[5]breeding line	.	[5]susceptible	[5]tolerant
Scab1	2x	*S. tuberosum, S. chacoense*	[6]parent of F2 population	.	[6]moderately susceptible	.
Scab14	2x	*S. tuberosum, S. chacoense*	[6]parent of F2 population	.	[6]moderately susceptible	.
524-8	2x	*S. chacoense*	[6]breeding line	[6]late	[6]resistant	.
US-W4	2x	*S. tuberosum*	[6]breeding line	.	[6]susceptible	.
Clones for Verticillium Wilt (VW) Evaluation						
Clone	**Ploidy**	**Species composition**	**Primary use**	**Maturity**	**Common Scab**	**Verticillium wilt**
Atlantic	4x	*S. tuberosum*	[2]fresh market; chipping	[3]mid-season	[3]tolerant	[3]tolerant
Ranger Russet	4x	*S. tuberosum*	[3]fresh market; frozen processing	[3]full season	[3]susceptible	[3]more resistant than Russet Burbank
Russet Burbank	4x	*S. tuberosum*	[2]boiling, baking, chipping, French frying; proce	[1]late to very late	[1]moderately resistant	[1]susceptible
Russet Norkotah	4x	*S. tuberosum*	[3]fresh market	[3]early to medium	[3]some tolerance	[3]very susceptible
Red Norland	4x	*S. tuberosum*	[1]fresh market; boiling, French frying	[3]early	[1]moderately resistant	[1]susceptible
Superior	4x	*S. tuberosum*	[3]early fresh market	[3]medium-early	[3]resistant	[3]highly susceptible
White Pearl	4x	*S. tuberosum*	[2]chipping after long-term storage	[2]medium-late to late	.	.

[1]Canadian Food Inspection Agency

[2]Washington State University

[3]The Potato Association of America

[4]Groza et al. 2007

[5]Navarro et al. 2012

[6]Jansky SH, unpublished data

Table 2. *Analysis of Variance (ANOVA) output for* Verticillium *colony counts considering all stems.* The covariance parameter estimates, type three fixed effects and p-value, and Tukey's LS means estimates are based on REML. The covariance parameter estimates represent the amount of variation that is partitioned into the parameter. Each residual covariance parameter estimate is provided by clone in the left-most ANOVA and is the stem-to-stem variation per clone. Each LS mean from the ANOVA of untransformed data is in italics and is the average number of *Verticillium* colonies per stem. The LS means from the ANOVA with transformed data (square root) is to the right, followed by the rank of the clone (based on LS means); within a column and box, numbers followed by the same letter are not significantly different at p=0.05.

	Units in model	All Stems (with error term broken out by clone)				All Stems (with one value for the error term)				All stems, *averaged per block*				Median of stems, *averaged per block*			
2011	**Covariance Parameter Estimates**																
	Block		4				4				43				0		
	Block*Clone		38				25				.				.		
	Plant(Block*Clone)		160				173				.				.		
	Residual - All		.				199				146				98		
	Residual - Atlantic	184				.				.				.			
	Residual - Ranger Russet	69				.				.				.			
	Residaul - Red Norland	403				.				.				.			
	Residual - Russet Burbank	74				.				.				.			
	Residual - Russet Norkotah	222				.				.				.			
	Residual - Superior	230				.				.				.			
	Residual - White Pearl	348				.				.				.			
	Type 3 Fixed Effects: p-value, df																
	Clone	0.12	6,12			0.10	6,12			0.64	6,12			0.05	6,12		
	Tukey's LSmeans Estimates (estimates, rank, group)																
	White Pearl	*1748*	35	1	A	*1736*	35	1	A	*1130*	32	5	A	*1273*	35	1	A
	Atlantic	*1624*	34	2	A	*1605*	34	2	A	*1533*	37	2	A	*1057*	29	3	A
	Russet Norkotah	*1367*	32	3	A	*1362*	32	3	A	*1602*	37	1	A	*984*	30	2	A
	Russet Burbank	*1193*	30	4	A	*1198*	29	4	A	*1122*	33	4	A	*507*	22	5	A
	Superior	*1020*	25	5	A	*1022*	25	5	A	*1133*	33	3	A	*777*	28	4	A
	Red Norland	*857*	19	6	A	*866*	19	6	A	*786*	25	6	A	*123*	9	7	A
	Ranger Russet	*335*	12	7	A	*335*	13	7	A	*591*	21	7	A	*184*	11	6	A
2012	**Covariance Parameter Estimates**																
	Block		0				5				2				0		
	Block*Clone		38				17				.				.		
	Plant(Block*Clone)		48				63				.				.		
	Residual - All		.				219				83				94		
	Residual - Atlantic	190				.				.				.			
	Residual - Ranger Russet	232				.				.				.			
	Residaul - Red Norland	211				.				.				.			
	Residual - Russet Burbank	284				.				.				.			
	Residual - Russet Norkotah	158				.				.				.			
	Residual - Superior	288				.				.				.			
	Residual - White Pearl	72				.				.				.			
	Type 3 Fixed Effects: p-value, df																
	Clone	0.55	6,12			0.55	6,12			0.73	6,12			0.10	6,12		
	Tukey's LSmeans Estimates (estimates, rank, group)																
	White Pearl	*949*	29	1	A	*949*	29	1	A	*1080*	31	1	A	*1101*	32	1	A
	Atlantic	*548*	16	7	A	*548*	16	7	A	*507*	21	7	A	*48*	7	7	A
	Russet Norkotah	*903*	26	2	A	*903*	26	2	A	*925*	29	2	A	*814*	27	2	A
	Russet Burbank	*893*	25	3	A	*893*	25	3	A	*839*	29	3	A	*513*	22	3	A
	Superior	*836*	21	4	A	*836*	21	5	A	*887*	29	4	A	*548*	21	4	A
	Red Norland	*618*	18	6	A	*618*	18	6	A	*541*	21	6	A	*355*	16	5	A
	Ranger Russet	*642*	20	5	A	*641*	20	5	A	*649*	25	5	A	*190*	13	6	A

Table 3. *Analysis of Variance (ANOVA) output for* Verticillium *colony counts considering the four most diseased stems.* The covariance parameter estimates, type three fixed effects and p-value, and Tukey's LS means estimates are provided using REML. The covariance parameter estimates represent the amount of variation that is partitioned into the parameter. The residual covariance parameter estimates are broken out by clone in the left-most ANOVA and is the stem-to-stem variation per clone. Each LS mean from the ANOVA of untransformed data is in italics and is the average number of *Verticillium* colonies per stem. The LS means from the ANOVA with transformed data (square root) is to the right, followed by the rank of the clone (based on LS means); within a column and box, numbers followed by the same letter are not significantly different at p = 0.05.

	Units in model	Four most diseased stems per block (with error term broken out by clone)				Four most diseased stems per block (with one value for the error term)				Four most diseased stems per block, *averaged per block*			
2011	**Covariance Parameter Estimates**												
	Block		32				23				32		
	Block*Clone		76				46				.		
	Plant(Block*Clone)		20				87				.		
	Residual - All		.				119				110		
	Residual - Atlantic	215				.							
	Residual - Ranger Russet	13				.				.			
	Residaul - Red Norland	450				.				.			
	Residual - Russet Burbank	120				.				.			
	Residual - Russet Norkotah	29				.				.			
	Residual - Superior	74				.				.			
	Residual - White Pearl	187				.				.			
	Type 3 Fixed Effects: p-value, df												
	Clone	0.10	6,12			0.15	6,12			0.14	6,12		
	Tukey's LSmeans Estimates (estimates, rank, group)												
	White Pearl	*3019*	53	1	A	*3027*	53	1	A	*3015*	55	1	A
	Atlantic	*2487*	46	4	A	*2495*	46	4	A	*2487*	48	3	A
	Russet Norkotah	*2810*	51	2	A	*2783*	51	2	A	*2842*	52	2	A
	Russet Burbank	*1916*	41	5	A	*1934*	42	5	A	*1903*	42	5	A
	Superior	*2210*	46	3	A	*2202*	45	3	A	*2218*	47	4	A
	Red Norland	*1692*	36	6	A	*1724*	36	6	A	*1674*	41	6	A
	Ranger Russet	*884*	27	7	A	*859*	27	7	A	*894*	28	7	A
2012	**Covariance Parameter Estimates**												
	Block		26				30				42		
	Block*Clone		96				53				.		
	Plant(Block*Clone)		0				68				.		
	Residual - All		.				122				119		
	Residual - Atlantic	228				.				.			
	Residual - Ranger Russet	170				.				.			
	Residaul - Red Norland	169				.				.			
	Residual - Russet Burbank	275				.				.			
	Residual - Russet Norkotah	14				.				.			
	Residual - Superior	193				.				.			
	Residual - White Pearl	64				.				.			
	Type 3 Fixed Effects: p-value, df												
	Clone	0.68	6,12			0.57	6,12			0.59	6,12		
	Tukey's LSmeans Estimates (estimates, rank, group)												
	White Pearl	*855*	31	5	A	*751*	28	6	A	*1080*	31	5	A
	Atlantic	*1134*	29	6	A	*1134*	29	5	A	*1129*	31	6	A
	Russet Norkotah	*1535*	37	4	A	*1530*	37	4	A	*1453*	37	4	A
	Russet Burbank	*1936*	40	2	A	*1967*	41	2	A	*1912*	43	2	A
	Superior	*1971*	42	1	A	*1923*	41	1	A	*2062*	44	1	A
	Red Norland	*1224*	29	7	A	*1228*	30	7	A	*1185*	31	7	A
	Ranger Russet	*1625*	38	3	A	*1603*	38	3	A	*1584*	40	3	A

Table 4. *Summary table of statistical analyses for Verticillium Wilt (VW)*. Comparisons within a column should be made above or below the dotted line for the CV, percent variability not explained by the model, and variation source. Models with the same letter groups (a through c) indicate a similar clonal ranking ($p < 0.05$) as determined by a Spearman-rank test of the LS means output from the model.

	CV (2011, 2012)	Percent variability not explained by the model (2011, 2012)	Greatest variation source	Least variation source	Recommended number of stems	Recommended number of plants	Shared clonal ranking (p<0.05), 2011	Shared clonal ranking (p<0.05), 2012
All stems	54, 69	28, 49	stem	block	9 to 11	7 to 8	a	c
4 most diseased stems	25, 32	14, 16	stem	plant	.	.	a	d
Mean of all stems per block	39, 34	53, 67	.	.	.	.	b	c
Mean of the 4 most diseased stems per block	24,30	38, 70	.	.	.	.	a	d
Median of all stems per block	43, 52	40, 51	.	.	.	.	a	c

Table 5. *Analysis of Variance (ANOVA) GLM model output for* Verticillium *colony counts to determine coefficient of variance (CV) and percent variability not explained by the model*. The percent variability not explained by the model is calculated by taking the error divided by the total sum of squares, then multiplying by 100. A low CV and low percent variability not explained by the model is desired.

	Experimental Units	All stems		All stems, averaged per block		Median of stems, averaged per block		Four most diseased stems per block		Four most diseased stems per block, averaged per block	
2011	**Coefficient of Variance (CV)**	**54**		**39**		**43**		**25**		**24**	
	Type I SS; p-value										
	Block	2975	0.0007	896	0.084	152	0.49	2597	0.0002	667	0.086
	Genotype	13266	< 0.0001	631	0.64	1801	0.05	5714	< 0.0001	1351	0.14
	*Block*Genotype*	9643	< 0.0001	.	.	.	.	5574	0.0006	.	.
	*Plant(Block*Genotype)*	38354	< 0.0001	.	.	.	.	7407	0.02	.	.
	Error	24608	.	1748	.	1221	.	3579	.	1319	.
	Percent of variability not explained by the model	**28%**		**53%**		**38%**		**14%**		**40%**	
2012	**Coefficient of Variance (CV)**	**69**		**34**		**52**		**32**		**30**	
	Type I SS; p-value										
	Block	2824	0.002	191	0.35	88	0.66	2790	0.0003	824	0.07
	Genotype	3207	0.03	297	0.73	1296	0.13	2266	0.02	568	0.59
	*Block*Genotype*	6516	0.005	.	.	.	.	5616	0.002	.	.
	*Plant(Block*Genotype)*	19955	0.004	.	.	.	.	6935	0.10	.	.
	Error	27814	.	992	.	3272	.	3272	.	1431	.
	Percent of variability not explained by the model	**49%**		**67%**		**70%**		**16%**		**51%**	

Table 6. *Resource allocation based on variance components of 2011 and 2012 Verticillium wilt (VW) data*. Variance was calculated for different combinations of number of stems, plants, and blocks were tested for variance to determine the optimal resource allocation for future experiments. The final variance number is based on the variance components observed in Table 2.

	Stems to collect per plant	Number of plants within a block	Number of blocks	Total sampling Units	variance
2011	1	2	3	6	70
	1	3	3	9	50
	1	**4**	**3**	**12**	**39**
	2	1	3	6	99
	2	2	3	12	54
	2	2	2	8	81
	2	3	2	12	58
	1	6	1	6	87
	2	6	1	12	70
2012	1	2	3	6	53
	1	3	3	9	37
	1	**4**	**3**	**12**	**29**
	2	1	3	6	63
	2	2	3	12	34
	2	2	2	8	52
	2	3	2	12	37
	1	6	1	6	64
	2	6	1	12	46

Table 7. *Analysis of variance (ANOVA) output for Common Scab (CS), percent surface area (PSA)*. The analyses were conducted using restricted maximum likelihood (REML). The covariance parameter estimates represent the amount of variation that is partitioned into the parameter. The residual covariance parameter estimates are broken out by clone. The LS means from the ANOVA of untransformed data are in italics. The LS means from the ANOVA with transformed data (square root) are to the right, followed by the rank of the clone, then the letter group that indicates significant differences. "All tubers" indicates that all tubers from a plot where used in the analysis, "four most diseased tubers" indicates that the four most diseased tubers for PSA were used in the analysis. The "mean of all tubers", "median of all tubers" and "mean of the four most diseased tubers" indicates that the mean of all, median of all, or mean of the four most diseased tubers for PSA per plot were averaged, and then used in the analysis.

