Examining Potato Virus Y (PVY) in the First Field Season of the University of Minnesota Potato Breeding Program

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Dedication

This theis is dedicated to my parents and grandparents, who taught me to value education and hard work.

Abstract

Potato virus Y (PVY) is a threat to the potato industry because potatoes infected with PVY have reduced yield and, in some cases, reduced tuber quality. Furthermore, PVY infection can cause seed potato lots to be downgraded or rejected. In the potato industry, the use of certified seed is the main control method used to reduce the incidence of PVY. In a potato breeding program, PVY infection can compromise the process of evaluating germplasm by masking the true genetic potential of potato genotypes. If PVY incidence is high in the first field season, when genetic variation of germplasm is at its maximum, high numbers of genotypes with potentially desirable traits may be discarded because of PVY infection; thus, reducing the genetic variability in this population. Prior to this study, the University of Minnesota Potato Breeding Program had not tested first field season breeding potatoes (nuclear seed) for PVY. This study investigated three different field locations and different time points of PVY testing during the first field season in the University of Minnesota Potato Breeding Program from 2007 to 2009 to estimate PVY incidence in the U of M Potato Breeding Program's nuclear seed. Greenhouse-grown minitubers were planted in three locations (Becker, MN; Grand Forks, ND; and Williston, ND) for three years. The minitubers were organized into seven populations based on minituber source and market type: Colorado Reds, Colorado Russets, Colorado Whites, North Dakota Reds, North Dakota Russets, North Dakota Whites, and Oregon Mix. During the growing season, leaf samples were taken at random and tested for PVY using serological methods. There were high percentages (above 0.5%) of PVY in the first field season at all locations in all years. Over all locations and years, 326 of 3532 samples (9.2% of the samples) tested positive for PVY. The average PVY incidence was highest in leaf samples collected late in the growing season. The Grand Forks, ND location (leaves collected 30 Sept. 2008) had the highest percentage (32.7%) of leaf samples testing positive for PVY. The Becker, MN location (leaves collected 9 July 2009) had the lowest percent (0.8%) of leaf samples testing positive for PVY. Within the populations, the highest PVY incidence estimated in this 3-year study occurred in Grand Forks, MN; where 65.6% of leaves collected from the Colorado red population tested positive for PVY. Those leaves were collected late in the growing season (30 Sept. 2008) and were planted in the field near advanced breeding lines that may have been a source of inoculum. Considering these results, strategies

for managing PVY in breeding programs are necessary. Strategies such as isolation planting, using barrier crops and using techniques to remove viruses from infected potato plants may be needed to reduce and eliminate PVY from a breeding program's germplasm. Since PVY is such problem for the potato industry, it is important for breeding programs to develop PVY-resistant cultivars of various market types and uses that are acceptable to growers and potato processors.

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Literature review

Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important crop by production in metric tonnes worldwide, after maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aestivum* L.), and the United States is the fifth largest producer by metric tonnes (FAO 2012). Potatoes are the number one vegetable crop in the U.S. (USDA 2012) and Minnesota ranks seventh for potato production (National Potato Council 2012). Potatoes are cultivated mainly for fresh consumption, processed foods, production of starch, and for seed tubers. Many characteristics are considered in selecting new potato varieties. Some of the most important traits are tuber shape, tuber skin color, tuber skin texture, flesh color, shallow eyes, yield, maturity, starch content, lack of bruising, resistance to cold sweetening, processing qualities, taste, and resistance to pathogens.

It can be difficult to avoid reductions in the quality of a vegetatively propagated crop like potato when field-grown plants are used for propagation because diseases in those plants can be further multiplied each year. The accumulation of diseases in vegetatively propagated crops is known as degeneration, and the cause of degeneration is often viral disease (Franc 2001). Potato is one of the oldest recorded victims of viral diseases (Bawden 1964). Cultivars that are resistant to a problematic virus or show clear symptoms (i.e. infected plants are easy to detect and remove) are critical to minimizing virus spread. One such problematic virus is potato virus Y (PVY), whose symptoms may vary depending on cultivar, strain of the virus, and even the physical environment (Draper et al. 2002; Mollov and Thill 2004).

Genotypes that are selected for cultivar release must meet objectives determined by plant breeders. Knowing if disease incidence differed by time or location, or both, helps a breeder choose planting locations and management options during field testing that best meets their objectives. Depending on the objective, a breeder may want to increase or decrease the chances that breeding lines may be subjected to PVY.

To screen for resistant plants or susceptible plants that express visual symptoms clearly, it would be best to maximize PVY incidence in early generations because genetic variation in the

breeding population is at its maximum from which to select. When evaluating quality traits, such as yield, it is best to minimize PVY infection over the years of breeding because PVY reduces yield and can compromise quality traits (Nolte et al. 2004; Whitworth et al. 2006). If quality traits are compromised, then the true genetic potential of the genotype may not be known. This study investigated three different field locations and different times of PVY testing during the first field season in the University of Minnesota (U of M) Potato Breeding Program with the purpose of 1) estimating PVY incidence in the U of M Potato Breeding Program's field-grown nuclear seed, 2) determining if PVY incidence differed by location, and 3) determining if PVY incidence differed by sampling time.

Potato Virus Y

Particle structure and composition

Potato virus Y (PVY) is the type species of the *Potyviridae* family and the *Potyvirus* genus. The *Potyvirus* genus includes 142 approved and 32 tentative species, making *Potyvirus* the largest group of plant viruses (Adams et al. 2011). Virons (complete infectious particles) of Potyviruses are filamentous, nonenveloped flexous rod-shaped particles. The PVY viron is about 730nm long (Delgado-Sanchez and Grogan 1966) and 11nm wide (Varma et al. 1968). Coat protein makes up about 95% of the mass of virons of Potyviruses (Hollings and Brunt 1981). PVY has a monopartite genome composed of single-stranded positive sense RNA, approximately 10 kb in length (Makkouk and Gumpf 1974). The genome encodes a large polyprotein that is cleaved by virus-encoded proteinases into nine or more functional proteins (Urcuqui-Inchima et al. 2001). The functions of these proteins are not all known, but some are known to have multiple functions. Functional proteins are critical to facilitating genome replication, cell-to-cell movement using plasmodesmata, and transport through a plant's vascular system (Carrington et al. 1996; Maule et al. 2002; Nelson and Citoysky 2005; Scholthof 2005).

Distribution and host range

PVY is distributed worldwide. Most members of the *Potyviridae* family have restricted or very restricted host ranges, but a few occur naturally in a wide range of dicot and monocot species. In addition to potato, members of *Potyviridae* infect many economically important crop species, including tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annum* Linn.); thus, PVY infections have attracted the attention of plant pathologists. PVY was first described in the 1930s in potato (Smith 1931). The host range of PVY is mainly members of the *Solanaceae* family, although some members of *Amaranthaceae*, *Chenopodiaceae*, *Asteraceae,* and *Fabaceae* are also susceptible. PVY has been shown to be transmitted by mechanical inoculation with sap to about 120 species (Edwardson, 1974; Horváth, 1979). In most U.S. potato seed production areas, there are few known alternative cultivated hosts (Gray et al. 2010). However, solanaceous weeds, especially the nightshades, can be sources of virus in potato production settings. This has been demonstrated in the Pacific Northwest where hairy nightshade (*Solanum sarrachoides*) has been shown to be an important reservoir of PVY (Cervantes and Alvarez 2011).

Seed certification programs' role in managing PVY

Seed potato certification is a formal, impartial system where a state agency sanctioned by federal and state law certifies that participating growers follow certification requirements and meet standards for disease levels, clonal/varietal purity, and tuber condition (USDA 2010; United States Potato Board 2007). Minnesota is one of 15 states that produce seed potatoes, and all of these states have seed certification programs (United States Potato Board 2007). All of the seed producing states are in the northern part of the United States because, in the past, northern regions had fewer problems with aphid-transmitted potato viruses than southern regions (Radcliffe et al. 2008). Important aspects of managing PVY with seed certification protocols include limiting the number of generations that potatoes can be grown as seed, summer field inspections, and post-harvest testing.

The certification of U.S. seed potato lots is based on a *limited generation* scheme, also referred to as a *flush out* scheme. A lot of seed potatoes is a group of potatoes consisting of one

variety, from one or more fields, grown on the same farm, and stored in physical separation from other lots (MN Department of Agriculture 2008a). In this limited generation scheme, certified seed potato lots can only be increased in the field for up to seven years (USDA 2010). The assumption for this is that over multiple years, there is a greater risk of potatoes acquiring diseases and if seed tubers have disease, vegetative propagation increases the number of seed tubers that have disease. In this limited generation scheme, *generation* does not necessarily refer to how many years a seed lot has been field grown, but rather to where a seed lot stands in terms of meeting tolerances for disease, varietal purity, and defects; thus, diseased lots may be downgraded to a higher generation or be totally rejected for certification if tolerances are exceeded. In other words, the number of field generations is as important as the disease level in determining a seed lot's generation classification. While states do not all use the same terminology for the generational classes, they all begin with a class where seed is produced in a controlled environment under sanitary conditions, followed by seven or fewer generations of field production and multiplication (Potato Association of America 2010). In Minnesota, the main classes for certified seed are nuclear class, generations 1 to 5, and certified class (MN Department of Agriculture 2011a) (Table 1). Tolerances for PVY in Minnesota certified seed potato lots are 0% for nuclear class seed, 0.5% for generations 1 to 5, and 1% for certified class seed (Table 1) (MN Department of Agriculture 2011a).

All states that produce certified seed potatoes require at least two field inspections during the growing season and one post-harvest evaluation (United States Potato Board 2007). For PVY, these inspections in Minnesota are mostly done visually. For varieties that have mild or no visible symptoms of PVY, seed potatoes undergo laboratory tests (Minnesota Department of Agriculture 2011b). If PVY (or any of the diseases a certification agency monitors) is found in a seed lot, but is below the tolerance level, Minnesota rules require that diseased or defective potato plants be rogued (removed) before the final field inspection (MN Department of Agriculture 2008b).

Winter post-harvest testing is important for detecting viruses that have latent symptoms and/or viruses that were acquired late in the summer growing season and therefore did not have time to show foliar mosaic symptoms. Tuber samples from seed lots that are to be

recertified for the next growing season are winter tested in a location where potatoes can be planted and grown from November to January. Minnesota does winter testing in Hawaii and uses ELISA (enzyme-linked immunosorbent assay) to supplement the visual testing of cultivars with mild or latent symptoms.

Not every plant can be visually inspected for PVY and/or tested by ELISA during the growing season or in post-harvest testing. During the growing season, inspections are typically done by examining 100 plants per acre, and 1000 is the minimum number of plants inspected per seed lot. A seed lot can be up to 40 acres (Potato Association of America 2004). In post-harvest tests, the number of tubers per lot required for testing varies widely by state (Potato Association of America 2004). Post-harvest tests in Minnesota require a minimum of 400 tubers per seed lot.

From the mid-1950's to the mid-1980's, it was rare for a Minnesota seed potato lot to be rejected for recertification (Radcliffe et al. 2008). During that time period, typically less than 5% of seed lots were rejected, and PVY was not a big concern (Radcliffe et al. 2008). Unfortunately, this situation changed in 1988 when 8.2% of seed lots were above the tolerance for PVY in the winter grow-out evaluations and that number was almost twice as high in 1989 and 1990 (Radcliffe et al. 2008). Then in 1991, an unprecedented 32.1% of seed lots entered into the Minnesota Seed Potato Certification Program winter grow-out tests were rejected for recertification because of PVY (Fig. 1) (Data courtesy of MN Department of Agriculture). With the exception of 1993, every year since 1991 has seen over 5% of seed lots entered into the Minnesota Seed Potato Certification winter grow-out tests rejected for recertification because of PVY (Fig. 1) (Data courtesy of MN Department of Agriculture). From 1998 to 2012, there were nine years that had more than 40% of seed lots rejected due to PVY incidence above the 0.5% certification standard (Fig. 1). In Minnesota, some of the worst years for recertification rejections in winter grow-out trials were 2004 and 2011, when 62% and 65% of lots were rejected, respectively, because of PVY. The high level of rejections in 2011 may have been due to unusually high numbers of an important vector, the soybean aphid, present in many upper Midwest fields that year (Ian MacRae, personal communication). In 2012, the number of seed lots rejected dropped to 43%.