Trait		Percent Surface Area (PSA)															
	Experimental Units	All tubers				Four most diseased tubers				Mean of all tubers				Median of all tubers				Mean of the four most diseased tubers			
2011	**Covariance Parameter Estimates**																
	Block	0.0				0.0				0.0				0.1				0.0			
	Block*Clone	0.0				0.0				0.0				0.0				0.0			
	Plot(Block*Clone)	1.8				2.3				.				.				.			
	Residual - 524-8	0.8				0.5				2.8				2.3				3.4			
	Residual - Atlantic	1.8				0.9				3.1				3.9				3.3			
	Residual - Megachip	1.4				0.5				1.7				2.3				2.0			
	Residual - Scab1	3.0				1.1				3.3				3.5				3.6			
	Residual - Scab14	3.6				1.7				2.2				3.1				2.6			
	Residual - Superior	1.4				0.8				1.9				3.4				2.1			
	Residual - W2324-1	2.3				1.1				1.4				2.0				1.6			
	Residual - US-W4	4.4				1.3				2.3				3.4				3.5			
	Type 3 Fixed Effects: p-value, df																				
	Clone	0.0018	7,7			0.0011	7,7			0.0011	7,7			0.0018	7,7			0.0009	7,7		
	Tukey's LSmeans Estimates (estimates, rank, group)																				
	W2324-1	*40*	6.2	1	A	*56*	7.4	1	A	*40*	6.3	1	A	*38*	6.0	1	A	*56*	7.4	1	A
	Scab14	*36*	5.7	2	AB	*49*	6.8	2	AB	*36*	5.9	2	AB	*34*	5.6	2	AB	*49*	6.8	2	AB
	Scab1	*33*	5.4	3	ABC	*43*	6.3	3	AB	*34*	5.6	3	ABC	*31*	5.3	3	ABC	*43*	6.3	3	ABC
	US-W4	*28*	4.9	4	ABCD	*42*	6.3	4	AB	*28*	5.1	4	ABC	*27*	4.9	4	ABC	*42*	6.2	4	ABC
	Megachip	*13*	3.4	5	BCDE	*19*	4.3	5	BC	*13*	3.4	5	CD	*12*	3.2	5	BCD	*19*	4.1	6	CD
	Atlantic	*14*	3.3	6	CDE	*21*	4.2	6	BC	*14*	3.3	6	BCD	*12*	2.9	6	BCD	*21*	4.2	5	BCD
	Superior	*12*	3.1	7	DE	*15*	3.7	7	C	*12*	3.1	7	CD	*10*	2.7	7	CD	*15*	3.7	7	CD
	524-8	*5*	2.1	8	E	*6*	2.2	8	C	*5*	1.5	8	D	*4*	1.5	8	D	*6*	1.7	8	D
2012	**Covariance Parameter Estimates**																				
	Block	0.1				0.1				0.1				0.2				0.1			
	Block*Clone	0.0				0.1				0.0				0.0				0.1			
	Plot(Block*Clone)	1.1				1.6				.				.				.			
	Residual - 524-8	0.5				0.4				0.7				0.5				1.0			
	Residual - Atlantic	2.3				0.7				2.5				3.0				4.1			
	Residual - Megachip	1.6				0.8				1.7				2.0				2.2			
	Residual - Scab1	2.4				0.6				0.7				1.1				1.0			
	Residual - Scab14	3.0				1.8				2.1				2.5				1.3			
	Residual - Superior	0.8				0.5				0.6				1.6				0.6			
	Residual - W2324-1	2.6				0.5				2.1				2.8				2.9			
	Residual - US-W4	3.4				1.3				1.9				2.4				2.0			
	Type 3 Fixed Effects: p-value, df																				
	Clone	0.0001	7,7			0.0004	7,7			<0.0001	7,7			<0.0001	7,7			<0.0001	7,7		
	Tukey's LSmeans Estimates (estimates, rank, group)																				
	W2324-1	*28*	5.0	3	ABC	*42*	6.3	4	AB	*28*	5.1	4	AB	*29*	5.1	3	ABC	*42*	6.3	4	AB
	Scab14	*42*	6.4	2	AB	*48*	6.9	2	AB	*46*	6.6	2	A	*44*	6.5	2	AB	*49*	6.9	2	AB
	Scab1	*49*	6.9	1	A	*61*	7.8	1	A	*49*	6.9	1	A	*48*	6.9	1	A	*61*	7.8	1	A
	US-W4	*28*	5.0	4	ABC	*45*	6.6	3	AB	*28*	5.2	3	AB	*25*	4.9	4	ABC	*45*	6.6	3	AB
	Megachip	*21*	4.3	5	BC	*30*	5.2	5	BC	*21*	4.3	5	B	*20*	4.2	5	BC	*30*	5.2	5	B
	Atlantic	*18*	3.9	6	CD	*27*	4.9	6	BC	*18*	3.9	6	BC	*18*	3.8	6	CD	*27*	4.8	6	BC
	Superior	*5*	2.1	7	DE	*8*	2.9	7	CD	*5*	2.1	7	C	*4*	1.6	7	DE	*8*	2.7	7	CD
	524-8	*1*	1.2	8	E	*1*	1.4	8	D	*1*	0.5	8	D	*1*	0.4	8	E	*1*	0.6	8	D

Table 8. *Analysis of variance (ANOVA) output for Common Scab (CS), lesion type (LT)*. The analyses were conducted using restricted maximum likelihood (REML). The covariance parameter estimates represent the amount of variation that is partitioned into the parameter. The residual covariance parameter estimates are broken out by clone. The LS means from the ANOVA are on the left, followed by the rank of the clone, then the letter group that indicates significant differences. "All tubers" indicates that all tubers from a plot where used in the analysis, "four most diseased tubers" indicates that the four most diseased tubers for PSA were used in the analysis. The "mean of all tubers", "median of all tubers" and "mean of the four most diseased tubers" indicates that the mean of all, median of all, or mean of the four most diseased tubers for PSA per plot were averaged, and then used in the analysis.

Trait		Lesion Type (LT)														
	Experimental Units	All tubers			Four most diseased tubers			Mean of all tubers			Median of all tubers			Mean of the four most diseased tubers		
2011	**Covariance Parameter Estimates**															
	Block	0.0			0.0			0.0			0.0			0.0		
	Block*Clone	0.2			0.3			0.1			0.2			0.2		
	Plot(Block*Clone)	0.4			0.6			.			.			.		
	Residual - 524-8	0.7			0.8			0.6			0.6			0.7		
	Residual - Atlantic	2.0			1.7			0.8			1.3			0.9		
	Residual - Megachip	1.2			0.4			0.7			0.8			0.8		
	Residual - Scab1	1.0			0.5			0.4			0.3			1.0		
	Residual - Scab14	1.2			0.7			0.4			0.5			1.0		
	Residual - Superior	0.8			0.8			0.4			0.5			0.6		
	Residual - W2324-1	0.7			0.1			0.3			0.4			0.2		
	Residual - US-W4	2.4			0.6			1.7			2.3			1.1		
	Type 3 Fixed Effects: p-value, df															
	Clone	0.0066	7,7		0.0056	7,7		0.0010	7,7		0.0034	7,7		0.0026	7,7	
	Tukey's LSmeans Estimates (estimates, rank, group)															
	W2324-1	4.3	1	A	4.9	1	A	4.3	1	A	4.5	1	A	4.9	1	A
	Scab14	2.3	3	BC	2.9	3	ABC	2.3	3	BC	2.4	3	AB	2.9	3	ABC
	Scab1	1.8	5	B	2.3	7	CD	1.8	5	BC	1.5	6	B	2.3	6	BC
	US-W4	3.2	2	AB	4.1	2	BC	3.3	2	AB	3.4	2	AB	4.1	2	AB
	Megachip	2.0	4	BC	2.6	5	ABC	2.0	4	BC	2.3	4	AB	2.6	5	ABC
	Atlantic	1.8	6	BC	2.8	4	ABC	1.8	6	BC	1.7	5	B	2.8	4	ABC
	Superior	1.2	7	C	1.6	7	C	1.2	7	C	1.0	7	B	1.6	7	C
	524-8	0.8	8	C	0.9	8	C	0.8	8	C	0.8	8	B	0.8	8	C
2012	**Covariance Parameter Estimates**															
	Block	0.0			0.0			0.0			0.0			0.0		
	Block*Clone	0.0			0.0			0.0			0.0			0.0		
	Plot(Block*Clone)	0.2			0.4			.			.			.		
	Residual - 524-8	0.4			0.4			0.1			0.1			0.3		
	Residual - Atlantic	1.9			0.4			0.7			0.9			1.0		
	Residual - Megachip	0.8			0.3			0.1			0.1			0.1		
	Residual - Scab1	0.8			0.4			0.7			0.7			0.9		
	Residual - Scab14	0.9			0.6			0.3			0.4			0.4		
	Residual - Superior	1.6			1.2			0.3			0.9			0.4		
	Residual - W2324-1	1.7			0.1			0.2			0.2			0.0		
	Residual - US-W4	2.6			0.7			1.1			1.4			1.4		
	Type 3 Fixed Effects: p-value, df															
	Clone	0.0001	7,7		<0.0001	7,7		<0.0001	7,7		<0.0001	7,7		<0.0001	7,7	
	Tukey's LSmeans Estimates (estimates, rank, group)															
	W2324-1	4.2	1	A	5.0	1	A	4.2	1	A	4.6	1	A	5.0	1	A
	Scab14	2.9	6	AB	3.1	6	BC	2.9	4	B	3.0	5	B	3.1	6	BC
	Scab1	2.9	5	B	3.4	5	BC	2.9	6	B	2.8	6	B	3.4	5	BC
	US-W4	2.9	4	AB	4.3	2	AB	2.9	5	AB	3.0	4	AB	4.3	2	AB
	Megachip	3.1	3	AB	3.6	4	BC	3.1	3	B	3.3	3	B	3.6	4	B
	Atlantic	3.2	2	AB	4.0	3	AB	3.1	2	AB	3.3	2	AB	4.0	3	AB
	Superior	1.2	7	C	2.2	7	C	1.2	7	C	0.9	7	C	2.2	7	C
	524-8	0.2	8	C	0.3	8	D	0.2	8	D	0.1	8	C	0.3	8	D

Table 9. *Summary table of statistical analyses for Common Scab (CS)*. Models with the same letter groups (a through c) indicate a similar clonal ranking ($p < 0.05$) as determined by a Spearman-rank test of the LS means output from the model.

Percent Surface Area (PSA)	CV (2011, 2012)	Percent variability not explained by the model (2011, 2012)	Greatest variation source	Least variation source	Number of plots	Shared clonal ranking (p<0.05), 2011	Shared clonal ranking (p<0.05), 2012
All tubers	36, 33	38, 35	tuber	block	.	a	a
Four most diseased tubers	**19, 16**	**14, 11**	plot	block	.	a	a
Mean of all tubers	35, 27	45, 27	plot	block	.	a	a
Median of all tubers	43, 32	51, 30	plot	block	.	a	a
Mean of the 4 most diseased tubers	**32, 26**	**42, 28**	plot	block	7 to 8	a	a
Lesion Type (LT)							
All tubers	49, 43	39, 49	tuber	block	.	ab	c
Four most diseased tubers	**30, 20**	**20, 17**	plot	block	.	ab	bc
Mean of all tubers	36, 25	30, 26	plot	block	.	ab	bc
Median of all tubers	41, 28	30, 26	plot	block	.	ab	bc
Mean of the 4 most diseased tubers	**31, 22**	**30, 25**	plot	block	7 to 8	ab	bc

Table 10. *ANOVA GLM model output for Common Scab (CS) to determine coefficient of variance (CV) and percent variability not explained by the model*. The percent variability not explained by the model is calculated by taking the error divided by the total sum of squares, then multiplying by 100. A low CV and low percent variability not explained by the model is desired. "All tubers" indicates that all tubers from a plot were used in the analysis, "four most diseased tubers" indicates that the four most diseased tubers were used. The "mean of all tubers", "median of all tubers", and mean of the four most diseased tubers" indicates that the mean of all, median of all, or the mean of the four most diseased tubers per plot for each trait were averaged, and then used in the analysis.

	Trait	Percent Surface Area (PSA)									
	Experimental Units	All tubers per plot		Four most diseased tubers per plot		Mean of all tubers per plot		Median of all tubers per plot		Mean of the four most diseased tubers per plot	
2011	Coefficient of Variance (CV)	36		19		35		43		32	
	Type I SS; p-value										
	Block	0	0.80	1	0.44	1	0.61	4	0.27	0	0.85
	Clone	1247	< 0.0001	919	< 0.0001	225	< 0.0001	228	< 0.0001	302	< 0.0001
	Block*Clone	84	< 0.0001	76	< 0.0001	21	0.27	22	0.42	24	0.27
	Plot(Block*Clone)	1324	< 0.0001	888	< 0.0001	.	.	.	.	.	.
	Error	1630	.	297	.	203	.	267	.	240	.
	Percent variability not explained by the model	38%		14%		45%		51%		42%	
2012	Coefficient of Variance (CV)	33		16		27		32		26	
	Type I SS; p-value										
	Block	33	0.0001	7	0.003	6	0	9	0.04	6	0.07
	Genotype	1823	< 0.0001	1235	< 0.0001	355	< 0.0001	384	< 0.0001	405	< 0.0001
	Block*Genotype	91	< 0.0001	69	< 0.0001	14	0	19	0.25	20	0.18
	Plot(Block*Genotype)	1016	< 0.0001	609	< 0.0001	.	.	.	.	.	.
	Error	1582	.	231	.	138	.	179	.	171	.
	Percent variability not explained by the model	35%		11%		27%		30%		28%	
	Trait	Lesion Type (LT)									
	Experimental Units	All tubers per plot		Four most diseased tubers per plot		Mean of all tubers per plot		Median of all tubers per plot		Mean of the four most diseased tubers per plot	
2011	Coefficient of Variance (CV)	49		30		36		41		31	
	Type I SS; p-value										
	Block	3	0.16	2	0.14	0	0.49	0	0.67	1	0.26
	Clone	833	< 0.0001	523	< 0.0001	113	< 0.0001	140	< 0.0001	143	< 0.0001
	Block*Clone	133	< 0.0001	81	< 0.0001	16	0.002	26	0.0002	20	0.002
	Plot(Block*Clone)	399	< 0.0001	265	< 0.0001	.	.	.	.	.	.
	Error	872	.	214	.	57	.	72	.	69	.
	Percent variability not explained by the model	39%		20%		30%		30%		30%	
2012	Coefficient of Variance (CV)	43		20		25		28		22	
	Type I SS; p-value										
	Block	0	1	6	0	0	0.84	0	0.95	1	0.29
	Clone	755	< 0.0001	489	< 0.0001	109	< 0.0001	152	< 0.0001	141	< 0.0001
	Block*Clone	42	0	23	< 0.0001	4	0.24	5	0.34	5	0.20
	Plot(Block*Clone)	304	< 0.0001	172	< 0.0001	.	.	.	.	.	.
	Error	1076	.	142	.	39	.	55	.	48	.
	Percent variability not explained by the model	49%		17%		26%		26%		25%	

Figure 1. *Effect of number of stems sampled on standard deviations for* Verticillium *colony counts by stem, 2011.*