Economic importance of PVY in potato

PVY is economically harmful to the potato industry for multiple reasons. For all growers, PVY reduces yield (Nolte et al. 2004; Whitworth et al. 2006). Seed growers have the additional concern of having their seed lots downgraded or deemed ineligible for recertification if PVY incidence exceeds the 0.5% certification tolerances. This not only causes loss of farm income for the seed grower but can also result in shortages of certified seed. PVY remains viable in potato tubers. Therefore, when PVY levels are high in a seed producer's field, there is an increased initial inoculum level for next year's seed potato crop, thus increasing the chances of that year's lots being downgraded or rejected. Another issue is rejection of raw potatoes at potato processing and shipping plants. If strains of PVY that cause tuber symptoms become more common, growers may also face rejections from the companies that process and ship potatoes because of poor quality tubers. Tuber symptoms are discolored rings on the skin and necrotic tissue under the discolored rings that may spread into the tuber flesh (USDA 2013a).

Strains of PVY

Strain is an important variable to consider as different PVY strains may have different symptom expression in their hosts. This is problematic in cases where some strains have mild symptoms in the foliage or tuber symptoms, or both mild foliage symptoms and tuber symptoms. It was once the case that symptoms of PVY were easily observed in foliage; therefore, PVY was effectively managed by seed certification programs and growers doing visual inspections. Tuber symptoms were not a concern in North America until 1990 when PVY^{N} was found in seed potatoes in Prince Edward Island and New Brunswick, Canada (Singh 1992). PVY^{N} is important because a variant of PVY^{N}, PVY^{NTN}, is associated with tuber symptoms (Beczner et al 1984; Le Romancer et al. 1994). Since the 1990s, strains associated with mild foliage symptoms and/or tuber symptoms have increased and are displacing PVY^{O} (Baldauf et al. 2006; Crosslin et al. 2002; Gray et al. 2010; Piche et al. 2004).

Historically, PVY had three main strains: PVY^O, PVY^N, and PVY^C. PVY^O is known as the *common* or *ordinary* strain. It has a worldwide distribution (De Bokx and Huttinga 1981). PVY^O may induce mild to severe leaf mottling in potato, as well as rugosity or leaf-drop streak. PVY^O causes systemic mottling in tobacco. PVY^N, the *tobacco veinal necrosis strain*, has been reported in Europe, North America, Africa and South America (Brunt 2001). PVY^N usually causes a very mild leaf mottling in most potato cultivars and induces severe systemic veinal necrosis in tobacco. PVY^C is known as the *stipple streak strain*. Its distribution includes Australia, India, and in some parts of UK and continental Europe (De Bokx and Huttinga 1981). Many potato cultivars have a hypersensitive response to strains in the PVY^C group (Calvert et al. 1980). Susceptible cultivars may show systemic mosaic or stipple streak. In tobacco, symptoms of PVY^C are similar to those caused by PVY^O strains. PVY^Z (Jones 1990) and PVY^E (Kerlan et al. 1999) are sometimes included in this group of historic strains.

Nomenclature of the PVY strain groups

Nomenclature of PVY strain is based upon: 1) host response/genetic classification, 2) presence of tuber symptoms, and 3) molecular classification. Historically, potato isolates of PVY were divided into strain groups based on the systemic and local symptoms they induced on potato and tobacco using *Nicotiana tabacum* (cvs. White Burley and Samsun NN), *Physalis floridana,* and *Solanum tuberosum* cv. Duke of York (De Bokx 1961; De Bokx and Piron 1978). Other potato cultivars with different resistance genes that induced hypersensitive resistance were used as a set of indicators, distinguishing between the PVY^O, PVY^C, PVY^N, PVY^Z and PVY^E strains (Baldauf et al 2006; Blanco-Urgoiti 1998a; De Bokx 1961; Dykstra 1939; Jones 1990; Kerlan et al. 1999; Nie and Singh 2002; Singh et al. 2008). Second, when isolates of PVY^N with the potato tuber necrotic ringspot disease (PTNRD) phenotype emerged from within the PVY^N strain group, these strains became known as PVY^{NTN} (Beczner et al. 1984; Glais et al. 2002; Le Romancer et al. 1994). The third aspect of virus research that has driven PVY strain nomenclature, molecular classification, is very important because most recombinant strains cannot be accurately classified by other methods. For example, most recombinants induce veinal necrosis in tobacco, making them indistinguishable from PVY^N (Barker et al. 2009; Lorenzen et al. 2006; Singh et al. 2008). While serotype is a molecular classification, serotype

currently only classifies a strain as PVY^{O} or PVY^{N}. Therefore, serology doesn't detect any recombinants. Reverse transcription polymerase chain reaction (RT-PCR) assays and genome sequencing are the molecular techniques now used to classify recombinant strains by determining the number and position of recombinant junctions in a PVY genome (Karasev and Gray 2013).

Recombinant strains of PVY

Recombinant strains are made from segments of the PVY^{N} and PVY^{O} genomes (Gray et al. 2010; Hu et al. 2009a; Karasev and Gray 2013). PVY^{N} and PVY^{O} are the parents of at least nine recombinant genomes (Hu et al. 2009b). Currently, the most common recombinant strain groups in potato production are $PVY^{N:O}$, $PVY^{N\text{-}Wi}$, and PVY^{NTN} (Karasev and Gray 2013). Sequencing has helped determine that some strains once thought to be different are actually the same. For example, $PVY^{N\text{-}Wi}$ has also been known as $PVY^{N:O}$, $PVY^{N\text{-}Wilga}$, $PVY^{N\text{-}W}$, and $PVY^{N\text{-}Wi\text{-}P}$ (Blanco-Urgoiti et al. 1998b; Chrzanowska 1991; Glais et al. 2002; Kerlan et al. 1999; Nie and Singh 2002, 2003). Considering how fast strain composition has shifted from PVY^{O} toward recombinants, determining taxonomic relationships among PVY strains is likely to be an ever-changing endeavor(Karasev and Gray 2013).

Strains of PVY that have mild symptoms

An important complication to managing this PVY epidemic is the increasing prevalence of the PVY strain N-Wi also known as the recombinant strain N:O. In 2002, visual observations in seed lots of the North Central Plains were consistent with ELISA for 83% of PVY infections. In 2004, however, only 50% of visual observations identified ELISA-detected infections (Davis et al. 2006a). Therefore, as $PVY^{N\text{-}Wi}$ has increased in prevalence, visual detection has become more difficult. In virus surveys in 2003, 2005, and 2007, $PVY^{N\text{-}Wi}$ accounted for 30%, 56% and 75% of PVY infected plants, respectively (Davis and Radcliffe 2008).

PVY virus-vector pathosystem

PVY is non-persistently transmitted by more than 50 aphid species (Radcliffe and Ragsdale 2002). Aphids select a host plant by probing into a plant's phloem with a mouthpart called a stylet. When probing a PVY-infected plant, an aphid can acquire the virus on its stylet.

If the aphid moves to an uninfected plant, the virus may be transmitted. If this occurs, the aphid would need to reacquire the virus in order to transmit PVY again.

Colonizing and non-colonizing aphids and Transmission efficiency

Upon probing, aphids will colonize or not colonize depending on whether or not the plant is recognized as a preferred host. *Myzus persicae* (green peach aphid) and *Macrosiphum euphorbiae* (potato aphid) will colonize potato and are important and efficient vectors of PVY (Radcliffe and Ragsdale 2002). Non-colonizing aphids are typically inefficient vectors; however, they can still be important in virus spread if their population density is high. *Aphis glycines* (soybean aphid), *Acyrthosiphon pisum* (pea aphid), and *Rhopalosiphum padi* (bird cherry-oat aphid) are some common non-colonizing aphids (Radcliffe and Ragsdale 2002); however, if thousands of aphids move through a potato field, the risk of PVY transmission is significant in the presence of adequate inoculum because these aphids may sample many potato plants before leaving the field.

Aphid species vary in their ability to transmit PVY. Davis and Radcliffe (2008) determined that PVY^{O}, $PVY^{N:O}$, and PVY^{mixed} (infected with both PVY^{O} and $PVY^{N:O}$) strains differed in their transmission based on the efficiency of four important aphid vectors: *Myzus persicae* (green peach aphid), *Aphis glycines* (soybean aphid), *Rhopalosiphum padi* (bird cherry-oat aphid), and *Acyrthosiphon pisum* (pea aphid). The green peach aphid was the most efficient PVY vector and transmitted all PVY strains equally well (30% average efficiency). The soybean aphid also transmitted all PVY strains equally, but had a lower transmission efficiency (12% efficiency) than the green peach aphid. The pea aphid did not transmit PVY^{O}, but it was able to transmit $PVY^{N:O}$ and PVY^{mixed} with 3% and 7% efficiency, respectively. The transmission of PVY by the bird cherry-oat aphid had an 8% transmission efficiency from PVY^{O} and $PVY^{N:O}$ sources, but this was reduced to 2% when acquiring from a PVY^{mixed} source (Davis and Radcliffe 2008).

Aphid migration and dispersal

There is an association between aphid-transmitted potato viruses and aphid migration (Boiteau and Parry 1985; Sigvald 1989, 1992; Zhu et al. 2006). Most aphid species presumed to be important PVY vectors do not survive cold winters; therefore, each spring, these aphids repopulate the northern Great Plains by migrating from lower latitudes (Zhu et al. 2006). Aphids

are weak fliers (Haine 1955). In order to move across a continent, migratory aphids depend on the movement of air currents such as low-level jet streams (Pedgley et al. 1995; Zhu et al. 2006). In the spring, air moves from the subtropics, and organisms that are at a dispersal-ready life stage can be transported on low-level jet streams across the Central U.S between the Rocky and Appalachian Mountains (Johnson 1995; Westbrook and Isard 1999). In most mid-latitude North American agricultural regions, continental-scale weather systems such as low-level jet events occur each spring; therefore, aphid population density can suddenly and dramatically increase (Stakman and Harrar 1957).

There are several ways that aphid migration is terminated. Gravity, cold, fatigue, impaction, and precipitation can all end or contribute to the end of aphid migration (Westbrook and Isard 1999). The northern edge of low-level jet streams usually results in precipitation in the Great Plains (Pedgley 1982) and these precipitation events are potential deposition mechanisms for aphids migrating on low-level jet streams (Zhu et al. 2006). Also, winged aphids can fly down, thus they have some control in choosing their destination (Radcliffe and Ragsdale 2002).

The asymptomatic expression of potato virus Y

In 1974, De Bokx and Mooi stated: "Looking for symptoms caused by a pathogen and counting the diseased plants per unit is the oldest way of quality assessment. Although no exact figures are known, it must be considered as a reliable method for producing certified seed. Visual observation for the presence of virus symptoms during the growing season will give a good impression of the health of the crop as far as viruses are concerned." While this may have been true in 1974, with the emergence and spread of PVY strains that have mild symptoms and the use of cultivars that have poor expression of mosaic symptoms, visual observation is becoming a less reliable indicator of plant health. More recently, researchers have reported that it is difficult or impossible to prevent virus incidence increase by visually observing foliage (Davis 2006; Singh et al. 1999; Sturz et al. 1997). Potato genotypes that have poor expression of mosaic symptoms in the foliage when infected with PVY are often referred to as asymptomatic (Mollov and Thill 2004); however, they are not truly asymptomatic because infected genotypes

still may exhibit yield loss (Nolte et al. 2004; Whitworth et al. 2006). Nevertheless, in the potato research community, the term *asymptomatic* is commonly used to refer to potato genotypes that have poor mosaic expression in the foliage. If breeders release varieties that are susceptible to disease and do not show visual symptoms, these symptomless plants will be problematic for the potato industry because 1) virus-infected plants would be difficult to detect and rogue, 2) they may provide an inoculum source to spread PVY, and 3) additional diagnostic testing may be required by certification agencies to identify these plants. Furthermore, in breeding programs, asymptomatic plants can result in incorrect selection decisions and the erroneous utilization of asymptomatic plants as parents in crossing. In potato, it has been demonstrated that using such plants as parents is unwise because the asymptomatic trait for PVY is highly heritable (Mollov and Thill 2004).

Asymptomatic cultivars

Sturz et al. (1997) were the first researchers to evaluate and publish research about the erroneous use of mosaic symptom expression as a measure of the incidence of PVY^{O}. They chose 'Shepody' since it was known to have poor expression of mosaic symptoms when infected with PVY. Their objective was to determine the accuracy of visual inspections in Prince Edward Island, Canada. From 1993 to 1995 they performed visual inspections at 60 and 82 days after planting, analogous to the 'early' and 'late' inspections done by seed inspectors in their region, and immediately after each visual inspection, leaves were collected and tested for PVY^{O} by ELISA. Compared to ELISA, the visual assessments had some percentage of false negatives and false positives for all three years at both inspections. The incidence of false positives (situations where PVY symptoms were observed visually but not detected by ELISA) were typically quite low. In 1994 and 1995 all samples were less than 4%, but the early season evaluation in 1996 had a 10% false positive. The percentages of visual false negatives (no visual observation of PVY^{O} but a positive ELISA) were much higher, and this is a troublesome situation because infected plants went undetected. The visual false negatives ranged from 6.7% to 100%. One of the more disturbing results occurred in the late season evaluation of 1994, when 32 of 36 plants

tested positive by ELISA had no visual symptoms, an 89% visual false negative. These results strongly recommend that 'Shepody' be evaluated by ELISA rather than visual observations.

Singh et al. (1999) came to a similar conclusion when they evaluated 'Atlantic', 'Norchip', 'AC Novachip', 'Red Pontiac', 'Russet Burbank', 'Russet Norkotah', 'Shepody', and 'Superior' for PVY^{O}. They compared nucleic acid spot hybridization (NASH), reverse-transcription polymerase chain reaction (RT-PCR), visual diagnosis, and ELISA. Using NASH and RT-PCR, dormant tubers can be assayed immediately after harvesting, which is an advantage over ELISA because ELISA is not accurate in detecting PVY in tubers (De Bokx and Cuperus 1987; De Bokx and Maat 1979). In total, 1538 tubers were harvested and tested by NASH and RT-PCR (Singh et al. 1999). After the natural dormancy break, a seed piece was removed from each tuber and grown in the greenhouse in order to observe visual symptoms on the foliage and to test by ELISA. Of the eight cultivars evaluated, only 'Red Pontiac' and 'Russet Burbank' were accurately assessed based on the visual symptoms. Much disagreement was found for PVY^{O} diagnosis among visual symptoms and other detection procedures for 'Russet Norkotah' and 'Shepody'. They concluded that supplementing current certification systems with NASH or RT-PCR post-harvest testing of tubers for PVY can help reduce virus incidence in future generations by more accurately determining which seed lots have low virus incidence

Draper et al. (2002) investigated factors affecting mosaic symptom expression of PVY infection in 'Russet Norkotah', 'Shepody', and 'Red LaSoda'. 'Red LaSoda' expresses severe mosaic symptoms when infected with PVY, while 'Russet Norkotah' and 'Shepody', as mentioned earlier, may not express typical mosaic symptoms. The objective was to categorize 'Shepody' and 'Russet Norkotah' as PVY-resistant or susceptible relative to 'Red LaSoda'. The experiments were on plants with primary, rather than secondary (tuber-borne) infection. This means the researchers inoculated foliage with PVY rather than beginning the experiment with infected tubers. They used quantitative ELISA to measure the relative rate of replication of PVY in these three cultivars, and observed that virus titers in these cultivars were all similar (Draper et al. 2002). They also investigated the effects of light intensity and infections of PVY and *potato virus X* (PVX), alone and as mixed infections, on the effects of symptoms in these three cultivars. In examining the three-way interaction of cultivar, virus infection, and light intensity, plant

height of healthy control plants was significantly greater than virus-infected plants in low light intensity (except in 'Shepody'). At high light intensity there was not much effect of cultivar-virus combinations on plant height. When cultivars were scored for mosaic symptoms on a numerical scale, low light intensity significantly increased the expression of disease symptoms in 'Red LaSoda' and 'Shepody' for PVX, PVY, and the combination of PVX and PVY; however, this was not true for 'Russet Norkotah' which looked healthy. This study concluded that 'Russet Norkotah' was susceptible to infection and replication of PVY but was resistant to foliar symptom expression.

Davis (2006) examined the extent of viruses and PVY strains in Minnesota seed lots and determined if visual virus indexing correlated with ELISA virus indexing. His work included a comparison of visual and ELISA results for virus detection in seed lots. The 2004 seed lots from the Minnesota Department of Agriculture's (MDA) Seed Potato Certification Program were visually scored for expression of the virus. Then, 1132 potato leaflets were collected from plants that were scored visually positive by MDA personnel. In addition, 971 leaflets from plants visually scored negative for PVY were collected and tested by ELISA. For the 2004 seed lots, Davis calculated the predictive value for estimating viral infection using visual indexing and the overall PVY score for 35 cultivars was 0.50. In other words, if a plant was assessed a positive visual reading, there was a 50% probability that the plant was actually infected with PVY. Davis (2006) concluded: "Visual virus indexing is no better than flipping a coin. Our research suggests that virus indexing on the basis of visual symptoms alone is unlikely to achieve the level of diagnostic accuracy required for seed potato certification to be truly effective."

At present, Oregon State University maintains a list of cultivars having poor PVY symptom expression based on observations from certification agencies and information shared at national meetings (Oregon State University 2011). Currently, this list has 21 cultivars and notes which U.S. states have difficulty in the visual evaluation of a particular cultivar. 'Shepody' and 'Russet Norkotah' stand out on the list, with eight and eleven states, respectively, noting poor symptom expression in these two cultivars.

Reasons PVY has become a major problem in potato

In North America, prior to 1990, PVY had obvious symptoms in the field and PVY was effectively managed by seed certification programs (Gray et al. 2010). There are now three major reasons why this is no longer the case: 1) certain recombinant strains of PVY are spreading, 2) asymptomatic cultivars have been widely planted, and 3) increased late season infections have been attributed to soybean aphids, a vector identified in 2002 (Alleman et al. 2002; Gray et al. 2010). In the Northern Great Plains, it can also be said that increased canola and soybean production as well as some changes in pesticide applications have contributed to the PVY problem by favoring aphid populations (Radcliffe et al. 2008). Also, the trend of warming temperatures could be beneficial to the green peach aphid (Davis et al. 2006b).

Before 1990, PVY^{O} was the only strain of PVY known to occur in North America (Singh 1992). The trend that recombinants like $PVY^{N:O/N-Wi}$ and PVY^{NTN} are displacing PVY^{O} means that strains with poor symptom expression are replacing a strain that generally has good symptom expression (Gray 2007; Lorenzen et al. 2006). While the reason for this displacement is not known, it suggests the recombinant strains may have a competitive advantage (Gray et al. 2010; Karasev and Gray 2013). Whatever the reason(s), strains inducing poor symptom expression have compromised the efficiency of seed certification procedures, possibly resulting in increased virus incidence in seed stocks (Karasev and Gray 2013).

Asymptomatic cultivars are another contributing factor to the ongoing PVY problem. These cultivars demonstrate that plant breeding and pathology have not always been well synchronized (Gray et al. 2010). It is critical that breeders release cultivars that are resistant to PVY or, at minimum, show clear symptoms to PVY. Evaluating these traits early in breeding can prevent the advancement and release of asymptomatic clones (Mollov and Thill 2004). Some cultivars with poor symptom expression have been widely adopted and used by the potato industry (i.e. ‘Shepody’ and ‘Russet Norkotah’). ‘Shepody’ was released in 1980 (Young et al. 1983) and ‘Russet Norkotah’ in 1987 (Johansen et al. 1988). It is possible that the adoption and use of these cultivars that were released in the 1980’s contributed to the PVY epidemic that reached unprecedented levels in Minnesota in 1991. Although it has been documented since the late 1990s that ‘Shepody’ and ‘Russet Norkotah’ can be asymptomatic, these clones are

desired by the potato industry; therefore, seed growers (locally and nationally) still produce certified 'Shepody' and 'Russet Norkotah' seed (Fig. 2 and 3). The potato industry can be slow to stop growing cultivars they have embraced; therefore, potato breeding programs now recognize the need to be cautious when releasing cultivars, avoiding germplasm that lacks PVY symptom expression (Mollov and Thill 2004).

For late season infection, the soybean aphid is an especially important consideration. The soybean aphid, a native of eastern Asia, was first detected in the U.S. in July 2000 in Wisconsin (Alleman et al. 2002). By the end of that summer, soybean aphids were present in 10 North Central U.S. states (Venette and Ragsdale 2004). Soybean aphids move through potato fields in mid to late summer, which is later than some other non-colonizing aphids (Gray et al. 2010; Schramm et al. 2011). Soybean aphid populations can reach very high densities (Ragsdale et al. 2004), so although they are inefficient vectors of PVY, they can still dramatically increase virus incidence in potato fields (Gray et al. 2010). When potato plants are infected with PVY late in the growing season by soybean aphids and/or other migrating aphids, there may not be time for foliar symptoms to manifest in the potato plant, yet tuber infection can still occur (Gray et al. 2010). It is in this way that late-season infections play a part in the discrepancy between acceptable summer field inspection ratings and postharvest test results that do not meet standards for recertification (Gray et al. 2010).

Potato Breeding

Advancing clones

A potato breeding program typically starts with selecting parents with traits of interest and making crosses to obtain botanical seed, also called *true potato seed*. Botanical seed is sown in a greenhouse during the summer so that minitubers will be ready for field planting the following spring. Alternatively, botanical seed may be planted in a greenhouse during late winter so that seedlings will be ready to be transplanted directly into the field by spring. In either case, this is the F_1 generation, where each plant is a different genotype. From this generation on, all plants will be produced asexually and *seed* refers to an asexual propagule (i.e. daughter tubers). In the field, each minituber or transplant is planted in a *single hill*. The single-hill plants produce multiple tubers (clones) from each genotype. The single-hill plants are

planted such that there is enough space, within row, between plants that the genotypes will not be mixed when tubers are mechanically harvested. At harvest, the tubers are evaluated visually in the field for desirable market traits. Only a small percentage of single-hill plants will be selected for advancement in the breeding program.

In the second field season, there are usually enough tubers from a genotype to be planted in a minimum of a *four-hill unit* (4 adjacent hills originating from the same source tuber) and planted at multiple locations. If selected for advancement to a third field season, a clone should have produced enough seed for replanting in multiple location trials and various disease screenings the following year. Each year, clones are either dropped or advanced depending on their performance. Developing and releasing a new variety can take 15 years.

Some breeding programs use the terminology of seed certification agencies when advancing clones. This terminology can vary by state. In Minnesota seed potato terminology, tubers from greenhouse-grown plants produce *nuclear class seed*. The first field increase can be called the *nuclear* generation until tubers come out of the ground. Those tubers are called *G1 class seed*. G1 seed is used to plant a G1 field that produces G2 seed, and so on. In a breeding program, those clones are *experimental* and, unlike certified seed, the number of generations allowed for experimental clones is unlimited.

Practices that influence PVY in a breeding program

Due to the number of years that potato breeding programs evaluate field-grown, asexually propagated clones, breeding programs use various strategies in order to maintain or regenerate clean seed. For controlling PVY, these strategies include tuber indexing, early harvest, tissue culture, increasing seed at a seed farm located far from commercial production, visually roguing symptomatic plants, and barrier crops (Davidson et al. 2013; Franc 2001). Unfortunately, some of these strategies may do little to minimize PVY in a breeding program. Worse yet, some strategies may do more harm than good.

Effective strategies include tuber indexing, early harvest, tissue culture and barrier crops. Tuber indexing (also called eye indexing) is done between growing seasons to identify and remove infected plants so that clones progressing to the next growing season will likely be

virus free. On a potato tuber, an eye is a sprout emerging from the tuber. Tuber indexing involves taking an eye from each genotype (or even each tuber) and testing the eye for virus or planting the eye in a greenhouse so that other phenotypic evaluations can be done along with virus testing (Franc 2001). Any clone or tuber that fails tuber indexing evaluation may be discarded from the breeding program before the next growing season. Early harvest involves manipulating the planting and vine kill dates so that tubers can be harvested before aphid arrival. The early harvest strategy may have limited value in a breeding program, such as the University of Minnesota's breeding program, which has multiple, distant field locations and differing maturities in genotypes. Nevertheless, early harvest is an option that could be used for breeding lines of special importance. Using barrier crops, also called border crops, to control PVY is a strategy where a secondary crop that is not a host to PVY is planted around potatoes to act as a virus sink where viruliferous winged aphids land and probe the barrier crop. This can remove PVY from an infected aphid's stylet before the aphid moves into potatoes (DiFonzo et al. 1996). Due to space constraints of a breeding program, the use of this strategy would likely be limited to genotypes of special importance. Tissue culture is used to store and multiply virus-free clones. Also, virus-free clones can be generated from infected plants using tissue culture protocols that involve heat, anti-viral drugs, meristem excision, and cryotherapy (Kunkel 1943; Quak 1961; White 1934; Kaczmarczyk et al. 2010). A breeding program will use virus clean-up procedures for advanced selections that have the potential to be released as commercial cultivars. Virus-free clones can be multiplied so that clean potato plants can be evaluated by growers or for evaluations in regional trials, national trials, and processor trials. Since 2010, the University of Minnesota Potato Breeding Program has used border crops around advanced selections that have been returned to field trials after having had viruses removed by clean-up procedures (Christian Thill, personal communication).