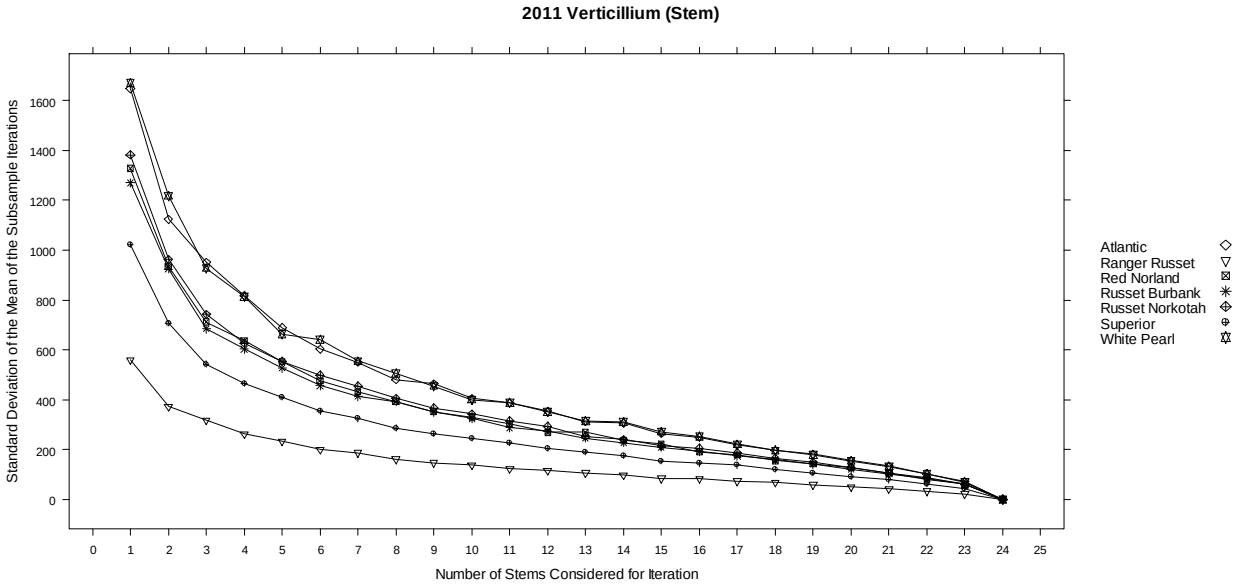


Figure 2. *Effect of number of stems sampled on standard deviations for* Verticillium *colony counts by stem, 2012.*

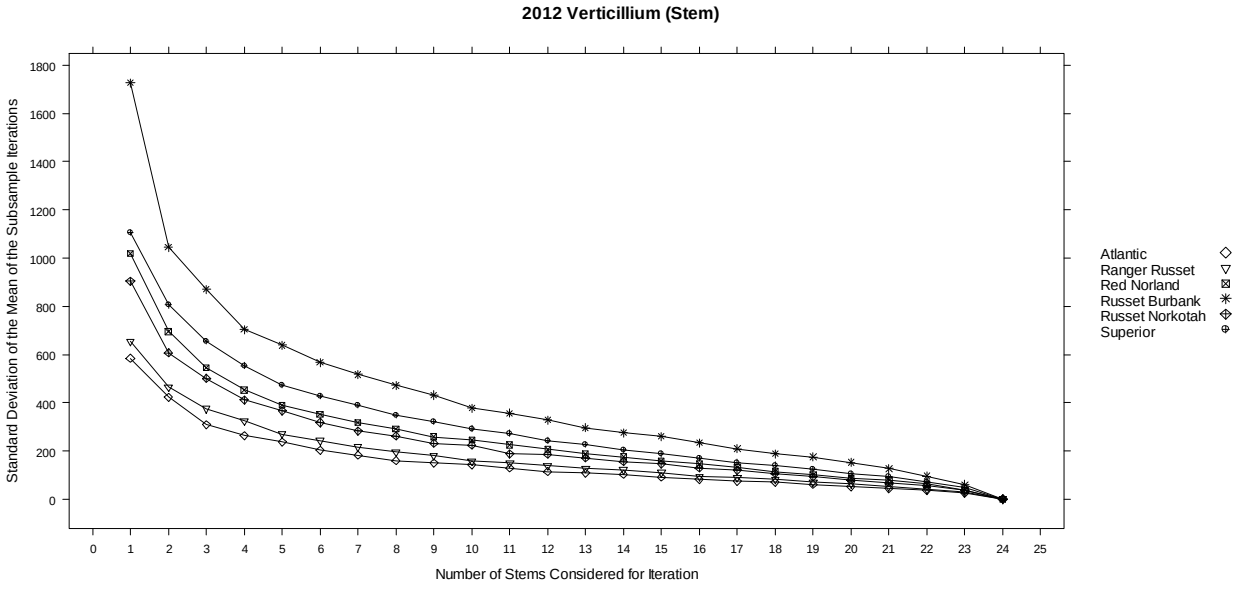


Figure 3. *Effect of number of stems sampled on standard deviations for* Verticillium *colony counts by plant, 2011.*

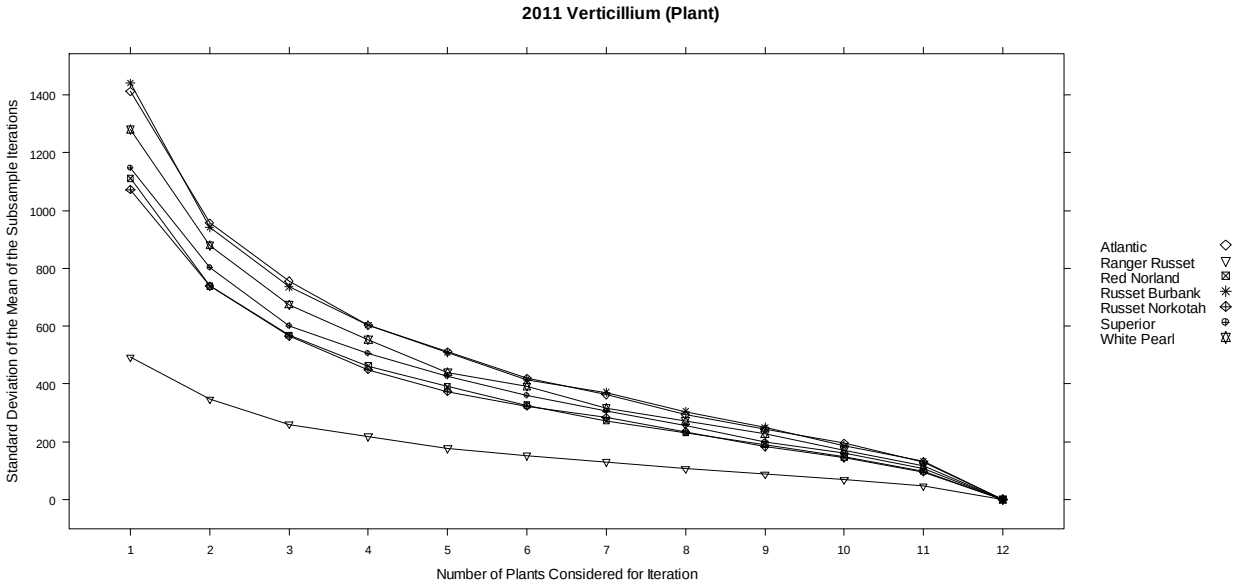


Figure 4. *Effect of number of stems sampled on standard deviations for* Verticillium *colony counts by plant, 2012.*

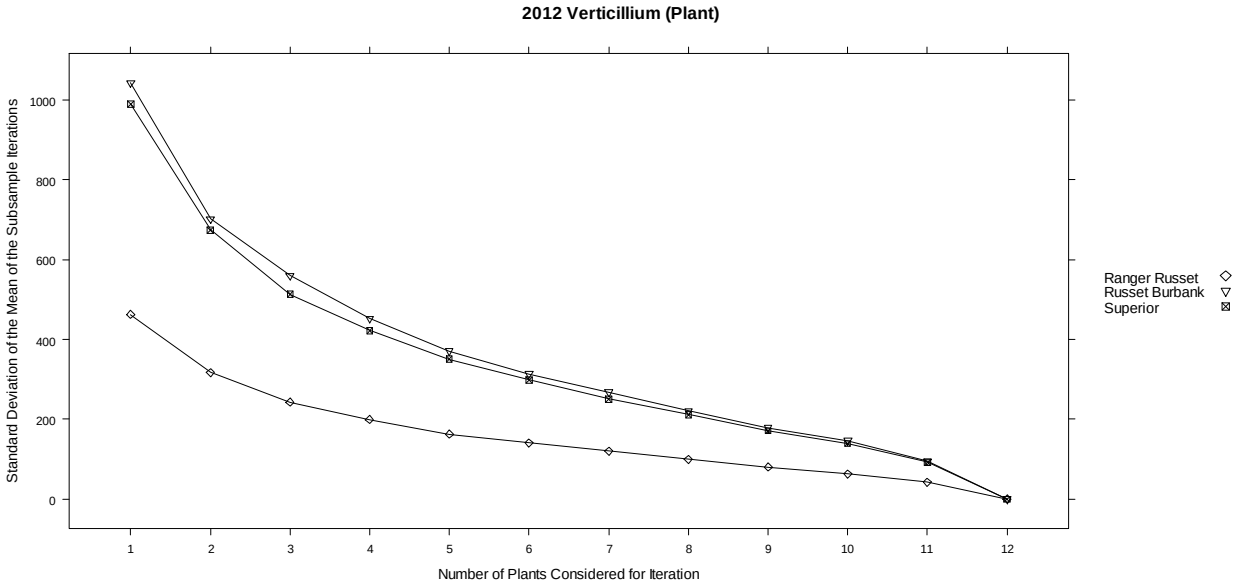


Figure 5. *Effect of number of plots sampled on standard deviations for Common Scab (CS), percent surface area (PSA) 2011*.

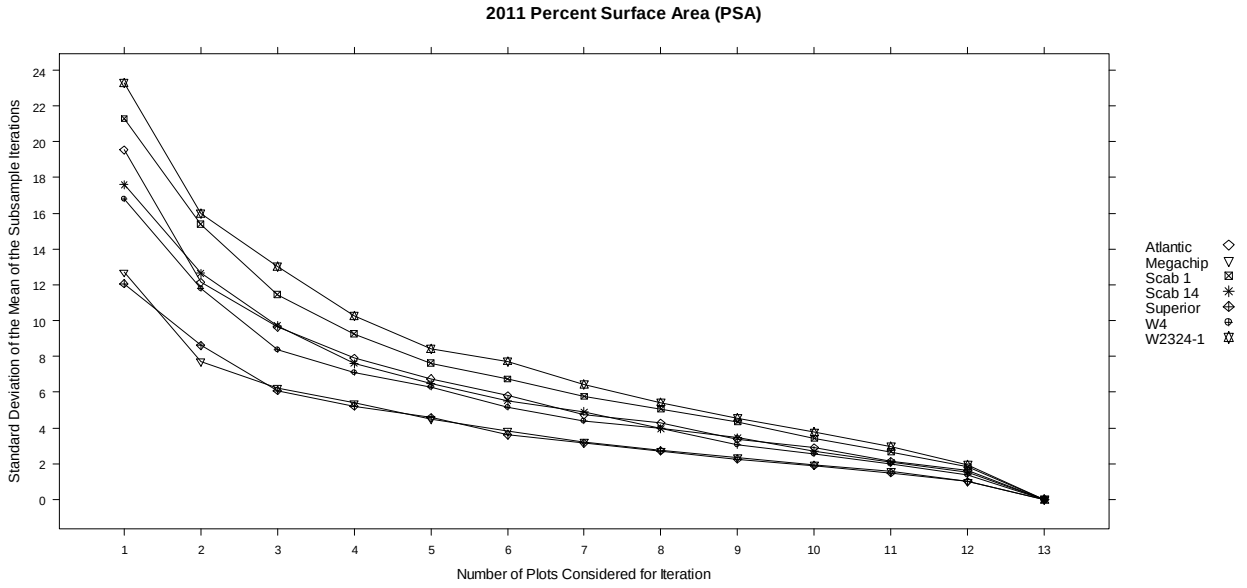


Figure 6. *Effect of number of plots sampled on standard deviations for Common Scab (CS), percent surface area (PSA), 2012*.

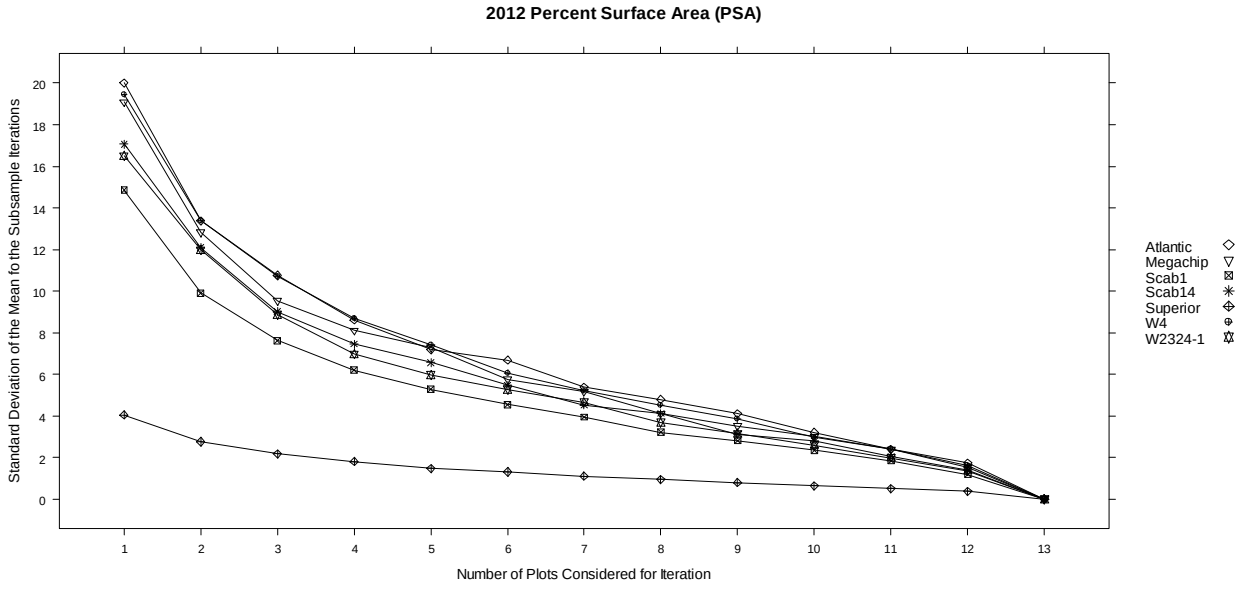


Figure 7. *Effect of number of plots sampled on standard deviations for Common Scab (CS), lesion type (LT) 2011*.

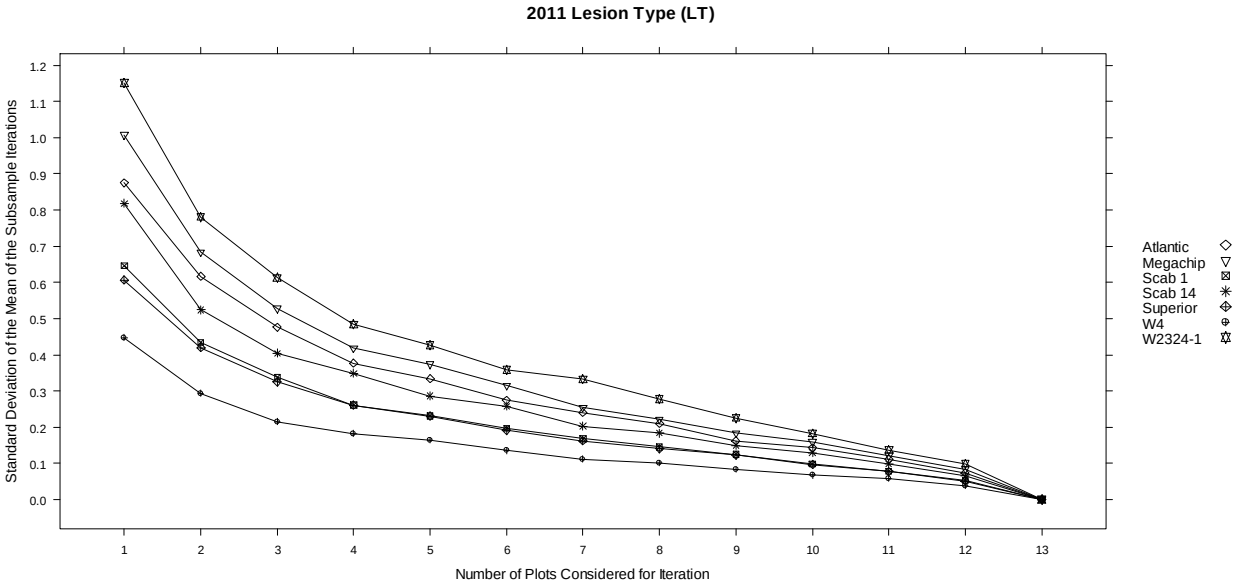
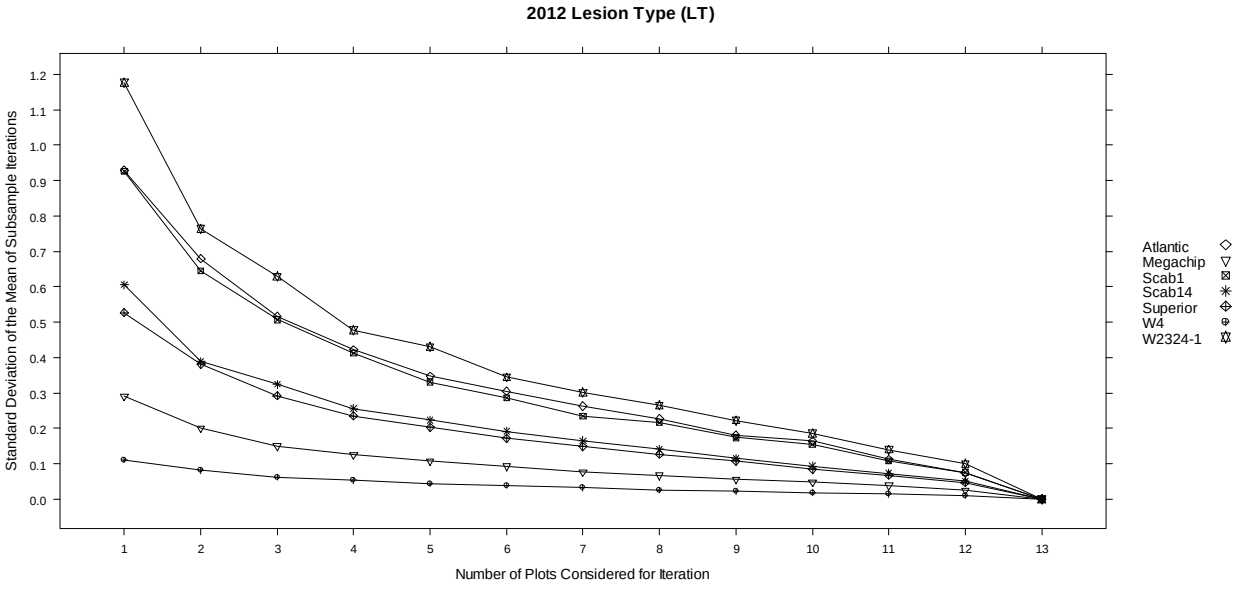


Figure 8. *Effect of number of plots sampled on standard deviations for Common Scab (CS), lesion type (LT) 2012*.



CHAPTER 3

MAJOR QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO COMMON SCAB AND COLD INDUCED SWEETENING IN DIPLOID POTATO

ABSTRACT

The development of germplasm with resistance to common scab (scab) is a high priority for potato breeders. A genetic map was constructed, quantitative trait loci (QTL) were identified, and gene action of significant QTL was characterized using 1311 single nucleotide polymorphisms (SNPs). The population used for the analysis was 98 diploid (2x) F2 progeny of two F1 progeny derived from the female *S. tuberosum* haploid (2x) parent US-W4 and male diploid *S. chacoense* parent 524-8. Progeny were evaluated in a greenhouse seedling trial (2008, 98 clones) and in replicated trials in Antigo, Wisconsin in a field high in scab disease pressure (2011, 51 clones; 2012, 95 clones). Up to ten tubers per plot were washed and scored for the scab phenotypes percent surface area (PSA) on a scale of 0 to 100% in increments of 5% and lesion type (LT) on a scale of 0 to 6 (0 = no lesions, 1 = superficial and small, 2 = superficial and coalescing, 3 = raised and small, 4 = raised and coalescing, 5 = pitted and small, 6 = pitted and coalescing). Clones were also assayed for chip color by storing tubers for 78 days at 4°C then cooking slices in vegetable oil at 191°C (375°F) until bubbling ceased (approximately 2 minutes), and visually evaluating them on a scale of one (very light) to nine (very dark). Other traits examined include vine maturity (1 = dead, or nearly so, 2 = prostrate, some dead stems, 3 = partially upright, some flowers, 4 = full flower, and 5 = pre-flower), plant vigor (1 = dead, 2 = small, but some vigor, 3 = medium, vigorous, 4 = big, vigorous for maturity, and 5 = very vigorous, big), tuber percent dry matter (PDM), and average tuber weight (ATW). Vine maturity and plant vigor was measured at one time in the growing season when segregation for vine maturity was at a peak. All significant QTLs were identified in 2012, except for one QTL in 2011 for CIS on chromosome 6. PSA and LT were present at the same location on chromosome eleven (percent variance explained by the QTL = 24.3% and 17.0%, respectively). The dominance ratio for PSA was 0.40 and LT was 0.28, with susceptibility derived from grandparent US-W4. Genes controlling CIS were present at two locations – one on chromosome six in 2011 (percent

phenotypic variance explained = 33.8%) and 2012 (percent phenotypic variance explained = 19.1%) and another on chromosome four in 2012 (percent phenotypic variance explained = 14.7%). Resistance to CIS from grandparent 524-8 had dominance ratios of -0.45 and -0.85 on chromosome six in 2011 and 2012 and a dominance ratio of 0.04 on chromosome four in 2012. Significant QTL were identified at the same location on chromosome five for maturity and vigor (percent phenotypic variance explained = 33.8% and 24.2%, respectively). Maturity had a dominance ratio of 0.18 and vigor had a dominance ratio of 0.26. US-W4 was the source of low maturity and vigor scores. Genetic control of ATW was identified on chromosome one (percent phenotypic variance explained = 20.2%) with a dominance ratio of 0.77 and high ATW derived from US-W4. Identification of QTLs is the first step toward developing molecular markers for breeders to efficiently integrate these desirable traits into cultivars.

INTRODUCTION

Potato common scab [*Streptomyces scabies* (Thaxt.) Waksman and Henrici] is a disease that displays symptoms of scab-like surface, raised, or pitted lesions (Loria et al. 1997), and can result in a substantial reduction in marketable yield for potato growers. Host plant resistance is considered to be one of the best options for managing scab (Mishra and Srivastava 2001; Jansky and Rouse 2003; Wanner and Haynes 2009). Clones with stable, high levels of resistance are desired (Haynes et al. 1997). However, little progress has been made toward the introgression of resistance into major cultivars (Douches et al. 1996).

Light-colored chips are desired by the potato industry and consumers. Cold induced sweetening (CIS) is induced at storage temperatures below 10°C (Sowokinos 2001). Reducing sugars cause dark color in fried products. Resistance factors have been identified for scab, CIS, and other desirable traits in conventional and wild germplasm (Bradshaw et al. 2008; Dionne and Lawrence 1961; Douches and

Freyre 1994; Haynes et al. 2009; Hosaka et al. 2011; Li et al. 2005a; Li et al. 2008; McCann et al. 2010; Menéndez et al. 2002). The wild tuber-bearing species *S. chacoense* has been identified as a source of resistance to scab and CIS (Dionne and Lawrence 1961; Douches and Freyre 1994; Hosaka et al. 2011; McCann et al. 2010).

The recent availability of the single nucleotide polymorphism (SNP) array developed by the SolCAP project has enabled genetic analysis with broad and dense genome coverage for potato (Hamilton et al. 2011; Felcher et al. 2012). Thus, genotypic information may be gathered efficiently for populations segregating for traits of interest, such as scab resistance. Quantitative trait locus (QTL) analysis is used to associate phenotypic and genotypic variation (Broman and Sen 2009). To date, only one study has identified QTLs for resistance to scab (Bradshaw et al. 2008). The purpose of this study was to identify QTL in an F2 population derived from the grandparents haploid (2x) *S. tuberosum* US-W4 and wild diploid *S. chacoense* 524-8.

MATERIALS AND METHODS

The 98 diploid clones used in the QTL analysis were generated by crossing two diploid F1 parents (Scab1 and Scab14). The F1 clones were generated from a cross between the *S. tuberosum* haploid US-W4 (2n=2x) and an S7 inbred clone of the diploid wild species *S. chacoense* 524-8. Grandparent US-W4 is susceptible to common scab (scab), and has early vine maturity, low plant vigor, low percent tuber dry matter (PDM), and moderate average tuber weight (ATW) (Table 2). Grandparent 524-8 has demonstrated resistance to common scab and CIS, late vine maturity, high plant vigor, high dry matter content, and low tuber weight (Table 2). Seed tubers of the 98 F2 clones and their parents and grandparents were generated in the greenhouse for 2011 and 2012 replicated field trials.

In 2008, all 98 clones and their parents and grandparents were evaluated for scab in a greenhouse seedling assay. In 2011 and 2012, clones were evaluated in a randomized complete block design consisting of two blocks near Antigo, Wisconsin, in a field that is maintained for high scab disease pressure. In 2011, the field was planted on May 25th and harvested on October 3rd. In this trial, 51 of the 98 F2 clones were planted in one replication per block. The remaining 47 clones were not planted due to a lack of seed. In 2012, the field was planted on May 11th and harvested on October 2nd. In this trial, 95 clones were evaluated with three replications in each block. In both 2011 and 2012, four check varieties with varying levels of susceptibility (Atlantic, Superior, Megachip, W2324-1) and the parents and grandparents (524-8, US-W4, Scab1, and Scab14) were planted in seven replications in each of the two blocks. All plots were evaluated for vine maturity and vigor on September 9th in 2011 and August 22nd in 2012. The vigor scale ranged one to five where 1 = dead, 2 = small, but some vigor, 3 = medium, vigorous, 4 = big, vigorous, and 5 = very vigorous, big. The maturity scale ranged from one to five where 1 = dead, or nearly so, 2 = prostrate, some dead stems, 3 = partially upright, some flowers, 4 = full flower, and 5 = pre-flower.

The ten largest tubers from each plot (or all tubers if less than ten were available) were washed and scored for scab. Two measures were used, percent surface area (PSA) (on a scale of 0 to 100%, with increments of 5%) and lesion type (LT) (0 = no lesions, 1 = superficial and small, 2 = superficial and coalescing, 3 = raised and small, 4 = raised and coalescing, 5 = pitted and small, 6 = pitted and coalescing). For the QTL analyses of PSA and LT, all scored tubers within a plot were averaged. PSA and LT were scored separately because the correlation between these two traits is variable, ranging from 0.30 to 0.93 (Bjor and Roer 1980; Loria 1981; Lambert et al. 2006).

For the 2011 and 2012 field trials, tubers were kept at room temperature, 22°C (72°F), for 21 days after harvest while they were scored for scab. They were then placed in a cooler at 4°C for 78 days

and then chipped to determine CIS resistance. The four largest tubers from each plot were weighed. Two of the tubers were cut in half from stem to bud end, and one 4 mm slice from each tuber was fried in vegetable oil at 191°C (375°F) and visually evaluated on a scale of one (very light) to nine (very dark). In 2011, one half of one of the sliced tubers was weighed to obtain the fresh weight, and in 2012, one half of each of two tubers was weighed to obtain the fresh weight. The dry weight was obtained after lyophilizing the tissue, and percent dry matter (PDM) was calculated [(weight of tuber tissue after lyophilization/weight of tuber tissue before lyophilization)*100)]. The average tuber weight (ATW) was determined by weighing the four largest tubers from the plot.

Analyses of variance were performed with SAS software (ver. 9.2) using the mixed model procedure (SAS Institute Inc., Cary, NC). Genotype was considered fixed, while the block and the block by genotype interaction were considered random. Grouping of clones was determined with the pdmix800 package in SAS using Tukey-Kramer ($p < 0.05$) (Saxton 1998). Pearson correlations between traits were calculated in R (ver 2.15.2). The R statistical package was used for all other analyses (Sarkar 2008).

Data normality assumptions were assessed and were satisfactory when PSA and ATW were square root transformed. The ANOVA output from the 98 genotyped clones was used to develop the map and QTL analyses, generate phenotypic distribution figures and selection indices, and for all other analyses, unless noted otherwise. Figures and tables that illustrate the phenotypic distributions, means, and medians are based on LS means of untransformed data.

DNA was extracted using the cetyl(trimethyl)ammonium bromide (CTAB) protocol (International Potato Center (CIP) 1999). 8303 SNPs for each clone were run on the Illumina platform developed by the SolCAP consortium. The SNPs were developed from Sanger or Illumina transcriptome sequencing of six potato cultivars. Biallelic, high confidence SNPs were chosen for the Illumina platform (Felcher et al.

2012; Hamilton et al. 2011). Physical positions of the markers were obtained from BLAST sequence alignments of the Potato Genome Sequencing Consortium (PGSC) *Solanum phureja* doubled monoploid (DM) pseudomolecule (ver 2.1.10).

Mapping was conducted in Joinmap (ver. 3.0) using Kosambi's mapping function and as an F2 population structure. Markers that fit the F2 distribution based on the Chi-square test ($p > 0.001$) were used in the analysis. Markers with LOD scores of at least 16.0 were assigned to linkage groups. The map used for QTL analysis was derived from the map one or map two functions provided by Joinmap for each linkage group using the regression mapping algorithm. QTL and map quality data checks were performed in R (ver. 2.15.2) using the rqtl package (Broman et al. 2003). Linkage groups were assigned a chromosome in accordance with the physical position on the DM pseudomolecule.