Increasing seed at a location far from commercial production can be ineffective. For example, the University of Minnesota potato breeding program has often used a research farm in Grand Forks, ND as a place to increase seed for the next year's trials. However, in this study, when random leaf samples were tested from Minnesota's single-hill fields in 2007, 2008, and 2009, high levels of PVY were detected.

Visual roguing of symptomatic plants in the field is also an ineffective strategy for managing PVY. This strategy also has multiple negative consequences in a breeding program. First, if care is not taken when roguing, viruliferous aphids may escape from plants that are being removed and land on healthy plants that may then become infected. Recommendations for roguing include carrying plants out of the field in a closed container, moving plants at least 150m away from the field, and covering the pile to prevent aphids from escaping the cull pile (Franc 2001).

Another negative aphid-related consequence of roguing is the creation of gaps in the field. Aphids tend to enter fields at the interface of foliage and fallow ground (Smith 1969, 1976). Thus, roguing might not reduce virus spread and can even increase virus spread (Bell 1989; Davidson et al. 2013; Davis et al. 2009). In experiments that created planter skips and impaired stands, Davis et al. (2009) concluded that a gap equivalent to three or more missing plants favored PVY spread in potato fields and that seeding the gaps with oats to reduce the contrast between potato foliage and soil did not have significantly less virus spread than leaving the gaps between potato plants fallow.

Three of the worst and potentially most far-reaching negative consequence of roguing are 1) the selective pressure roguing puts on strains of the virus, 2) roguing reduces genetic variation in a breeding population, and 3) rogueing may leave behind clones that fail to show clear viral symptom expression. If a field contains the PVY^{O} strain, which generally shows clear foliage symptoms, and recombinant strains with mild foliage symptoms, then roguing will likely shift the PVY population towards the more undesirable recombinant PVY strains. In a field of early generation breeding lines, the PVY reactions of each genotype are not known. Therefore, roguing symptomatic plants from fields can leave remaining breeding populations with an abundance of asymptomatic clones. These asymptomatic clones are troublesome in a breeding program because previous research on PVY in the University of Minnesota Potato Breeding program determined the asymptomatic phenotype is a heritable trait (Mollov and Thill 2004).

Research Objectives

The objectives of this research were to: 1) estimate the PVY incidence in the University of Minnesota Potato Breeding Program's nuclear seed grown in three locations (Becker, MN; Grand Forks, ND; and Williston, ND), 2) determine if PVY incidence differed by location, 3) determine if PVY incidence differed by time.

Materials and Methods

Material

Seven populations of nuclear seed were planted in 2007, 2008, and 2009 in three locations (Becker, MN; Grand Forks, ND; and Williston, ND) with seed that originated from greenhouses at Colorado State University (CSU), North Dakota State University (NDSU) and Oregon State University (OSU) (Table 2.). Nuclear seed was produced from greenhouse-grown botanical seed (true potato seed), resulting in minitubers with different genotypes. Plants grown from nuclear seed are at their first field increase. The populations were generated by first combining minitubers from families (progeny from a crosses) having the same market type (fresh market red, round white for chip processing, or russet for fry processing) and origin (CSU, NDSU, and OSU). The Oregon population was a mixture of market types because of the amount of available seed. There were seven populations: Colorado Reds, Colorado Russets, Colorado Whites, North Dakota Reds, North Dakota Russets, North Dakota Whites, and Oregon Mix (Table 2.). New, genetically different, minitubers were produced each year. The minitubers were then weighed and divided by weight for planting at the three locations. There was not enough seed to plant every population at each location in every year (Table 2.). The experimental units were single hills, each hill being a different genotype. Minitubers were planted in rows. Rows were 0.91 m apart.

Location descriptions

The three locations differ in terms of soil type, average precipitation, and average frost-free period (Table 3; USDA 2013b). The Becker location is on the University of Minnesota's Sand

Plain Research Farm in Becker, MN. Potatoes grown there are irrigated. Of the three locations, Becker has the highest mean annual precipitation and potentially has the longest frost-free period. The Grand Forks location is at the Red River Valley Potato Research Farm, a grower-owned potato research farm, located eight kilometers south of Grand Forks, ND (Northern Plains Potato Growers Association n.d.). The soil is somewhat poorly drained and potatoes are not irrigated. The Williston location is at the Williston Research Extension Center in the Nesson Valley, 40 kilometers west of Williston, ND. It is typically the driest location.

Weather data

Weather data from 2007 to 2009 (April to September) were obtained from the National Climatic Data Center (NOAA 2013) from the nearest weather station that had temperature and precipitation data available. For Becker, Grand Forks and Williston these weather stations were USC00217502 Santiago 3 E, USC00323621 North Dakota State University, and USW00094014 Williston Sloulin Field International Airport, respectively. Station USC00217502 Santiago 3 E is approximately 25 kilometers northeast of the Sand Plain Research Farm in Becker, MN. Station USC00323621 at North Dakota State University is approximately 8 kilometers north of the Red River Valley Potato Research Farm. USW00094014 at Williston Sloulin Field International Airport is approximately 40 kilometers west of the Williston Research Extension Center, Nesson Valley Farm.

Leaf collection and PVY testing

At each location, leaf samples were taken at random from every population. For each population, the number of leaf samples was substantially greater than the number of observations a seed certification agency would require for inspection based on field size. In 2007, fifty leaves were collected from each population, regardless of the population size. In 2008 and 2009, the random sampling was adjusted to be proportional to the size of the populations. For every 6.8 linear meters, one leaf sample was collected and tested for PVY. The total number of random samples taken in 2007, 2008, and 2009 was 1050, 903, and 1579, respectively. It should be noted that in 2007 the Becker and Grand Forks locations were rogued before leaf samples were collected while the Williston field was not rogued. In each case,

roguing was the removal of plants with mosaic symptoms in the foliage. In 2008 and 2009, none of the fields were rogued.

All leaf samples were tested for presence or absence of PVY by ELISA. Three leaf discs were punched from each leaf sample and inserted into a 1.2mL cluster tube, which was covered and stored at -80 °C until tests were performed. The ELISA was conducted according to manufacturer's directions (Agdia, Elkhart, IN) with the polyclonal kit for PVY. Leaf grinding was done with a Tissuelyser II (Qiagen, Valencia, CA). Plates were read with a BioRad 380 plate reader (BioTek, Winooski, VT) at 405 nm absorbance. A sample was considered positive if the OD_{405} reading was equal to or greater than twice the mean of five negative controls. An exception was for the 2008 Williston samples, which were tested using immunostrips for PVY (Agdia, Elkhart, IN). Immunostrips are a type of serological test that do not yield an absorbance value, rather a positive or negative value.

Data Analysis

Weather analysis

Since the Aphid Alert system was not operating from 2004 to 2012, precipitation and temperature data were used to infer if conditions were favorable for aphid arrival, survival, and reproduction. Precipitation data for May and June were analyzed for each location to infer favorable conditions for early season arrival of aphids. Temperature data were examined to look for temperature conditions reported to favor or impede the survival and/or reproduction of a major colonizing aphid, the green peach aphid (Davis et al. 2006b).

Location analysis

To examine the effect of location on PVY, the proportion of samples positive for PVY was compared among locations using a 3 x 2 chi-square contingency table for each year of data. Dependence was determined by comparing the chi-square value to the critical chi-squared value at $\alpha = 0.05$. These calculations were done using SAS (SAS 9.3, Cary, NC). To examine the nature of any dependence detected by the chi-square test, percentages were calculated to determine the proportions of samples positive for PVY by location and year.

In order to test for general association between location and the presence of PVY while controlling for the different years, the Cochran-Mantel-Haenszel test for repeated tests of independence was calculated in SAS (McDonald 2009). The null hypothesis was that PVY was independent of years across locations. The Cochran-Mantel-Haenszel test is a good choice for circumstances where different subjects are used in an experiment that is repeated multiple times, as in this experiment where genetically different minitubers were planted in three locations over three years with new minitubers each year. This test is good for examining the overall effect of location on PVY, but controls for the possibility of different levels of PVY in different years with different plants.

Locations were compared two at a time in 2 x 2 chi-square contingency tables to determine if there were significant differences in PVY incidence between the two locations in each year (18 comparisons) using SAS. Odds ratios were calculated in SAS so that comparisons could be made between locations, such that the odds of a location's PVY incidence could be compared to another location.

Time analysis across locations

To examine the effect of time on PVY, linear regressions were modeled. For all regressions, years were pooled. The cases where fields were rogued were excluded.

The first set of regressions fit percentages of PVY to the *number of days from planting to leaf collection*. One regression fit the *average PVY at a location/timepoint* to the *number of days from planting to leaf collection*. The other regression fit the *percent PVY in each population* to the *number of days from planting to leaf collection*. There were seven time points for the *number of days from planting to leaf collection*. The time points were: 44, 61, 64, 83, 91, 99, and 122 days from planting to leaf collection.

The second set of regressions fit percentages of PVY to the *number of days from July 1st to leaf collection*. One regression fit the *average percent PVY at a location/timepoint* to the *number of days from July 1st to leaf collection*. The other regression fit the *percent PVY in each population* to the *number of days from July 1st to leaf collection*. There were seven time points for the *number of days from July 1st to leaf collection*. The time points were: 8, 29, 36, 42, 77, and 91 days past July 1st.

Time points were also classified as *early*, *middle*, or *late* season leaf collection times. The proportion of samples positive for PVY were compared to the *early*, *middle*, or *late* leaf collection times using a 3 x 2 chi-square contingency table as described for the location analysis. Percentages were calculated for PVY by *early*, *middle*, or *late* collection times to examine the nature of any dependence detected by the chi-square test. The null hypothesis for this test is that PVY is independent of the *early*, *middle*, or *late* leaf collection times. *Early* season leaf collection time points ranged from 8 to 29 days past July 1st. *Middle* season leaf collection time points ranged from 36 to 42 days past July 1st. *Late* season leaf collection time points ranged from 77 to 91 days past July 1st.

Field maps

Maps of each single-hill field were drawn. The maps included any known feature that may have impacted the incidence of PVY. These features include field gaps, alleys, known sources of inoculum, likely sources of inoculum, compass directions, and adjacent plants that may have functioned as a border. The percentage of PVY in the random sample from each population was plotted on each field map.

Results

Overall, there was a high incidence of PVY in the first field season of the University of Minnesota Potato Breeding Program's nuclear seed grown in Becker, MN; Grand Forks, ND; and Williston, ND. Over all locations and years, 326 of 3532 samples (9.2% of the samples) tested positive for PVY (Table 5). This was statistically significant by the Cochran-Mantel-Haenszel statistics that showed general association between location and PVY when controlling for year (χ^2_{CMH}=263.3, 2 d.f., *P*-value <0.0001; Table 5).

Weather

Normal precipitation occurred in all locations (Fig. 4). Average monthly temperatures were similar for all locations in all years from June through September (Table 4). Both Williston and Grand Forks had days with minimum temperatures below 0 °C into May. On 1 and 2 June

2009 at Williston there was a late spring freeze with temperatures of -11 °C and 0 °C, respectively.

Locations

Dependence was found between location and PVY in 2007, 2008 and 2009 (χ^2, 2 *df* = 6.5, *P*=0.0388; χ^2, 2 *df* = 61.4, *P*<0.0001; χ^2, 2 *df* = 311.7.5, *P*<0.0001, respectively; Table 5). The nature of this association was Grand Forks having the highest percentages of PVY in all three years and Williston having the lowest percentage of PVY, except in 2009 when Becker was slightly lower than Williston (Fig. 5, Table 6).

When locations were compared two at a time, some did not have significantly different levels of PVY. In 2007, only Williston and Grand Forks were significantly different (χ^2, 1 *df* = 5.96, *P*=<0.015), with the proportion of samples having PVY at Grand Forks being 2.17 times greater that of Williston (Table 7), even though the Grand Forks field had been rogued of symptomatic plants while the Williston field was not rogued. The Becker field was also rogued that year; regardless, samples from Becker were 1.35 times more likely to have PVY than those from Williston (Table 7). In 2008, all locations had significant differences for PVY, with Grand Forks and Williston having the largest difference (χ^2, 1 *df* = 59.05, *P*<0.0001). The odds for a leaf sample testing positive for PVY were almost tenfold higher in Grand Forks than Williston in 2008 (Table 7). Becker samples were 3.672 times as likely to have PVY compared to Williston samples (Table 4). Grand Forks samples were 2.565 times more likely to have PVY than the Becker samples in 2008 (Table 7).

The most dramatic results for PVY incidence occurred in 2009, when Becker and Williston were quite low and Grand Forks was high (Table 6). The proportion of samples that had PVY at Grand Forks was 40.4 and 47.6 times that of Williston and Becker, respectively (Table 7). PVY levels in the Becker and Williston fields were not significantly different in 2009 (χ^2, 1 *df* = 0.07, *P*= 0.8; Table 7). The Chi-square tests for each year confirm that the data have evidence against the null hypothesis that there is no relationship between being PVY positive and location (Table 5).

Time

To examine the effect of time on PVY incidence, linear regressions were used. Linear regression between *average PVY at a location/timepoint* and the n*umber of days from planting to leaf collection* was not significant (R^2 = 0.119, *P*= 0.449; Fig. 6). A linear regression between *percent PVY in each population* and the *number of days from planting to leaf collection* was significant (R^2 = 0.145, *P*=0.024; Fig. 7); however the correlation was low. A linear regression between *average PVY at a location/timepoint* and the *number of days from July 1st to leaf collection* was significant (R^2 = 0.577, *P*= 0.047; Fig. 8), as was the linear regression between *percent PVY in each population sample per timepoint/location* and the *number of days from July 1st to leaf collection* (R^2 = 0.5403, *P*<0.0001; Fig. 9).

Dependence was found between the time of season of leaf collection (*early*, *middle* or *late*) and PVY (χ^2, 2 *df* = 217.7, *P*<0.0001; Table 8). The nature of this association is that *late* season had the highest percentages of PVY while *early* season had the lowest (Fig. 10 and 11). The Chi-square tests for the times of season confirm that the data have evidence against the null hypothesis that there is no relationship between leaf samples positive for PVY and whether leaves were collected in early, middle, or late season (Table 8).

Field Maps

Maps were diagramed (Figs. 12-20). In seven of nine instances, there was high PVY (over 5%) in single-hill population samples that were close to G1 plots or other advanced lines. The exceptions were Becker 2009 (Fig. 18) and Williston 2009 (Fig. 20). These cases had the earliest leaf collection times. The highest percent PVY at Becker 2009 was the Colorado russets with 1.9% PVY (Fig. 18). The Colorado russets were next to the G1 field (Fig. 18). Single-hill populations further from the Becker 2009 G1 field all had 0% PVY (Fig. 18). The situation at Williston 2009 was similar. Colorado russets were next to the G1 field on one side, and next to other advanced lines on 2 sides (Fig. 20). Those Colorado russets had the highest PVY with 2.1%. Colorado Reds and Oregon mix in Williston 2009 had 0% PVY in samples (Fig. 20). Those populations were mostly surrounded by other single-hill populations (Fig. 20).

Discussion

The objectives of this research were to: 1) estimate the PVY incidence in the University of Minnesota Potato Breeding Program's nuclear seed grown in Becker, MN; Grand Forks, ND; and Williston, ND; 2) determine if disease pressure differed by location, and 3) determine if disease pressure differed by time. Prior to this study, the University of Minnesota Potato Breeding Program had not tested single-hill breeding genotypes grown from nuclear seed for PVY. Compared to tolerances for PVY in certified seed (0.5%), the percent of PVY incidence was high in samples from U of M single-hill fields. Of the nine fields tested, all had an average PVY incidence over 0.5%, with the lowest being 0.8% (Fig. 5, Table 6). In 2007, two of the fields (Becker and Grand Forks) were rogued for virus symptoms, yet still had high levels of PVY, with 5.7% in Becker and 8.9% in Grand Forks (Fig. 5, Table 6). From the data collected in this study, it was not possible to determine if PVY pressure differed by location. After the exclusion of the two rogued fields (Becker and Grand Forks in 2007) and the remaining data pooled across years, there was an increase in PVY as the growing season progressed.

The importance of seed potato certification and the importance to crop health have been discussed by Gray et al. (2010). Existing seed potato certification guidelines allow for a small, but measurable, amount of PVY (0.5%) for recertification. That being said, there seems to always be opportunity for PVY inoculum to be present in potato production. It is thought that ware potato crops (crops for end use rather than seed) are the main source of inoculum (DiFonzo et al. 1997). In Minnesota, seed potato production is restricted to certain locations mainly near the Red River Valley (Minnesota Department of Agriculture 2012), yet both PVY and aphid vectors have been reported in restricted seed potato growing areas (DiFonzo et al. 1997. While seed potato production is restricted to certain areas, ware production is not isolated from seed production (DiFonzo et al. 1997). The Becker and Williston experiment stations are surrounded by ware production, and both seed potato and ware production occur around Grand Forks. There is uncertainty about how much separation is required between potato seed fields and ware production (or any known PVY inoculum source) to effectively reduce PVY spread. A study done in England suggested 800 meters as a minimum separation from potential sources of PVY (Harrington et al. 1986). Physical separation doesn't guarantee that a crop that enters a

field free of PVY will be harvested PVY free; however, adjacent ware production and seed production can perpetuate PVY inoculum (DiFonzo et al. 1997). The situation can be similar (but more extreme) as in this study where, due to space constraints and various breeding objectives, there was little or no separation between the nuclear seed and advanced lines known to have or likely to have PVY. Also, in a breeding program there may be fairly limited choices for field locations due to large field space requirements if each breeding generation is grown in isolation.

Much was also said in the introduction about PVY strains with mild symptoms, asymptomatic cultivars, and late season infections. These topics are important in the big picture of the PVY problem. In this study, PVY strains with mild symptoms and asymptomatic genotypes can be considered as possible causes of the high levels of PVY found in the 2007 rogued fields (Becker and Grand Forks). The Becker field was rogued July 18, 2007 and leaf samples were collected one month later. The average PVY infection was 5.7%(Fig. 5, Table 6). The exact date that the Grand forks field was rogued is not known, but occurred sometime before the August 20th leaf collection (personal communication, Jeff Miller January 2008). The average PVY infection was 8.9% (Fig. 5, Table 6). Alone or in combination, PVY strain, genotype of the potato, and late season infection could have contributed to high levels of PVY found in these fields that were rogued for mosaic symptoms in the foliage.

Aphid migration and dispersal and weather - what aphids like

While no aphid scouting or trapping was done in this study and Aphid Alert data were not available from 2004 through 2011 (MacRae and Koch 2013; Radcliffe et al. 2008) the conditions were favorable for aphid arrival (Zhu et al. 2006), reproduction and survival in every year of the study (Davis et al. 2006b). Zhu et al (2006) found association between low-level jet duration and green peach aphid migration. During their nine year study, 29 of 30 low-level jet events were associated with rain in May and June and rain is a potential deposition mechanism for aphids migrating on low-level jet streams (Zhu et al. 2006). While normal precipitation occurred every spring at all locations in the present study, the confounding issues of roguing and widely varied leaf collection times, made it impossible to determine if there was correlation between current season PVY and spring rain. Another thing to consider with wind is the origin of the jet streams. Williston gets more wind from western jet streams while Becker and Grand

Forks may get more wind from low-level jet streams originating in the Gulf (Ian MacRae, personal communication). Different jet stream origins may contribute to different species composition and abundance of aphid vectors at Williston Becker and Grand Forks.

Comparing monthly average temperatures (Table 4) to the fluctuating temperature regime reported in the experiment of Davis et al. (2006b), green peach aphids should have survived and reproduced from June to September at all locations in the present experiment across all years. There were late freezes in both Williston and Grand Forks with minimum temperatures below 0 °C into May each year of this study. These late spring freezes could have killed any aphids that may have arrived or hatched by that time. The latest spring freeze in Williston was on 1 and 2 June. Despite late spring freezing temperatures in both Williston and Grand Forks, that may have killed aphids present at the time, it is reported that aphid populations are typically most numerous between mid-July and mid-August (DiFonzo et al. 1997).

Aphids are attracted to the contrast between fallow ground and foliage (Smith 1969, 1976). An example of a small soil-to-foliage interface would be a field with few, if any, missing plants and little or no fallow ground around the field. A large soil-to-foliage interface would have noticeable fallow ground in and around the field. Three things contributed to soil-to-foliage interfaces in this study: 1) all fields had alleys around and through the fields, 2) in most cases there were at least some gaps within fields, and 3) In 2009, the Grand Forks field had standing water next to the single-hill field which contributed to a poor field stand and greater soil-to-foliage interface than non-flooded areas. The present study found high incidence of PVY in fields that had large areas of soil-to-foliage interface. The three-foot gaps used to separate each minituber in single-hill fields resulted in a checkerboard-like pattern that can induce aphid landing (Carroll 2005; Davis et al. 2009; Kennedy et al. 1961). The single-hill plants in this study were planted so that there was enough space between plants that genotypes would not be mixed up when tubers were mechanically harvested. To aphids, this type of planting with wide spacing and different genotypes can have the appearance of an impaired stand that is attractive to aphids and favorable to the spread of PVY (Davis et al. 2009). In Grand Forks, another cause of impaired stands is frequent floods that can cause seed piece decay or plant loss due to

hypoxia or root rot. In 2009 the Grand Forks field had standing water in the G1 field adjacent to some of the single hills. By design, as far as aphid behavior is concerned, single-hill fields function as poor stands and this can be worsened by planter skips, poor emergence, or anything that increases the contrast between foliage and soil (Davis et al. 2009).

Confounding issue

One objective of this study was to determine if disease pressure differed by location. However, this could not be determined because *locations* were confounded with *order of leaf collection within year*. The *location* variable can be changed to *order of leaf collection within year* and the results do not change. In each year, the leaf samples that were collected first, regardless of location, had the lowest percent PVY incidence. Likewise, whichever leaf samples were collected last, regardless of location, had the highest percent PVY incidence. To determine if there was a trend for differences in PVY at Becker, Grand Forks and Williston, PVY testing would need to be done at multiple time points at all locations across a number of years. In this study of PVY incidence in nuclear seed, it was not feasible to test at multiple points in time at all locations due to travel distances and a limited number of people involved in leaf collection and testing. Nevertheless, when years were pooled, the leaf collection times spanned the growing season and it was clear that samples collected later in the growing season were more likely to have PVY than samples collected earlier in the growing season. It should be noted that pooling years may have some risk because there can be differences in aphid counts by year. In a study from 1992 through 1994, Difonzo et al. (1997) found differences in PVY that were related to aphid numbers and species composition. In that study, 1993 had low aphid numbers and few experimental plants became infected with PVY. They did not examine why 1993 had low aphid numbers.

The number of days until leaf collection was determined, with 1 July as the initial day (0 d). Counting the number of days starting at 1 July was biologically important because most aphids will have arrived in the North Central Plains by then and will have had sufficient time to reach populations sizes likely to find PVY inoculum and begin to spread it to uninfected plants. The early part of the growing season (April to June) is not nearly as important for the spread of

PVY because aphids will most likely not have had sufficient time to arrive, reach population sizes likely to find PVY inoculum, and begin to spread it to uninfected plants (DiFonzo et al. 1997).

Conclusions

A practical implication of this study pertains to methodology for PVY resistance breeding. First, starting from the premise that growing nuclear seed is important to breeders so they can have disease-free potato plants for subsequent years, and that genetic variation is at its maximum in early generations and selection among early breeding generations would provide the greatest opportunity for selecting for disease resistance; then a modified breeding procedure similar to that proposed by Mollov and Thill (2004) studying PVY expression could be developed for selecting for PVY resistance. To screen for resistance, it would be best to maximize PVY pressure in early generations. Single-hill fields have too many plants to test each genotype individually for PVY by serology or RT-PCR and few clones advance to the next year. Second field-season clones (G1) likely have small enough numbers that all genotypes could be tested for PVY infection by serology or tested for PVY resistance genes with molecular markers so that infected or susceptible clones would not advance to the third year. With this method, by year three (G2), all clones would be either resistant or have escaped infection. Currently, evaluating PVY symptoms and PVY infection during year three is standard in the Minnesota Potato Breeding Program.

Future PVY research by the U of M Potato Breeding Program could include: 1) solanaceous weeds, and 2) field stand (spacing of plants). Hairy nightshade has been implicated as a PVY reservior in the Pacific Northwest (Cervantes and Alvarez 2011). This plant is widespread in many U.S. and Canadian potato seed production areas and is present in Minnesota (USDA 2013c), yet investigating the role of solanaceous weeds as reservoirs for PVY in Minnesota has not been done. Single-hill fields are excellent places to study field stand because of gaps and spacing that result in many areas with soil-to foliage contrast. Davis et al. (2009) studied seeding gaps with oats in order to reduce plant vs. soil contrast. They created a gap by roguing out a 3.3 m^2 area before row closure and found that covering the gap with oats reduced PVY spread but the difference was not statistically significant compared to insecticide

treatments and suggested that further research would be necessary to determine the effects of seeding gaps with oats to reduce PVY spread. Seeding parts of the U of M's single-hill fields with oats or another plant to reduce the plant to soil contrast may be another future research area.