QTL analysis was conducted using Haley-Knott regression. The percent phenotypic variance explained by the QTL (also referred to as the heritability of the QTL) was calculated from the following equation: $1 - 10^{-2\,LOD/n}$ for traits with a single QTL. 1000 permutations were performed to determine the LOD significance level for each trait and year separately. A QTL was considered significant at the 5% LOD permutation threshold for the year and trait. A peak was considered notable if the p-value was less than or equal to 0.08. If more than one QTL was detected, then a multiple QTL model was implemented and the LOD values and heritability of the QTL were reported from the model. Traits with more than one QTL were evaluated for epistatic effects. The flanking markers of significant QTL were calculated with 1.5x-LOD support and approximate Bayesian credible interval. The effect of an allelic substitution was calculated as follows from the LS means used in the QTL analyses for each trait (Fehr 1991):

$$dominance\ ratio = d/a$$

where

$$d = AB - \frac{AA + BB}{2}$$

and

$$a = \frac{AA - BB}{2}$$

RESULTS

Phenotypic Analysis of F2 Genotyped Clones.

In 2011, differences among clones were not significant ($p = 0.12$) for PSA, but significant for LT ($p = 0.0006$). In 2012, significant differences were detected among clones ($p < 0.0001$) for PSA and LT. A normal and wide distribution of individuals was observed in the population for LT and PSA, after square root transformation for PSA. The mean and median values for PSA were highest in 2012 (19.0 and 15.2, respectively, on a scale of 0-100%). The mean and median values for LT were highest in 2008 (2.1 and 1.8, respectively, on a scale of 0-6) (Table 1). Significant differences ($p < 0.05$) were detected among clones for cold induced sweetening (CIS), vine maturity (maturity), plant vigor (vigor), average tuber weight (ATW) and percent dry matter (PDM) in both 2011 and 2012. CIS, maturity, vigor, ATW, and PDM mean and median values were higher in 2011 than 2012 (Table 1). The mean values for the breeding clones and standard cultivars span the mean and median of the genotyped population used in the QTL analyses (Table 2). Significant differences ($p < 0.05$) were detected among these clones for all tested traits in 2012 and traits that were replicated in 2011 (Table 2).

There was a strong and significant correlation between PSA and LT in all three years (Table 3). There was a weak to moderate, yet significant correlation for PSA between years (Table 4). For LT, only the correlation between 2011 and 2012 was significant, yet it was weak. Correlations between 2011 and 2012 were significant but moderate for all other tested traits.

Other significant correlations were found between maturity and vigor in both 2011 and 2012, between ATW and PSA in both 2011 and 2012, and between ATW and LT in both 2011 and 2012 (Table 3). There was also a negative correlation between PDM and ATW in both 2011 and 2012. Negative correlations were also observed between PDM and PSA, and between PDM and LT. Vigor and PSA had a significant and positive correlation for both years. Other significant correlations were observed for only one year.

Six clones (51, 73, 93, 99, 112, and 113) were selected for low CS incidence (< 20% PSA, < 2 LT), and CIS resistance (chip color score ≤ 4) based on 2012 data. Three of these individuals had a score of less than two for maturity, and three had a score less than five (five representing the maximum for maturity). All had a low vigor score (< 2.5). The ATW for the selected clones ranged from 4.3 grams to 9.4 grams. This was closer to grandparent 524-8 than US-W4. PDM (21.7%) for all tested clones was above industry processing standards.

The top 10% of the 2012 tested clones for ATW had values ranging from 29.8 to 55.8 g. Of these clones, PSA ranged from 15.0 to 40.4 and LT ranged from 1.8 to 2.8. Ranges of scores were 4.6 to 8.3 for CIS (chip color), 1.3 to 3.3 for maturity, 1.4 to 3.7 for vigor, and 27.2 to 32.9 for PDM.

Clone 48 had a very low incidence of scab (PSA = 6.2%, LT = 1.0), moderate CIS (chip color) (5.7), early maturity (1.8), low vigor (1.8), high (top 25% of the population) PDM (35.6), and moderate ATW (13.2 grams) in the 2012 trial. Similar values were obtained in 2011. This clone produces 2n pollen and 2n eggs, and has been successfully crossed to tetraploid cultivars in both directions (data not shown).

Genetic Map. Of the 8303 SNP markers on the Illumina platform, 3015 were polymorphic in the F2 population. Of the polymorphic markers, 1511 fit the expected F2 distribution. Markers that did not fit the mapping model were eliminated, leaving a total of 1311 markers distributed across the genome for QTL mapping. Thirteen linkage groups on twelve chromosomes were identified; two linkage groups were identified for chromosome seven. The total genetic map length was 657.1 cM and the average length of a linkage group was 50.5 cM.

QTL Analysis. The evaluated traits and relevant results of the QTL analyses, including flanking markers, are reported in Tables 5 and 6. QTL peaks are illustrated in Figure 1. The SNPs for QTLs reported below are the markers at the maximum likelihood position.

QTL Analysis for Scab. One QTL was significantly above the LOD threshold for 2012 PSA (LOD = 5.7; p = 0.001). It was on chromosome eleven (SolCAP SNP c1_16581; 10.1 cM). The percent phenotypic variance explained by the QTL is estimated to be 24.3%. No other significant QTLs were identified at the 5% LOD significance level. A peak at the same position on chromosome eleven was identified for 2012 LT (LOD = 3.9; p = 0.033) and explained 17.0% of the phenotypic variance. No significant QTL were identified for PSA or LT in 2008 or 2011.

QTL Analysis for Cold Induced Sweetening (CIS). In 2011, one significant QTL (LOD = 4.5; p = 0.018) was detected on chromosome six (SolCAP SNP c2_52385; 33.6 cM). The percent phenotypic variance explained by this QTL is 33.8%. A non-significant, but notable peak (LOD = 3.7; p = 0.064), is on chromosome four at 5.0 cM. The nearest marker is 5.2 cM away (SolCAP SNP c2_11487). In 2012, the model explained 41.9% of the variation. One QTL was found on chromosome four (SolCAP SNP c1_11791; 15.8 cM; LOD = 4.6; p = 4.0e-05; percent phenotypic variance explained = 14.7%). As in 2011, a QTL was identified on chromosome six (SolCAP SNP c2_51774; 14.6 cM; LOD = 5.9; p = 2.8e-06; percent phenotypic variance explained = 19.1%). Additional QTL were examined, but not added to the

model because each accounted for less than 8% of the variation and had a LOD score of less than 3.2. No epistatic interactions of interest were detected. All tested interactions had LOD values below 1.3 and accounted for less than 2.6% of total variation.

QTL Analysis for Vine Maturity. No significant QTL were detected in 2011. In 2012, one significant QTL (LOD = 8.5; $p < 0.004$) was detected on chromosome five and explained 33.8% of the phenotypic variance. The nearest SNP marker is 0.1 cM away (SolCAP SNP c2_22957; 11.1 cM). Another notable peak (LOD = 3.6; $p = 0.065$) was on chromosome eight (SolCAP SNP c1_8282; 49.1 cM).

QTL Analysis for Vigor. In 2011, no significant QTL were detected at the 5% significance level. A notable peak (LOD = 3.6; $p = 0.063$) was detected on chromosome ten. The nearest SNP marker was SolCAP SNP c1_329 at a distance of 3.2 cM. A significant QTL was detected (LOD = 5.7; $p < 0.004$) in 2012 on chromosome five. The nearest SNP marker to this location was SolCAP SNP c2_22957 (11.1 cM). The percent phenotypic variance explained by the QTL was 24.2%.

QTL Analysis for Percent Dry Matter (PDM) and Average Tuber Weight (ATW). One significant QTL (LOD = 4.7; $p = 0.008$) was detected in 2012 for average tuber weight on chromosome one (SolCAP SNP c1_14931; 35.2 cM). The percent phenotypic variance explained by the QTL was 20.2%. No other QTLs were detected for 2011 average tuber weight, 2011 or 2012 percent dry matter.

Effect of an allelic substitution on a QTL. The effect of an allelic substitution of significant QTL was found to vary by trait (Table 6, Figure 2). The dominance ratio was largest for CIS (2012; chromosome six) (-0.85), followed by ATW (0.77). The next largest dominance ratio value was for CIS (2011; chromosome six) (-0.45), then PSA (0.40), then LT (0.28) and vigor (0.26). Maturity and CIS (2012; chromosome four) had the smallest dominance ratio values (0.18 and 0.04, respectively).

SNP genotypes in the extreme clones for significant scab QTL in 2012. Of the ten most diseased clones for PSA, there were three “AA” (US-W4) genotypes, seven “AB” (heterozygous) genotypes, and zero

"BB" (524-8) genotypes. Zero "AA", two "AB", and eight "BB" genotypes are found among the ten least diseased clones for PSA. Of the ten most diseased clones for LT, there were two "AA" genotypes, seven "AB", and one "BB". Of the ten least diseased clones for LT, there were zero "AA", four "AB", and six "BB" genotypes.

The ten clones with the greatest value for CIS on chromosome four, ten clones with the greatest values had five "AA" genotypes, five "AB", and zero "BB". The ten clones with the smallest values had one genotype of "AA", two with "AB", and seven of "BB". On chromosome six, seven had genotype "AA", two "AB", and one "BB" of the ten clones with the greatest values. Of the ten clones with the smallest values, zero were "AA", seven were "AB", and three were "BB" genotypes.

Of the ten clones with the greatest value for both maturity and vigor, zero had an "AA" genotype, two had "AB", and eight had "BB". The ten clones with the lowest values, six had an "AA" genotype, three have "AB" genotypes, and one had "BB". Of the ten clones with the greatest value for ATW, four had genotypes of "AA", six had genotypes of "AB", and zero had "BB". Of the ten clones with the smallest value, one had a genotype of "AA", two had "AB", and seven had genotypes of "BB".

ANOVA at marker of maximum likelihood position of significant QTLs detected in 2012 for 2008 and 2011. Significant differences were detected among genotype groups for scab (PSA and LT) in 2008, but not 2011 ($p < 0.05$) (Table 7). Significant differences were detected in 2011 for QTL on chromosomes four and six as well as maturity and ATW. Significant differences were not detected among genotype groups for vigor in 2011.

DISCUSSION

A single significant QTL for scab was identified in an F2 population derived from a cross between the susceptible *S. tuberosum* clone US-W4 and the resistant *S. chacoense* clone 524-8. Moreover, this QTL has been identified with the same SNP marker for both PSA and LT. Bradshaw et al. (2008) identified two QTLs for scab, but no single QTL explained greater than 8.2% of the phenotypic variance in the tetraploid population. Those QTL were identified using a tetraploid full-sib family of 227 clones from a cross between processing clone 12601ab1 and table cultivar Sterling. The QTLs were on chromosomes two and six. The population was effectively phenotyped in only one of three years of the study due to lack of disease pressure. A scale of one (susceptible) to nine (resistant) was used to assign the scab phenotype. In this study, we report a single QTL for PSA and LT on chromosome eleven, explaining 24.3% and 17.0% if the phenotypic variance, respectively.

Haynes et al. (1997) suggested the possibility of developing clones with stable, high levels of resistance to scab using new germplasm sources. Clone 524-8 has performed consistently across environments for both percent surface area (PSA) and lesion type (LT). The QTL on chromosome eleven can be used for marker-assisted selection (MAS) to improve the efficiency of selecting for scab resistance in potato breeding programs. For example, the ten most diseased clones for PSA or LT had either zero or one marker call of the resistant grandparent (524-8). Of the ten least diseased clones for either PSA or LT, zero marker calls were of the susceptible grandparent (US-W4). The use of markers is particularly important for traits that are time-consuming to screen and require specialized and limited field space, such as scab resistance. Markers improve genetic gain because resources previously spent on phenotypic screening in highly replicated trials can then be spent on increasing the number of test progeny.

A significant QTL was detected for PSA and LT in 2012, but not in the other years. This was likely due to differences in experimental design among years**.** In 2008, the screen for scab resistance was conducted on tubers of a single plant for each genotype in the greenhouse. In 2011, the screen consisted of a total of two replications per genotype in the field. In addition, only 51 of the 98 genotyped clones were assayed. This is in contrast to 2012, where a total of six replications of each of 95 of the 98 genotyped clones were present in the field. However, significant differences were detected among genotype groups for both scab traits (PSA and LT) in the 2008 data set at the marker of the maximum likelihood position detected in 2012 (Table 7).

In 2011 and 2012, a common QTL for CIS was detected on chromosome six. The QTL detected in 2012 is within the wide interval for that found in 2011. It is not surprising that the interval is narrower in 2012, since multiple QTL analyses provide a smaller region of interest (Broman and Sen 2009). The second QTL on chromosome four detected in 2012 may have not been detected in 2011 due to limited replication and fewer tested individuals. However, in 2011, a peak of interest is present on chromosome four near the statistically significant peak found in 2012. Using the 2011 phenotypic data, significant differences were detected among genotype groups at the maximum likelihood position detected in 2012 for QTL on both chromosome four and six (Table 7).

In contrast to most *S. tuberosum* cultivars, which cannot undergo cold storage without accumulating reducing sugars during storage, some potato wild species are excellent sources of CIS resistance (Douches and Freyre 1994; Groza et al. 2006; Hamernik 1998; McCann et al. 2010; Thill and Peloquin 1994). Many of these studies, including the current one, use *S. chacoense* as a source of resistance to CIS (Douches and Freyre 1994; Groza et al. 2006; Hamernik 1998; McCann et al. 2010; Thill and Peloquin 1994). In *S. tuberosum* diploid populations, using a candidate gene approach for sugar content, CIS was associated with many QTLs (Menéndez et al. 2002). QTL that explained greater than

10% of the variance for reducing sugars were identified on linkage groups one, three, seven, nine, and eleven. Li et al. (2005a) identified an association between chip color and DNA variation on chromosome nine at the invertase locus *invGE/GF*, a locus that is a candidate gene for tuber starch and sugar content on each of the twelve chromosomes (Gebhardt et al. 2005; Menendez et al. 2002; Schäfer-Pregl et al. 1998)

In a population derived from a *S. tuberosum* X *S. chacoense* hybrid female parent and *S. phureja* clone 84S10 male parent, Douches and Freyre (1994) identified six QTL with significant marker associations with the female parent by measuring chip color. Two QTL were identified on chromosome two, one on four, two on five, and one on ten. In this study, Douches and Freyre (1994) stored tubers at 15°C for three weeks, then at 10°C for 45 days before chipping. Bradshaw et al. (2008) identified four QTL for accounting for 30% of chip color variation under an additive model in a *S. tuberosum* tetraploid population. The QTL were identified on linkage groups XIa, A (possibly chromosome nine), and chromosome six in parent Sterling, and linkage group XIa in parent 12601. As in the current study, the chips were visually scored after storage at the same temperature (4°C), but for a longer time (166 to 182 days). The discrepancies observed in percent phenotypic variance explained by the QTL and number of QTLs among this study and studies by Menéndez et al. (2002), Douches and Freyre (1994), and Bradshaw et al. (2008) may be due to differences in experimental design, method of analysis, and most likely, germplasm source. This study has demonstrated a source for CIS and scab resistance in grandparent *S. chacoense* 524-8. The QTLs from this study were present on chromosomes four and six. Breeders would benefit from the ability to use molecular markers to predict progeny phenotypes from crosses made with this source of scab and CIS resistance.