Considering that 9.2% of the samples from three locations over three years tested positive for PVY, this disease is common and widespread; therefore, a main conclusion from this study is that breeding for PVY resistance has become extremely important. In Minnesota, prior to 1991, certification procedures were enough to keep PVY at low levels and breeders did not have to concern themselves with resistance to PVY (Gray et al. 2010). Considering everything involved in the ongoing national and regional PVY problem (mainly strains with mild and/or tuber symptoms, widely used asymptomatic cultivars, and late season infections), breeding for resistance is of utmost importance. If desirable resistant cultivars of various market types and uses were available and adopted by growers and industry, PVY inoculum could be greatly reduced and hopefully bring an end to the current PVY epidemic.

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Table 1. Requirements for the production of different class of certified seed potatoes in Minnesota (1510 §2330) (Minnesota Department of Agriculture, 2011a).

Minnesota Seed Class[1]	Seed Source Comments	MN Tolerance for PVY[2]
Nuclear class certified seed potatoes (N)	• Must be produced in a greenhouse or screenhouse under sanitary conditions free from insects and weeds that can harbor or transmit potato diseases or other conditions of possible disease contamination.	0.00%
Generation 1 class certified seed potatoes (G1)	• Seed source must be either nuclear tubers or plantlets. • 1st field increase	0.50%
Generation 2 class certified seed potatoes (G2)	• Must originate from G1 class seed • 2nd field increase	0.50%
Generation 3 class certified seed potatoes (G3)	• must originate from G2 class seed • 3rd field increase	0.50%
Generation 4 class certified seed potatoes (G4)	• Must originate from G3 class seed • 4th field increase	0.50%
Generation 5 class certified seed potatoes (G5)	• Must originate from G4 class seed • 5th field increase	0.50%
Certified class certified seed potatoes. (C)	• Must originate from Foundation or Generation classes of seed potatoes. • 6th field increase	1%

[1]Nuclear class seed tolerances are based on testing 200 samples per lot and for the other classes, 400 tubers per lot are tested and a lot can be up to 40 acres (Potato Association of America 2004).

[2]Tolerance means a specified allowance for variation from the standards provided for diseases and physical defects (1510§2305) (Minnesota Department of Agriculture, 2008a).

Table 2. Number of leaves randomly sampled for PVY incidence among nuclear seed populations representing different potato market types while grown in Becker, MN; Grand Forks, ND; and Williston, ND from 2007 to 2009.

				Market Type[1]							
				Red		Russet		White		Mix	Total
Year	Planting Date	Leaf Collection Date	Location	CO[2]	ND[2]	CO	ND	CO	ND	OR[2]	
2007	18-May-07	18-Aug-07	Becker, MN	50	50	50	50	50	50	50	350
	26-Jun-07	20-Aug-07	Grand Forks, ND	50	50	50	50	50	50	50	350
	10-May-07	9-Aug-07	Williston, ND	50	50	50	50	50	50	50	350
2008	17-May-08	16-Sep-08	Becker, MN	52	10	106	8	24	165	94	459
	23-Jun-08	30-Sep-08	Grand Forks, ND	32	-	52	8	12	95	-	199
	15-May-08	6-Aug-08	Williston, ND	43	-	53	22	30	76	21	245
2009	26-May-09	9-Jul-09	Becker, MN	72	-	213	-	128	-	80	493
	12-Jun-09	12-Aug-09	Grand Forks, ND	-	-	230	-	27	-	96	353
	27-May-09	30-Jul-09	Williston, ND	142	-	477	-	-	-	114	733
Total				349	160	1091	188	371	486	745	3532

[1]Market types: red, white, russet, mix.

[2]Seed origins: Colorado (CO), North Dakota (ND), Oregon (OR).

Table 3. Characteristics of the research locations (Becker, MN; Grand Forks, ND; and Williston, ND) where nuclear seed populations were grown and sampled.

	Location		
Site Characteristics[1]	Becker Sherburne County, MN	Grand Forks Grand Forks County, ND	Williston Williams County, ND
Mean annual precipitation	25 to 34 inches	19 to 24 inches	12 to 14 inches
Frost-free period	120 to 180 days	110 to 135 days	110 to 130 days
Drainage class	Excessively drained	Somewhat poorly drained	Well drained
Farmland classification	Not prime farmland	All areas are prime farmland	Farmland of statewide importance
Typical soil profile	0 to 19 inches: Loamy sand 19 to 80 inches: Sand	0 to 60 inches: Silty clay loam	0 to 6 inches: Loam 6 to 60 inches: Clay loam

[1]United States Department of Agriculture (2013)

Table 4. Mean Temperature (°C) data obtained from National Climatic Data Center (NOAA) for Becker, MN; Grand Forks, ND; and Williston, ND during 2007, 2008, 2009.

	2007		
	Becker, MN[1]	Grand Forks, ND[2]	Williston, ND[3]
June	19.6	19.6	18.1
July	22.3	22.1	24.4
August	20.4	18.8	20
Sept	16.7	14.6	14.3
	2008		
June	18.1	16.9	16.3
July	21.3	20.1	21.9
August	19.9	20.1	21.1
Sept	15.8	14.3	13.4
	2009		
June	18	16.9	16
July	18.8	18.5	18.8
August	18.9	18.2	18.5
Sept	17.6	17.8	18

Mean Temperature data from National Climatic Data Center (NOAA 2013) weather stations:
[1]USC00217502 Santiago 3 E (25 kilometers northeast of the Sand Plain Research Farm in Becker, MN).
[2]USC00323621 North Dakota State University 8 kilometers north of the Red River Valley Potato Research Farm).
[3]USW00094014 – Williston Sloulin Field International Airport (40 kilometers west of the Williston Research Extension Center).

Table 5. Dependence between PVY incidence and location at Becker, MN; Grand Forks, ND; and Williston, ND during 2007, 2008, 2009.

		Location			
	2007	Becker	Grand Forks	Williston	Total
PVY Positive[1]		20	31	15	66
PVY Negative		330	319	335	984
Total		350	350	350	1150
χ^2, 2 *df* = 6.5, *P*=0.0388					
	2008	Becker	Grand Forks	Williston[2]	
PVY Positive		73	65	12	150
PVY Negative		386	134	233	753
Total		459	199	245	903
χ^2, 2 *df* = 61.4, *P*<0.0001					
	2009	Becker	Grand Forks	Williston	
PVY Positive		4	99	7	110
PVY Negative		489	254	726	1469
Total		493	353	733	1579
χ^2, 2 *df* = 311.7.5, *P*<0.0001					
			Total		
PVY Positive		97	195	34	326
PVY Negative		1205	707	1294	3206
Total		1302	902	1328	3532
χ^2_{CMH}=263.3, 2 *df*, *P*-value <0.0001.					

[1] PVY positive by ELISA if a sample had an OD_{405} reading equal to or greater than twice the mean of five negative controls.

[2] The 2008 Williston samples were tested using immunostrips for PVY (Agdia, Elkhart, IN).

Table 6. Frequencies of PVY outcomes, and percent PVY positive samples at research locations (Becker, MN; Grand Forks, ND; and Williston, ND) during 2007, 2008, 2009.

		PVY		
Year	Location	PVY positive[1]	PVY negative	% PVY positive
2007	Becker	20	330	5.71
	Grand Forks	31	319	8.86
	Williston	15	335	4.29
2008	Becker	73	386	15.90
	Grand Forks	65	134	32.66
	Williston[2]	12	233	4.90
2009	Becker	4	489	0.81
	Grand Forks	99	254	28.05
	Williston	7	726	0.95

[1] PVY positive by ELISA if a sample had an OD_{405} reading equal to or greater than twice the mean of five negative controls.

[2] The 2008 Williston samples were tested using immunostrips for PVY (Agdia, Elkhart, IN).

Table 7. Comparisons between locations two at a time (Becker, MN; Grand Forks, ND; and Williston, ND) during 2007, 2008, 2009 by 2 x 2 chi-square contingency table and odds ratios.

Year	Comparison	sample size	Odds Ratio	95% Confidence Limits			χ2, 1 df	*P*
2007	B to W	700	1.354	0.68	to	2.69	0.75	0.386
	B to GF	700	0.624	0.11	to	2.56	2.56	0.110
	GF to W	700	2.170	1.15	to	4.10	5.96	0.015*
	GF to B	700	1.603	0.90	to	2.87	2.56	0.110
	W to GF	700	0.461	0.24	to	0.87	5.96	0.015*
	W to B	700	0.739	0.37	to	1.47	0.75	0.386
2008	B to W	704	3.672	1.95	to	6.91	18.23	<.0001***
	B to GF	658	0.390	0.26	to	0.57	23.52	<.0001***
	GF to W	444	9.419	4.91	to	18.07	59.05	<.0001***
	GF to B	658	2.565	1.74	to	3.78	23.52	<.0001***
	W to GF	444	0.106	0.06	to	0.20	59.05	<.0001***
	W to B	704	0.272	0.14	to	0.51	18.23	<.0001***
2009	B to W	1226	0.848	0.25	to	2.91	0.07	0.794
	B to GF	846	0.021	0.01	to	0.06	142.69	<.0001***
	GF to W	1086	40.424	18.54	to	88.16	198.52	<.0001***
	GF to B	846	47.649	17.34	to	130.96	142.69	<.0001***
	W to GF	1086	0.025	0.01	to	0.05	198.52	<.0001***
	W to B	1226	1.179	0.34	to	4.05	0.07	0.794

Significant at: *P<0.05, **P<0.01, ***P<0.001

Table 8. Dependence between PVY incidence and time of season (early middle, and late season leaf collection times)

	Time of Season leaf samples were collected			
	Early[2]	Midseason[3]	Late[4]	Total
PVY Positive[1]	11	126	138	275
PVY Negative	1215	822	520	2557
Total	1226	948	658	2832
Percent of samples PVY Positive	0.9%	13.3%	21%	35.2%
$\chi 2$, 2 *df* = 217.7, *P*<0.0001				

[1] PVY positive by ELISA if a sample had an OD_{405} reading equal to or greater than twice the mean of five negative controls.

[2]Early season leaf collection time points ranged from 8 to 29 days past July 1st.

[3]Middle season leaf collection time points ranged from 36 to 42 days past July 1st.

[4]Late season leaf collection time points ranged from 77 to 91 days past July 1st.

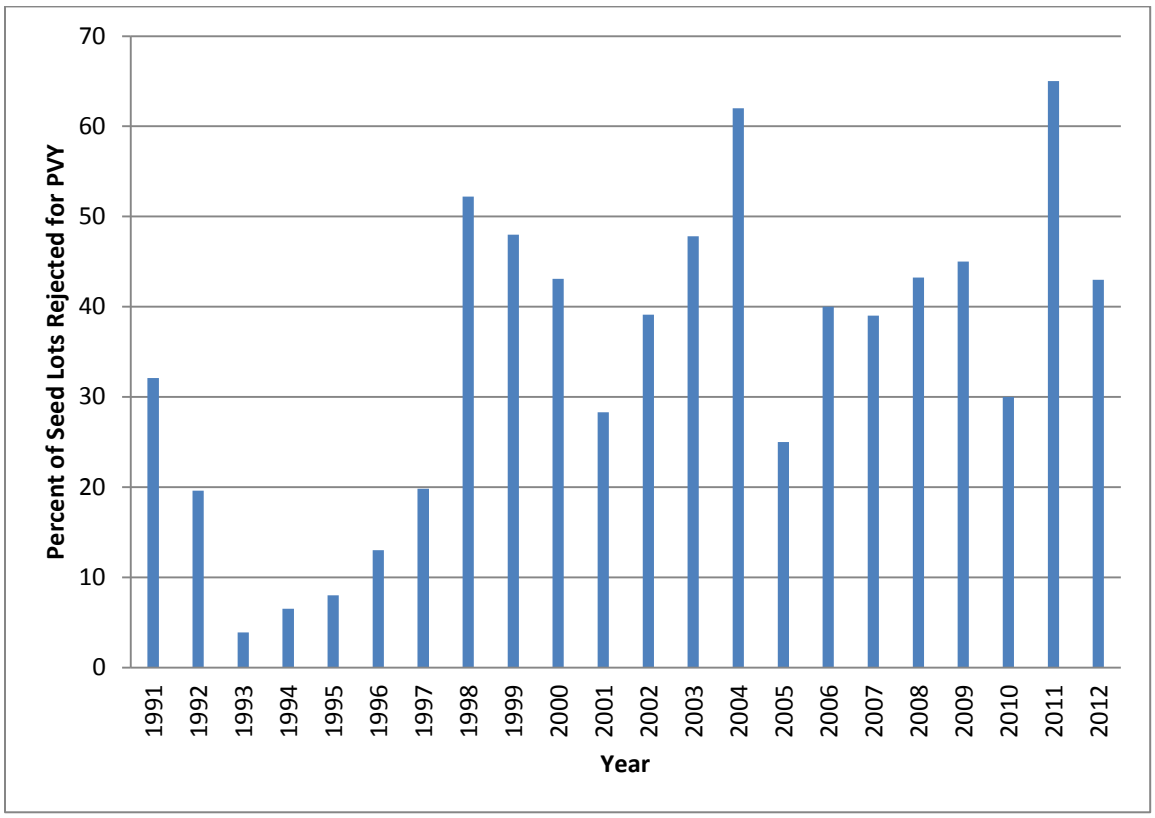

Figure 1. Percent of Minnesota potato seed Lots rejected due to PVY incidence above the 0.5% certification standard (Data courtesy of MN Department of Agriculture).