In this study, QTL for maturity and vigor were found at the same position on chromosome five, which is explained, at least in part, by the relationship between these two traits, particularly observed in

2012 (2011 r = 0.36, 2012 r = 0.75). In previous studies, QTLs for vine maturity were identified on chromosome five in tetraploid populations of *S. tuberosum.* The QTLs explained 56% of the phenotypic variance (Bradshaw et al. 2008), 9.6 to 15.6% of the phenotypic variance (McCord 2011a) and 9.6 to 18.7% of the phenotypic variance (McCord 2011b). The current study also identified a QTL on chromosome five that explained 34% of the phenotypic variance for vine maturity. Other studies have identified a QTL for this trait on chromosome five in diploid populations with wild species in the pedigree (Collins et al. 1999; Visker et al. 2005; Malosetti et al. 2006). Similarly, Van den Berg et al. (1996) detected eleven QTLs on seven chromosomes for tuberization in reciprocal backcrosses between *S. tuberosum* and *S. berthaultii*. The QTL that explained the most phenotypic variance (27%) was on chromosome five.

In this study, no major QTL were identified for percent dry matter (PDM). This may be because the two grandparents were not different enough to produce a range in progeny that are different from each other, despite the relatively high PDM value for 524-8 (Table 1 and 2). It may also be that the tubers from this trial were small, and that PDM may be more variable among small tubers than large tubers. For this trait, Bradshaw et al. (2008) estimated PDM from specific gravity. They identified a QTL explaining less than 10% of the variance on chromosome five. Similarly, McCord et al. (2011b) detected QTLs for both specific gravity and PDM in a *S. tuberosum* population. QTLs significant for more than one year for PDM were on chromosomes two and five, and linkage group seven. For specific gravity, QTLs on chromosome two and linkage groups seven, eight, and thirteen were significant for more than one year. Freyre and Douches (1994) reported ten QTL for specific gravity on six linkage groups in a (*S. tuberosum* x *S. chacoense*) x *S. phureja* diploid population. The percent variance explained by the QTL for individual loci ranged from 4.0 to 15.8%. The QTLs detected consistently across multiple populations and environments were on chromosomes one, two, five, and seven. Schäfer-Pregl et al. (1998) reported

eighteen QTL on all twelve chromosomes for tuber starch content (estimated from specific gravity) in a S*. tuberosum* population and a (*S. tuberosum* x *S. chacoense*) x *S. tuberosum* population.

The total genetic map length of this population was 657.1 cM. Previous studies report maps that range in size from 606 cM to 1120 cM using diploid populations of wild species and cultivars (Bonierbale et al. 1988; Jacobs et al. 1995). Despite reports of genetic maps and markers, marker-assisted selection has not been widely implemented in breeding potato (Chen et al. 2001; Gebhardt et al. 1989; Gebhardt et al. 2006; Hamilton et al. 2011; Li et al. 2005b; Li et al. 2008; Milbourne et al. 1998; Schäfer-Pregl et al. 1998; Tanksley et al. 1992; van Os et al. 2006). Markers that have been reported to date include resistance to late blight (Colton et al. 2006), Potato Virus Y (Heldak et al. 2007; Ottoman et al. 2007; Kasai et al. 2000), Verticillium wilt (Bae et al. 2008), and potato cyst nematode (Gebhardt et al. 1993). One of the goals of this study is to report a genomic region with QTL analysis that can be used to develop a molecular marker for resistance to scab. The advantage to the identification of a genomic region associated with scab resistance with SNPs used in this study is that the markers are at a single-nucleotide level, codominant, and high in density. This is different from markers that have been utilized in other studies to identify QTLs, which consist of markers that may be abundant, but dominant (Restriction Fragment Length Polymorphisms, Amplified Fragment Length Polymorphisms) or those that are codominant, but limited in number (Simple Sequence Repeats). Moreover, the markers used for other studies are used to report large segments of DNA associated with the trait.

A strong correlation was identified between PSA and LT for scab in each year (2008 r = 0.76, 2011 r=0.74, and 2012 r = 0.69). Similar relationships have been found, and range from 0.30 to 0.77 (Bjor and Roer 1980), 0.80 to 0.93 (Loria 1981), and 0.85 (Lambert et al. 2006). Given the high correlation between PSA and LT and a significant QTL at the same SNP marker, it will be possible to select for both PSA and LT simultaneously. In general, tubers with a smaller surface area infected have a

less severe lesion type (Leach 1938; Bjor and Roer 1980). This relationship between PSA and LT may be partially explained by the life cycle of the pathogen. After infection, the host cells die and provide nutrients for the bacterium. The living cells surrounding the lesion divide and produce cork cells. This cycle continues, and the cork cells push outward and result in a scab lesion (Agrios 1997; Loria 2001). Moreover, environmental factors and cultivar susceptibility appear to determine lesion type (severity) and incidence (Locci 1994; Loria et al. 1997). It may be that similar environmental conditions are conducive to both PSA and LT. In addition, overlapping regions of the genome may control these two phenotypes. Genotypes that do not fit the linear trend may have distinct resistance mechanisms against either PSA or LT, but not both.

In both 2011 and 2012, correlations between average tuber weight (ATW) and several traits were significant (Table 3). The high, positive correlations observed between ATW and PSA (2011 r = 0.53, 2012 r = 0.28) and LT (2011 r = 0.61, 2012 r = 0.35) suggest that clones that initiate tubers earlier in the growing season tend to have more time for the disease to progress (Table 3). This suggestion requires the assumption that all clones in this population have a similar tuber growth rate and are exposed to conditions favorable to infection throughout the growing season. ATW and PDM were negatively correlated for both years (2011 r = -0.69, 2012 r = -0.52) (Table 3). There was no significant negative relationship between the scab phenotypes (PSA and LT) and CIS, allowing for selection of breeding lines for both scab and CIS resistance. The significant, positive correlation between scab susceptibility and ATW is reflected in the six lines selected for scab and CIS resistance, as they have values that are lower than the mean (selected lines range from 4.3 to 9.4 g and the genotyped population mean is 16.9 g) (Table 3). The ATW range of the selected lines is comparable to the ATW of the scab resistant grandparent 524-8, further supporting the observed correlation (Table 2). The

selected clones span the maturity ranges, indicating that selections were made independent of the positive correlation between CIS and maturity (Table 3).

Significant, but weak to moderate correlations between years were identified for both scab phenotypes PSA and LT. The weak to moderate correlations may be explained by the experimental design differences among years. In 2012, six replications of each of 95 clones were assessed. This is in contrast to 2011, where two replications of 51 clones were placed in the field, and 2008, where seedling-generated tubers were assayed in the greenhouse. It may be that *S. scabies* was less pathogenic in the greenhouse (in terms of PSA) than outdoor experiments due to environmental influences, as observed by Archuleta and Easton (1981). Moreover, significant genotype by environment interactions for both PSA and LT have been reported (Haynes et al. 1997; Haynes et al. 2010). Screening for scab resistance is difficult because the environmental influence is large (Wiersema 1974; Bradshaw et al. 2008), making heavily replicated trials (such as those of the 2012 experiment) necessary to account for environmental influences on phenotype. It may also be that the pathogen load varied, as this can affect lesion development on tubers (Wanner 2004).

In this germplasm, scab susceptibility appears to be a partially dominant trait (dominant grandparent is susceptible US-W4), with LT (dominance ratio = 0.28) being closer to additive than dominant compared to PSA (dominance ratio = 0.40) (Table 6; Figure 2). Scheible et al. (2003) identified resistance to thaxtomin due to reduced uptake as a single, recessive mutation at a nuclear locus in *Arabidopsis*. It may be worth examining this as a candidate gene for resistance to scab.

In tetraploid crosses between *S. chacoense* and *S. tuberosum*, Dionne and Lawrence (1961) reported incomplete dominance for some scab resistance factors from *S. chacoense*. Dominant alleles for resistance to scab were detected by Bradshaw et al. (2008) in a tetraploid *S. tuberosum* population. Haynes et al. (2009) identified dominance variation for resistance to scab in a diploid hybrid *S. phureja-S.*

stenotomum population. Consequently, Haynes et al. (2009) suggest that it may be feasible to transmit resistance to the tetraploid level by using 4x-2x crosses in which the diploid parent produces 2n pollen. Haynes (1990; 1992) demonstrated that (4x-2x) and bilateral (2x-2x) sexual polyploidization can transmit a portion of the non-additive (dominance) genetic variance. Murphy et al. (1995) successfully transferred scab resistance to tetraploid offspring in 4x-2x crosses with the diploid sources *S. phureja*, *S. stenotomum*, and haploid *S. tuberosum*. 4x-2x crosses are more likely to transmit dominance than 2x-2x crosses because both alleles involved in the dominance interaction are often retained in the 2n gametes. In the 2x-2x crosses, the two alleles involved in the dominance interaction will be separated during meiosis. The Murphy et al. (1995) study supports the hypothesis developed by Alam (1972) that two independent loci are required for resistance to CS found in *S. phureja*. At one locus, a dominant allele confers resistance, while homozygous recessive alleles are necessary for resistance at a second locus. In this study, we have identified what appears to be recessive resistance to CS (Table 6; Figure 2). Other disease resistance traits, chipping quality, and high starch content from wild species have been successfully introgressed using the unilateral sexual polyploidization breeding scheme (Carputo et al. 2000; Zimnoch-Guzowska and Dziewonska 1989; Watanabe et al. 1992; Ortiz et al. 1997; Ortiz 1998). The transfer of ATW, vigor, and CIS resistance QTL via 4x-2x crosses may also be successful, as these traits are partially dominant (Table 6, Figure 2). Using this scheme, grandparent 524-8 is favorable for scab (PSA), CIS (QTL on chromosome six), and vigor. Either grandparent may be used for maturity, depending on the desired response (Table 6; Figure 2). However, considering that the QTL for maturity and vigor is in the same position and the positive correlation between the traits, the breeder will have to choose the phenotype to be later maturing and vigorous or earlier maturing and not as vigorous.

In the population under study, the QTL for CIS resistance on chromosome four appears to be additive (dominance ratio = 0.04) (Table 6, Figure 2). Douches and Freyre (1994) reported preliminary

results that suggested a significant contribution of additive effects for chip color variation. Maturity also appears to have additive characteristics (dominance ratio = 0.18) (Table 6, Figure 2). The phenotypic response to selection for additive traits can be observed from one generation to the next, under the germplasm and environmental conditions in which the experiment was conducted (Falconer and Mackay 1996).

Six clones have both scab and CIS resistance. Currently, there are no widely used cultivars that have both of these traits. Efforts are being made to further test the six selected clones and ideally, make them available as breeding lines with markers. Similarly, clone 48 has been crossed to widely-used tetraploid cultivars to produce clones with scab resistance.

CONCLUSIONS

A major QTL for resistance to scab for potato has been identified in the population derived from the diploid (2x) *S. chacoense* clone 524-8. The QTL is significant for both PSA and LT, which are two highly correlated traits. Therefore, selection can simultaneously be conducted for both PSA and LT scab phenotypes. The QTLs identified in this study could be leveraged to develop and validate a molecular marker to efficiently integrate resistance to scab into breeding lines using the 524-8 grandparent. The 4x-2x breeding scheme may also be used in conjunction with the marker since the 524-8 grandparent produces 2n gametes. A similar scheme could be used to transfer CIS resistance, given that the CIS QTLs are also found in 524-8. Resistance to both scab and CIS are important for several potato breeding programs, and the identification of QTLs is a step toward the development of molecular markers that accelerate breeding progress.

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TABLES AND FIGURES

Table 1. *Mean and median values of the population used for quantitative trait loci (QTL) analysis, based on 98 clones in 2008, 51 clones in 2011, and 95 clones in 2012*. Common scab was scored using the percent tuber surface area covered with lesions (PSA) and lesion type (LT) on a scale of zero (no lesions) to six (confluent, pitted lesions). Cold induced sweetening (CIS) was visually scored on chips, on a scale of zero (light) to nine (dark). Vine maturity and vigor were scored on a scale from one (early, low) to five (late, high). The average tuber weight (ATW) was obtained from the largest four tubers per plot. Percent dry matter (PDM) was obtained from the ratio between fresh and lyophilized tissue weights.

				Mean			
	PSA	**LT**	**CIS**	**Maturity**	**Vigor**	**PDM**	**ATW**
2008	11.8	2.1	.	.	.	.	.
2011	14.9	1.3	5.9	2.3	2.5	38.3	19.4
2012	19.0	1.7	5.4	2.2	2.0	32.7	16.9
				Median			
	PSA	**LT**	**CIS**	**Maturity**	**Vigor**	**PDM**	**ATW**
2008	7.5	1.8	.	.	.	.	.
2011	9.6	1.0	5.5	2.0	2.2	35.1	18.0
2012	15.2	1.6	5.4	2.0	2.0	32.1	14.8

Table 2. *Mean values of the standard cultivar checks, parents, and grandparents for traits examined in the F2 population for quantitative trait loci (QTL) analysis.* Common scab was scored using the percent tuber surface area covered with lesions (PSA) and lesion type (LT) on a scale of zero (no lesions) to six (confluent, pitted lesions). Cold induced sweetening (CIS) was visually scored on chips, on a scale of zero (light) to nine (dark). Vine maturity and vigor were scored on a scale from one (early, low) to five (late, high). The average tuber weight (ATW) was obtained from the largest four tubers per plot. Percent dry matter (PDM) was obtained from the ratio between fresh and lyophilized tissue weights. The means below are the Tukey-Kramer least square means from the analysis of variance that includes all F2 genotyped clones and clones listed below. The letter groups represent significant differences among clones within the population. The letter groups for PSA and ATW are based on transformed data. There are no letter groups for 2008 and some traits for 2011 because there was no analysis of variance due to lack of replication.

p-value, LS mean, and letter group (Tukey-Kramer at p < 0.05) for each trait and year															
		PSA		**LT**		**CIS**		**Maturity**		**Vigor**		**PDM**		**ATW**	
2008	**524-8**	2.5	.	0.4	.	.	.	.	.	.	.	.	.	.	.
	Superior	9.4	.	2.2	.	.	.	.	.	.	.	.	.	.	.
	Atlantic	23.4	.	4.0	.	.	.	.	.	.	.	.	.	.	.
	US-W4	31.0	.	3.3	.	.	.	.	.	.	.	.	.	.	.
	Scab1	11.3	.	1.7	.	.	.	.	.	.	.	.	.	.	.
	Scab14	9.6	.	2.7	.	.	.	.	.	.	.	.	.	.	.
2011		**p = 0.002**		**p = 0.0058**		.		**p = 0.0122**		**p = 0.0006**		.		.	
	524-8	4.9	D	0.7	C	4.0	.	2.1	A	1.8	C	45.8	.	11.8	.
	Superior	11.6	CD	1.2	BC	9.0	.	1.1	B	1.7	BC	21.0	.	124.2	.
	Atlantic	14.0	BCD	1.8	BC	7.0	.	2.1	AB	3.6	AB	24.2	.	299.5	.
	Megachip	13.1	BCD	2.0	BC	6.0	.	2.0	AB	3.6	AB	26.1	.	229.5	.
	US-W4	27.9	ABC	3.3	AB	8.0	.	2.2	A	2.4	C	26.0	.	34.1	.
	Scab1	34.0	ABC	1.8	BC	7.0	.	2.0	AB	4.0	AB	31.3	.	31.7	.
	Scab14	36.5	AB	2.3	ABC	6.0	.	2.7	A	3.8	AB	31.5	.	51.5	.
	W2324-1	40.4	A	4.3	A	6.0	.	2.1	AB	4.4	A	26.5	.	227.0	.
2012		**p = 0.0002**		**p = 0.0001**		**p = 0.0031**		**p < 0.0001**		**p < 0.0001**		**p < 0.0001**		**p < 0.0001**	
	524-8	0.7	D	0.2	C	6.0	ABC	2.7	AB	2.0	EF	39.4	A	4.1	D
	Superior	4.8	CD	1.2	C	8.8	A	1.1	D	1.1	F	20.5	D	183.3	AB
	Atlantic	17.6	BC	3.1	AB	6.5	ABC	1.9	C	3.2	CDE	25.4	CD	275.1	A
	Megachip	20.7	B	3.1	AB	7.4	AB	2.0	BC	4.5	AB	25.5	CD	295.1	A
	US-W4	28.0	AB	2.9	B	6.8	ABC	2.3	BC	2.4	DE	28.1	BC	22.4	CD
	Scab1	48.6	A	2.9	B	4.8	C	2.0	BC	3.9	BC	30.3	BC	76.6	BC
	Scab14	45.6	A	2.9	B	4.8	C	3.2	A	3.4	CD	32.5	B	58.7	CD
	W2324-1	28.1	AB	4.2	A	5.5	BC	2.0	BC	5.0	A	26.0	C	279.0	A

Table 3. *Significant Pearson correlation values between traits within a year for 2008, 2011, and 2012*. Common scab was scored using the percent tuber surface area covered with lesions (PSA) and lesion type (LT) on a scale of zero (no lesions) to six (confluent, pitted lesions). Cold induced sweetening (CIS) was visually scored on chips, on a scale of zero (light) to nine (dark). Vine maturity and vigor were scored on a scale from one (early, low) to five (late, high). The average tuber weight (ATW) was obtained from the largest four tubers per plot. Percent dry matter (PDM) was obtained from the ratio between fresh and lyophilized tissue weights. Variable 1 response is a considered a decrease. The only correlation tested in 2008 was between PSA and LT.

Variable 1	Variable 2	Variable 2 response	r (2008)	r (2011)	r (2012)	Favorable relationship for breeding program?
PSA	LT	decrease	0.76	0.74	0.69	Yes, selection can take place for both LT and PSA at the same time.
	ATW	decrease	.	0.53	0.28	No, low PSA and high ATW are desired.
	PDM	increase	.	-0.34	-0.20	Yes, low PSA and high PDM are desired.
	Vigor	decrease	.	0.37	0.21	No, low PSA and high vigor are desired.
LT	ATW	decrease	.	0.61	0.35	No, low LT and high ATW are desired.
	PDM	increase	.	-0.43	-0.37	Yes, low LT and high PDM are desired.
Maturity	Vigor	decrease	.	0.36	0.75	Undetermined - high vigor is desired, but maturity depends on the objective of the breeder.
	PDM	decrease	.	0.38	.	Undetermined - high PDM is desired, but maturity depends on the objective of the breeder.
	ATW	increase	.	-0.38	.	Undetermined - high ATW is desired, but maturity depends on the objective of the breeder.
ATW	PDM	increase	.	-0.69	-0.52	No, high ATW and high PDM are desired.
	Vigor	decrease	.	.	0.30	Yes, high ATW and high vigor are desired.
CIS	Maturity	increase	.	.	-0.38	Undetermined - low CIS is desired, but maturity depends on the objective of the breeder.
	Vigor	increase	.	.	-0.23	n/a

Note: r values only displayed for Pearson correlations that are significant ($p < 0.05$)

Table 4. *Pearson correlations for traits between years*. Common scab was scored using the percent tuber surface area covered with lesions (PSA) and lesion type (LT) on a scale of zero (no lesions) to six (confluent, pitted lesions). Cold induced sweetening (CIS) was visually scored on chips, on a scale of zero (light) to nine (dark). Vine maturity and vigor were scored on a scale from one (early, low) to five (late, high). The average tuber weight (ATW) was obtained from the largest four tubers per plot. Percent dry matter (PDM) was obtained from the ratio between fresh and lyophilized tissue weights.

Trait	Year	Year	Correlation (r)
PSA	2008	2011	0.28*
	2008	2012	0.33*
	2011	2012	0.50*
LT	2008	2011	0.27
	2008	2012	0.15
	2011	2012	0.35*
CIS	2011	2012	0.69*
maturity	2011	2012	0.43*
vigor	2011	2012	0.43*
PDM	2011	2012	0.58*
ATW	2011	2012	0.56*

* Denotes a significant Pearson correlation

Table 5. *Map features of significant QTL in an F2 population derived from US-W4 (S. tuberosum) and 524-8 (S. chacoense).* "Nd" indicates that no significant QTL was detected (LOD threshold at 5%).

Trait	Year	Chromosome	LOD	p-value	% Phenotypic variance explained by the QTL	SolCAP SNP marker at maximimum likelihood estimate of QTL location	Genetic Map Position (cM)	Bayes Flanking Marker1 (position in cM; LOD)	Bayes Flanking Marker2 (position in cM; LOD)	1.5-LOD Support Flanking Marker1 (position in cM; LOD)	1.5-LOD Support Flanking Marker2 (position in cM; LOD)	Physical Map Position (Mb) of maximum likelihood estimate of QTL
Percent Surface Area (PSA)	2008	nd	.	.	.	.	.	.	.	.	.	.
	2011	nd	.	.	.	.	.	.	.	.	.	.
	2012	11	5.7	0.001	24.3	c1_16581	10.1	c2_12333 (0.0; 3.0)	c2_22185 (22.7; 4.0)	c2_12333 (0.0; 3.0)	c2_22185 (22.7; 4.0)	327.41
Lesion Type (LT)	2008	nd	.	.	.	.	.	.	.	.	.	.
	2011	nd	.	.	.	.	.	.	.	.	.	.
	2012	11	3.9	0.033	17.0	c1_16581	10.1	c2_12333 (0.0; 2.5)	c1_4926 (26.0; 1.5)	c2_12333 (0.0; 2.5)	c1_4926 (26.0; 1.5)	327.41
Cold Induced Sweetening (CIS)	2011	6	4.5	0.018	33.8	c2_52385	33.6	c2_26501 (3.9; 3.0)	c2_5821 (49.4; 2.6)	c2_3104 (0.0, 2.0)	c2_5821 (49.4; 2.6)	370.41
	2012	4	4.6	4.02E-05	14.7	c1_11791	15.8	c2_58198 (9.1; 3.5)	c1_6036 (16.8; 3.1)	c2_39624 (8.1; 2.5)	c1_6036 (16.9; 3.1)	438.14
	2012	6	5.9	2.77E-06	19.1	c2_51774	14.6	c2_3104 (0.0; 4.3)	c2_54220 (16.3; 3.9)	c2_3104 (0.0, 4.3)	c2_54220 (16.3; 3.9)	338.27
Vine Maturity (Maturity)	2011	nd	.	.	.	.	.	.	.	.	.	.
	2012	5	8.5	< 0.004	33.8	c2_22957*	11.1	c2_23743 (0.0; 4.1)	c2_38163 (13.5; 6.9)	c2_23743 (0.0; 4.1)	c2_38163 (13.5; 6.9)	41.96
Plant Vigor (Vigor)	2011	nd	.	.	.	.	.	.	.	.	.	.
	2012	5	5.7	< 0.004	24.2	c2_22957*	11.1	c2_23743 (0.0; 3.5)	c1_15292 (19.8; 3.0)	c2_23743 (0.0; 3.5)	c1_15292 (19.8; 3.0)	41.96
Percent Dry Weight (PDW)	2011	nd	.	.	.	.	.	.	.	.	.	.
	2012	nd	.	.	.	.	.	.	.	.	.	.
Average Tuber Weight (ATW)	2011	nd	.	.	.	.	.	.	.	.	.	.
	2012	1	4.7	0.008	20.2	c1_14931	35.2	c2_51055 (26.1; 3.2)	c2_2463 (40.7; 2.9)	c2_54797 (22.6; 2.5)	c2_2463 (40.7; 2.9)	679.97

*** Indicates that the nearest SNP marker to the maximum likelihood estimate of the QTL location is used**

Table 6. *Effect of allelic substitution on significant quantitative trait loci (QTL) for percent tuber surface area (PSA) covered with scab lesions, cold*

induced sweetening (CIS), vine maturity (maturity), plant vigor (vigor), and average tuber weight (ATW). Significant differences are indicated by the number groups in the superscript.

Trait (year)	Scale of Trait	Marker at maximum likelihood position	Bayes flanking markers	Chromosome	Maximum likelihood position (cM)	Bayes flanking position of QTL (cM)	LOD	% Phenotypic variance explained by the QTL	Genotype Mean			Dominance Ratio[x]
									AA	AB	BB	
PSA (2012)	0 - 10	c1_16581	c2_12333 to c2_22185	11	10.1	0.0 to 22.7	5.7	24.3	4.8^{1}	4.3^{1}	2.9^{2}	0.40
LT (2012)	0 - 6	c1_16581	c2_12333 to c1_4926	11	10.1	0.0 to 26.0	3.9	17.0	2.1^{1}	1.8^{1}	1.2^{2}	0.28
CIS (2011)	1 - 9	c2_52385	c2_26501 to c2_5821	6	33.6	3.9 to 49.4	4.5	33.8	8.0^{1}	6.0^{2}	5.2^{2}	-0.45
CIS (2012)	1 - 9	c1_11791	c2_58198 to c1_6036	4	15.8	9.1 to 16.8	4.6	14.7	6.4^{1}	5.6^{2}	4.7^{3}	0.04
CIS (2012)	1 - 9	c2_51774	c2_3104 to c2_54220	6	14.6	0.0 to 16.3	5.9	19.1	6.3^{1}	5.1^{2}	5.0^{2}	-0.85
Maturity (2012)	1 - 5	c2_22957*	c2_23743 to c2_38163	5	11.1	0.0 to 13.5	8.5	33.8	1.6^{1}	2.1^{2}	2.9^{3}	0.18
Vigor (2012)	1 - 5	c2_22957*	c2_23743 to c1_15292	5	11.1	0.0 to 19.8	5.7	24.2	1.7^{1}	1.9^{1}	2.4^{2}	0.26
ATW (2012)	0 - 10	c1_14931	c2_51055 to c2_2463	1	35.2	26.1 to 40.7	4.7	20.2	4.2^{1}	4.1^{1}	2.9^{2}	0.77

*denotes the nearest SNP marker to the maximum likelihood estimate of the QTL used

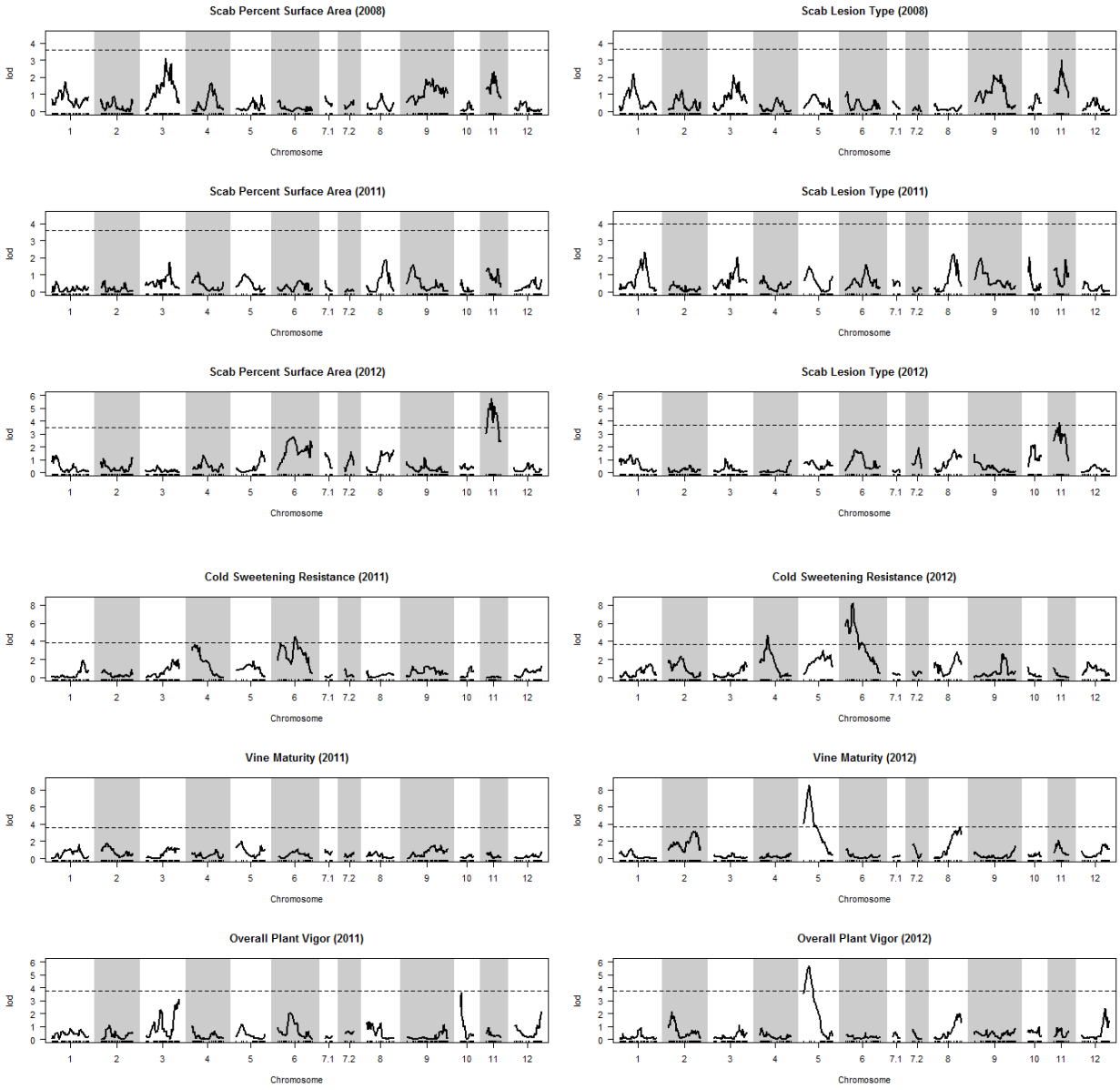
^{1,2,3}denotes group from the ANOVA of the trait and year

[x]Fehr 1991

Table 7. *Analysis of variance (ANOVA) for 2008 and 2011 at the maximum likelihood position of significant quantitative trait loci (QTL) detected in 2012.* Significant differences are indicated by the number groups in the superscript.