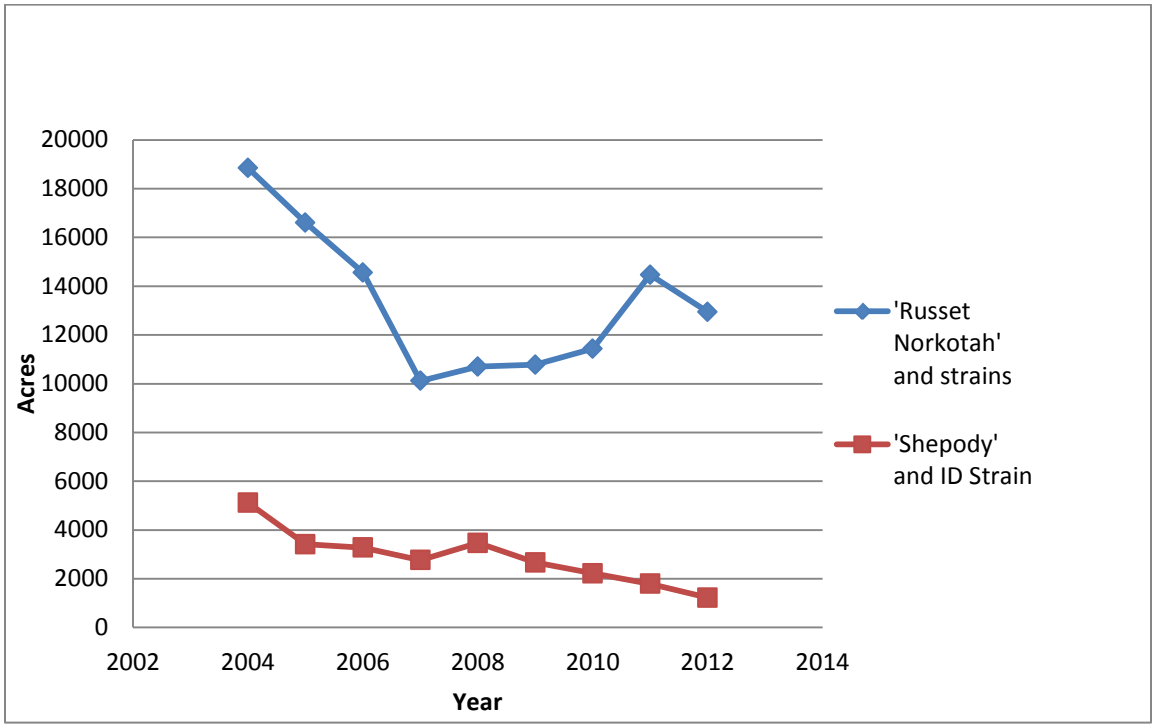

Figure 2. U.S. Certified Seed Potato Accepted Acres of 'Russet Norkotah' (blue line with blue diamonds) and 'Shepody' (red line with red squares) 2004 to 2012 (Potato Association of America 2012). Strains (intraclonal selections) of each cultivar are included with the original cultivar.

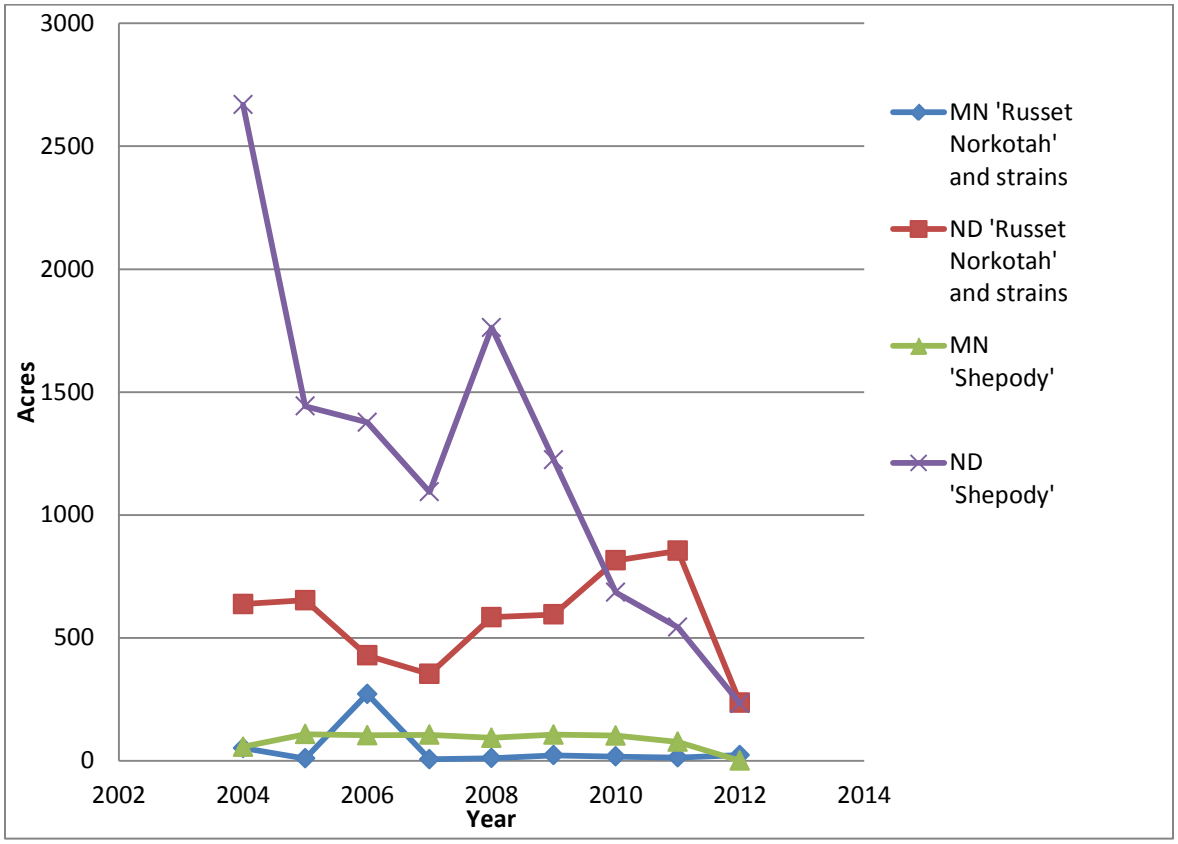

Figure 3. Minnesota and North Dakota Certified Seed Accepted Acres of 'Russet Norkotah' and 'Shepody' 2004 to 2012 (Potato Association of America 2012). Strains (intraclonal selections) of 'Russet Norkotah' are included with the 'Russet Norkotah' cultivar. Strains of 'Shepody' were not grown in MN or ND during 2004-2012. Acres of 'Russet Norkotah' and strains grown in Minnesota have a blue line with blue diamonds. Acres of 'Russet Norkotah' and strains grown in North Dakota have a red line with red squares. Acres of 'Shepody' grown in Minnesota have a green line with green triangles. Acres of 'Shepody' grown in North Dakota have a purple line with purple x's.

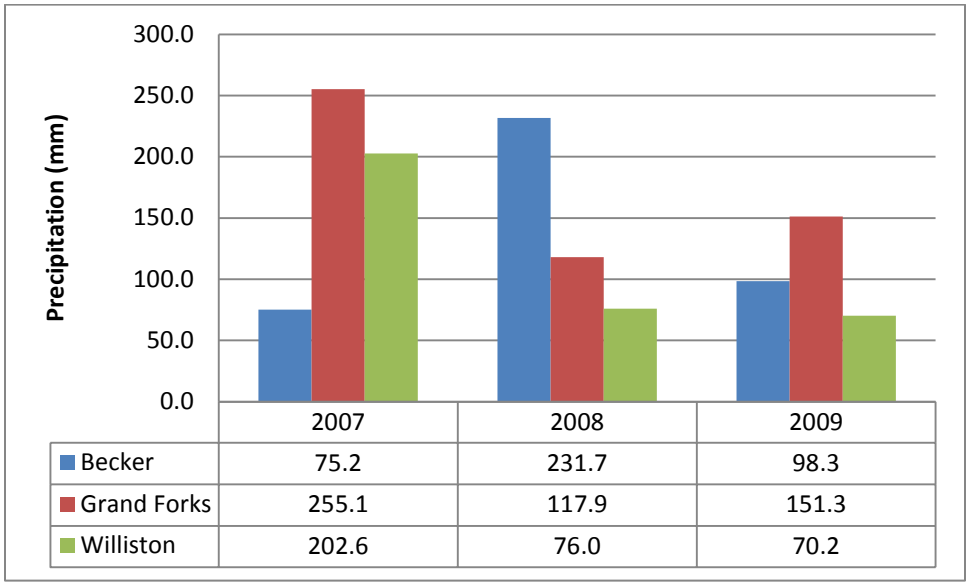

Figure 4. May and June Total Rainfall at 3 Locations (Becker, MN; Grand Forks, ND; and Williston, ND) from 2007 to 2009. Weather data were obtained from the National Climatic Data Center (NOAA 2013) for the following weather stations: USC00217502 Santiago 3 E (25 kilometers northeast of the Sand Plain Research Farm in Becker, MN), USC00323621 North Dakota State University (8 kilometers north of the Red River Valley Potato Research Farm), and USW00094014 – Williston Sloulin Field International Airport (40 kilometers west of the Williston Research Extension Center).

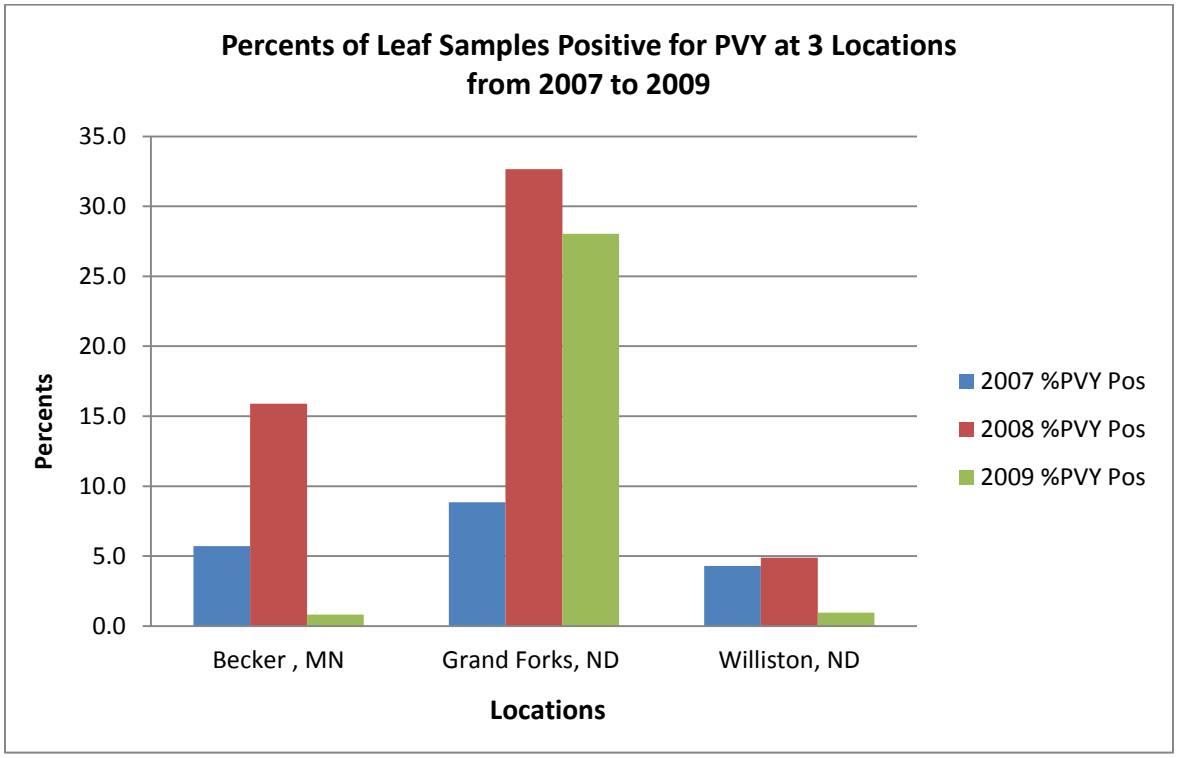

Figure 5. Percentages of Leaf Samples Positive for PVY at 3 Locations (Becker, MN; Grand Forks, ND; and Williston; ND) from 2007 to 2009.