Marker (2012)	**c1_16581**				**c1_11791**	**c2_51774**	**c2_22957**		**c1_14931**
Chromosome	**11**				**4**	**6**	**5**		**1**
Trait, year	**PSA, 2008**	**LT, 2008**	**PSA, 2011**	**LT, 2011**	**CIS, 2011**	**CIS, 2011**	**Maturity, 2011**	**Vigor, 2011**	**ATW, 2011**
p-value	**0.02**	**0.03**	**0.10**	**0.23**	**0.01**	**0.00**	**0.01**	**0.08**	**0.01**
LSmean (Tukey-Kramer)									
AA (US-W4)	4.0^{1}	2.9^{1}	4.2^{1}	1.7^{1}	7.2^{1}	7.2^{1}	1.6^{2}	1.6^{1}	4.4^{12}
AB (Het)	2.9^{12}	2.1^{12}	3.5^{1}	1.4^{1}	6.0^{12}	5.9^{2}	2.3^{12}	2.5^{1}	4.4^{1}
BB (524-8)	2.4^{2}	1.6^{2}	2.8^{1}	1.1^{1}	5.1^{2}	5.0^{2}	2.8^{1}	2.8^{1}	2.8^{2}

Figure 1. *QTL peaks by trait and year.* QTLs are based on data from 98 greenhouse-grown clones in 2008 (one replication), 51 field-grown clones in 2011 (two replications), and 95 field-grown clones in 2012 (six replications).



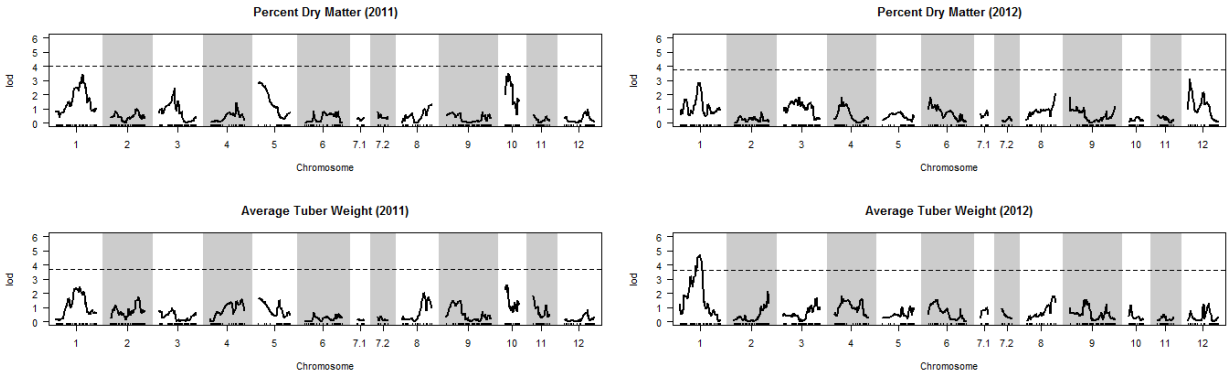
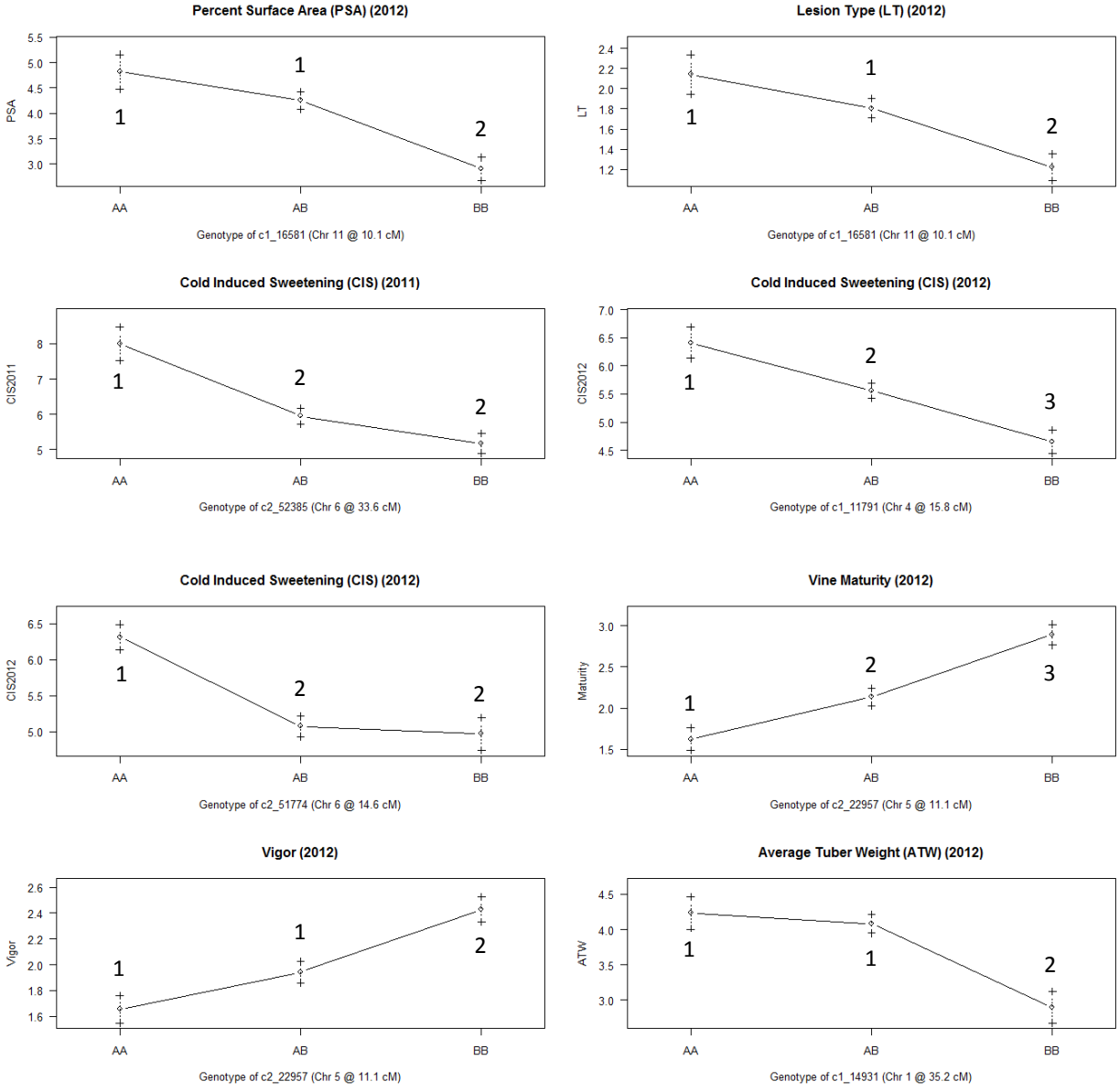

Percent Dry Matter (2011)
Percent Dry Matter (2012)
Average Tuber Weight (2011)
Average Tuber Weight (2012)
lod
Chromosome
1 2 3 4 5 6 7.1 7.2 8 9 10 11 12
0 1 2 3 4 5 6

Figure 2. *Effect of allelic substitution on QTL for percent tuber surface area (PSA) covered with scab lesions, cold induced sweetening (CIS), vine maturity, plant vigor, and average tuber weight (ATW).* The standard error of the mean at each genotype is represented by the by the "+" signs. "AA" is representative of grandparent 524-8, and "BB" is representative of grandparent US-W4. Significant differences are indicated by the number groups.



DISSERTATION SUMMARY

Common scab is a disease of the potato tuber that results in reduced quality and subsequent market loss to growers. Despite what is known about the pathogen, there is no single and efficient method known to control the disease (Lerat et al. 2009; Powelson and Rowe 2008). Host plant resistance to scab is a favorable strategy for managing the disease in potato (Mishra and Srivastava 2001; Jansky and Rouse 2003; Wanner and Haynes 2009). However, little progress has been made toward the introgression of resistance into major cultivars (Douches et al. 1996). Wild species show promise as sources of resistance, such as *S. chacoense* (Dionne and Lawrence 1961; Douches and Freyre 1994). Until recently, dense marker coverage and high-throughput screening has not been available to identify genomic regions of interest, such as those for resistance to scab. The recent availability of the single nucleotide polymorphism (SNP) array developed by the Solanaceae Coordinated Agricultural Project (SolCAP) consortium has enabled high-throughput genotyping. Moreover, software is readily available for genetic analysis of diploid populations. Such programs include JoinMAP to synthesize a genetic map of the population and Rqtl to identify quantitative trait loci.

Only one study in the current literature has identified quantitative trait loci in potato for resistance to scab (Bradshaw et al. 2008). However, the tetraploid *S. tuberosum* germplasm in that study was not a major source of resistance. Moreover, the phenotypic data set was collected over only one year and was based on one overall score per plot. An objective of this dissertation was to fill a gap in the literature and provide information regarding the genetic composition of a strong, stable source of resistance from the diploid wild species (*S. chacoense*) line 524-8. This research will likely contribute to the development of marker-assisted selection (MAS) methods.

Chapter one consists of a review of the literature on scab. It reviews the *Streptomyces* pathogens that cause the disease, conditions conductive to the disease, virulence factors of the

pathogen, life cycle, and screening methods. It also discusses the demand to find a stable and strong source of resistance (Haynes et al. 1997) and the work that has already been successful in transferring resistance using unilateral sexual polyploidization (USP) methods (Murphy et al. 1995).

Chapter two examines methods to reduce variability when screening for scab and Verticillium wilt (VW), another disease that requires labor-intensive phenotyping. The main objective of this research was to guide future experiments to make efficient use of resources in terms of sampling effort. The results of the VW trial revealed several interesting discussion points. As Frost (2004) reported, *the stem-to-stem variability is large*. In fact, my research found that some clones have more stem-to-stem variability than others, although no one particular clone had the greatest stem-to-stem variability from year to year. It is likely that this variation masks detection of significant differences among clones. An iterative process based on stem colony counts for each year and clone determined that *nine to eleven stems per clone should be collected*. This is based on the number of stems that reduces the standard deviation of the estimated CFU mean of a clone by 75%. Another iterative process was used to determine the approximate number of plants to sample. After averaging all stems collected per plant (up to four plants), the iterative process was conducted under the same parameter. The results suggested that *stems from seven to eight plants should be collected*. Based on these iterations, resource allocation calculations were performed to determine the optimal number of stems to collect from each plant and block. Calculations which resulted in the collection of a total of six to twelve stems were tested. The final sampling strategy which yielded the lowest variation (for both 2011 and 2012) and *suggestion for future collection of stems is to collect one stem from four plants in three blocks, totaling twelve stems per clone*.

The possibility of using the four most diseased stems per clone per block was also examined in screening for VW. *This sampling strategy resulted in a lower coefficient of variance (CV) and percent*

variability not explained by the model. While not observed in this study, lower CV and percent variability not explained by the model should increase the probability of detecting significant differences among clones. However, there are two caveats to this method. One is that differences in clonal ranking may be observed if one only considers the most diseased stems rather than all stems collected. Secondly, all stems need to be processed in order to identify the most diseased stems. One area of future research may be to look for a possible relationship between the appearance of disease in the stem and actual colony counts.

Similar to VW, the *tuber-to-tuber variation within a plot for CS was greater than other examined parameters*, although it was not as extreme as observed for VW. *Unlike VW, significant differences were detected among clones*. In addition, *the ranking of clones was generally interchangeable whether all tubers from a plot or the four most diseased tubers per plot are used in the analysis*. This is true for both scab phenotypes, percent surface area (PSA) and lesion type (LT*). Thus, the suggestion for a high-throughput method to identify levels of resistance or susceptibility is to score the four most diseased tubers per plot for each scab phenotype*. Unlike VW, the most diseased tubers can be identified at harvest, as soon as the tubers are washed and do not need to be identified after all tubers have been assayed. A similar iteration procedure was used to identify the suggested number of plots. For the iteration, the four most diseased tubers per plot were averaged for each scab phenotype. *Based on these data, five to eight plots per clone should be assayed*. Like VW, *the CV and percent variability not explained by the model is lowest when the four most diseased tubers are considered*.

Chapter three focuses on QTL analysis and the effect of an allelic substitution for scab. Other traits examined include cold induced sweetening (CIS), vine maturity (maturity), plant vigor (vigor), percent dry matter (PDM), and average tuber weight (ATW). The F2, diploid mapped population had 98 individuals derived from two F1s of a cross between female *S. tuberosum* US-W4 and male *S. chacoense*

524-8. 524-8 was the source of resistance to scab and CIS, has late maturity, high vigor, and low tuber weight relative to US-W4. *A significant QTL was identified for scab phenotypes PSA (percent phenotypic variance explained = 24.3%) and LT (percent phenotypic variance explained = 17.0%) on chromosome eleven in the same position (10.1 cM) in 2012*. *This is not surprising, since the Pearson correlation between the two scab traits was high (2008 = 0.76; 2011 = 0.74; 2012 = 0.69)*. It is likely that the lack of replication in 2008 (tubers generated from seedlings were assayed), the lack of individuals assayed from the population (51 of 98) and the lack of replication compared to the 2012 trial (two replications in 2011 compared to six replications per clone in 2012) prevented the detection of significant QTL. *The dominance ratio was 0.40 for PSA and 0.28 for LT. The source of dominance was susceptible grandparent US-W4*. Two significant QTLs were identified for CIS, one on chromosome six (2011 percent phenotypic variance explained = 33.8%; 2012 percent phenotypic variance explained = 22.5%) and one on chromosome four (2012 percent phenotypic variance explained = 10.1%). The dominance ratio for the QTL on chromosome six was -0.45 for 2011 and -0.85 in 2012. The QTL on chromosome four in 2012 had a dominance ratio of 0.04. QTLs for maturity and vigor were identified at the same position on chromosome five (maturity percent phenotypic variance explained = 33.8%; vigor percent phenotypic variance explained = 24.2%) in 2012. It is not surprising that they are found in the same position on the chromosome, since the Pearson correlation was high (r = 0.75). The dominance ratio for the maturity QTL was 0.18 and the dominance ratio for the vigor QTL was 0.26. One QTL was identified for ATW on chromosome one (percent phenotypic variance explained = 20.2%) and had a dominance ratio of 0.77 for large size.

The scab QTL, and other QTLs identified in this study can be used to develop a marker to improve efficiency in selecting scab-resistant clones in breeding programs. This is particularly useful for traits such as scab that are time-consuming to phenotype and require many replications in a field

specifically managed to be conductive to the disease. Additional information about the level of dominance for significant QTLs is useful. Traits with QTL that tend to be dominant may be most effectively transferred using a unilateral sexual polyploidization scheme. Genetic gain can be made from parent to offspring with the QTL that have additive gene action.

In summary, this dissertation work provides clarity on optimizing resources to efficiently screen for scab and VW and the tools to develop a marker for scab resistance. In addition, fertile clones have been identified with both scab and CIS resistance. Resistance to scab and CIS can be pursued for marker development and released with the *S. chacoense* germplasm line 524-8. Ultimately, the goal of this work was to contribute to breeding progress for potato and help to provide scab-resistant varieties that reduce or eliminate marketable yield losses due to common scab.

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