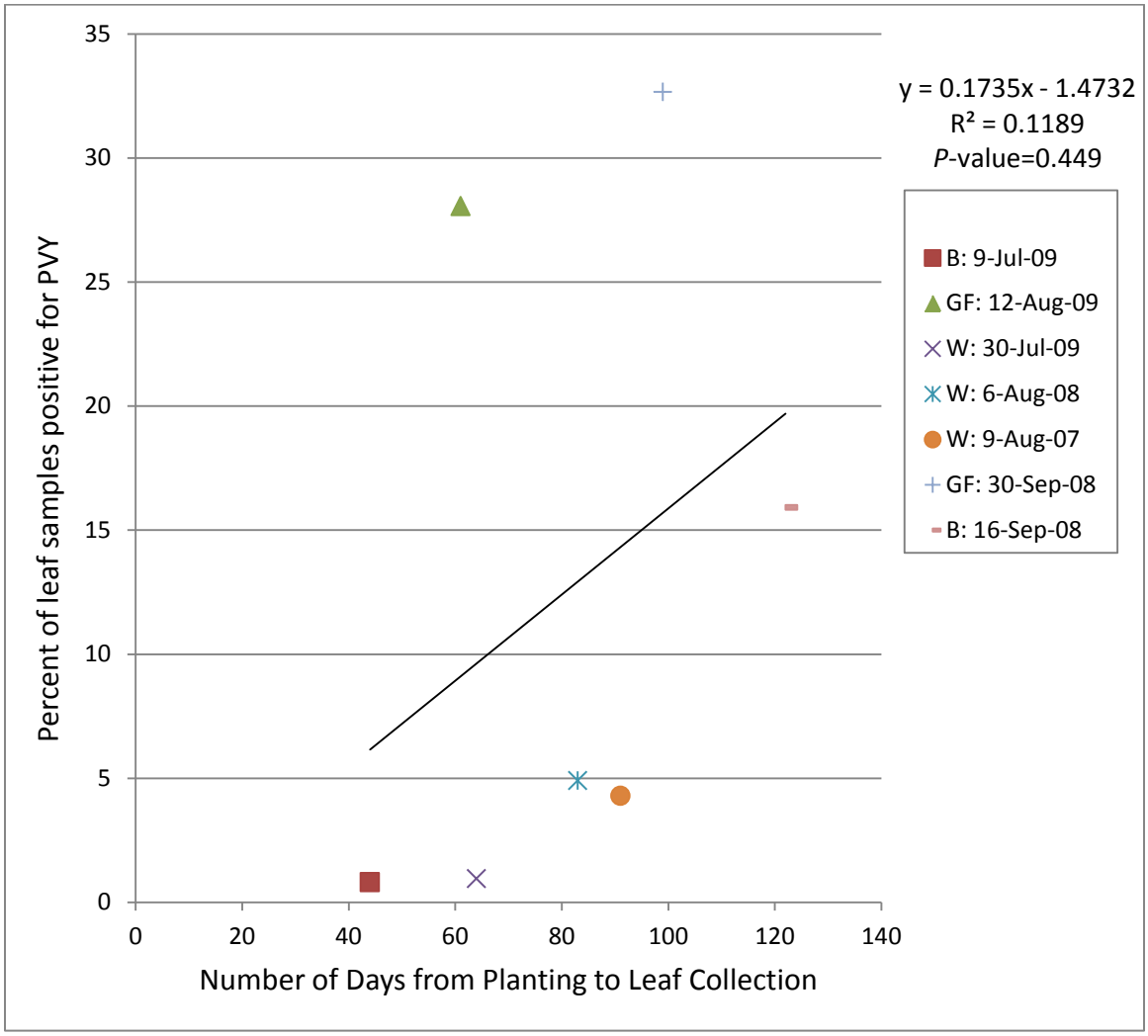

Figure 6. *Average percent PVY positive at a timepoint/location* vs. n*umber of days from planting to Leaf Collection*. This regression fits the *average percent PVY at a location/timepoint* to the *number of days from planting to leaf collection*. Legend abbreviations: B= Becker, MN; GF= Grand Forks, ND; W= Williston, ND. The dates in the legend are leaf collection dates.

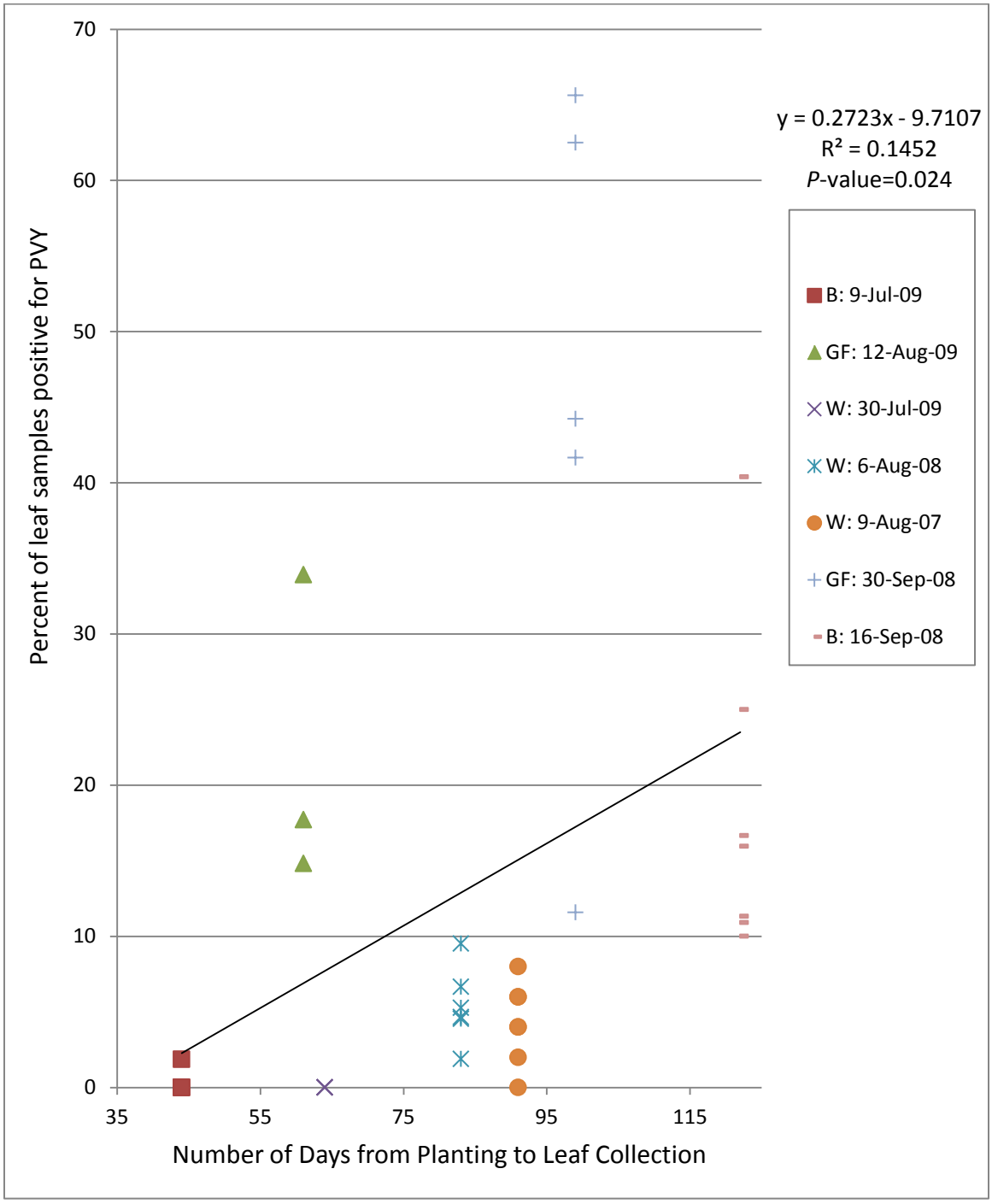

Figure 7. Percent PVY positive by population at each timepoint/location vs. number of days from planting to leaf collection. This regression fits the percent PVY in each population to the number of days from planting to leaf collection. Legend abbreviations: B= Becker, MN; GF= Grand Forks, ND; W= Williston, ND. The dates in the legend are leaf collection dates.

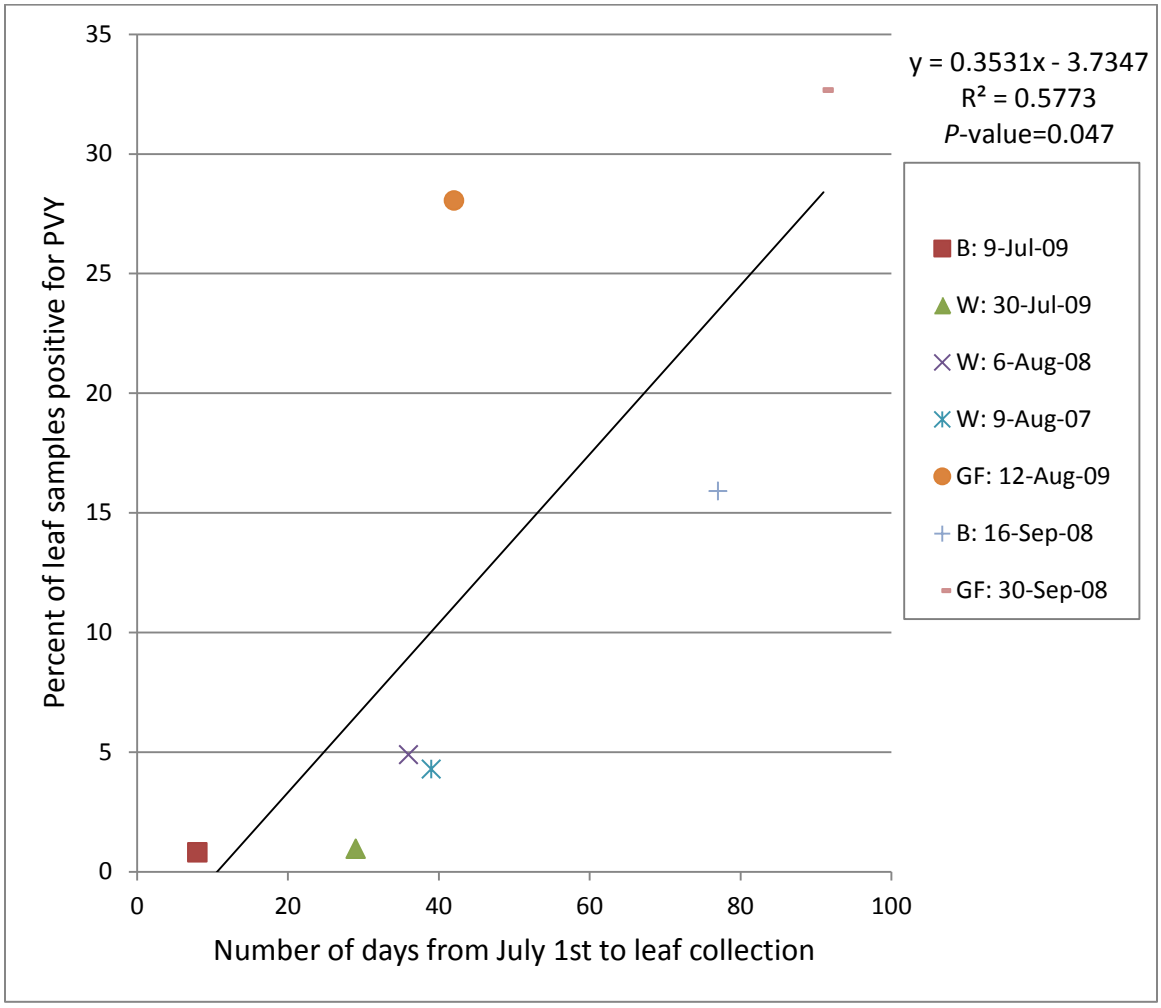

Figure 8. *Average percent PVY positive samples per timepoint/location* vs. *number of days from July 1st to leaf collection.* This regression fits the *average percent PVY at a location/timepoint* to the *number of days from July 1st to leaf collection*. Legend abbreviations: B= Becker, MN; GF= Grand Forks, ND; W= Williston, ND. The dates in the legend are leaf collection dates.

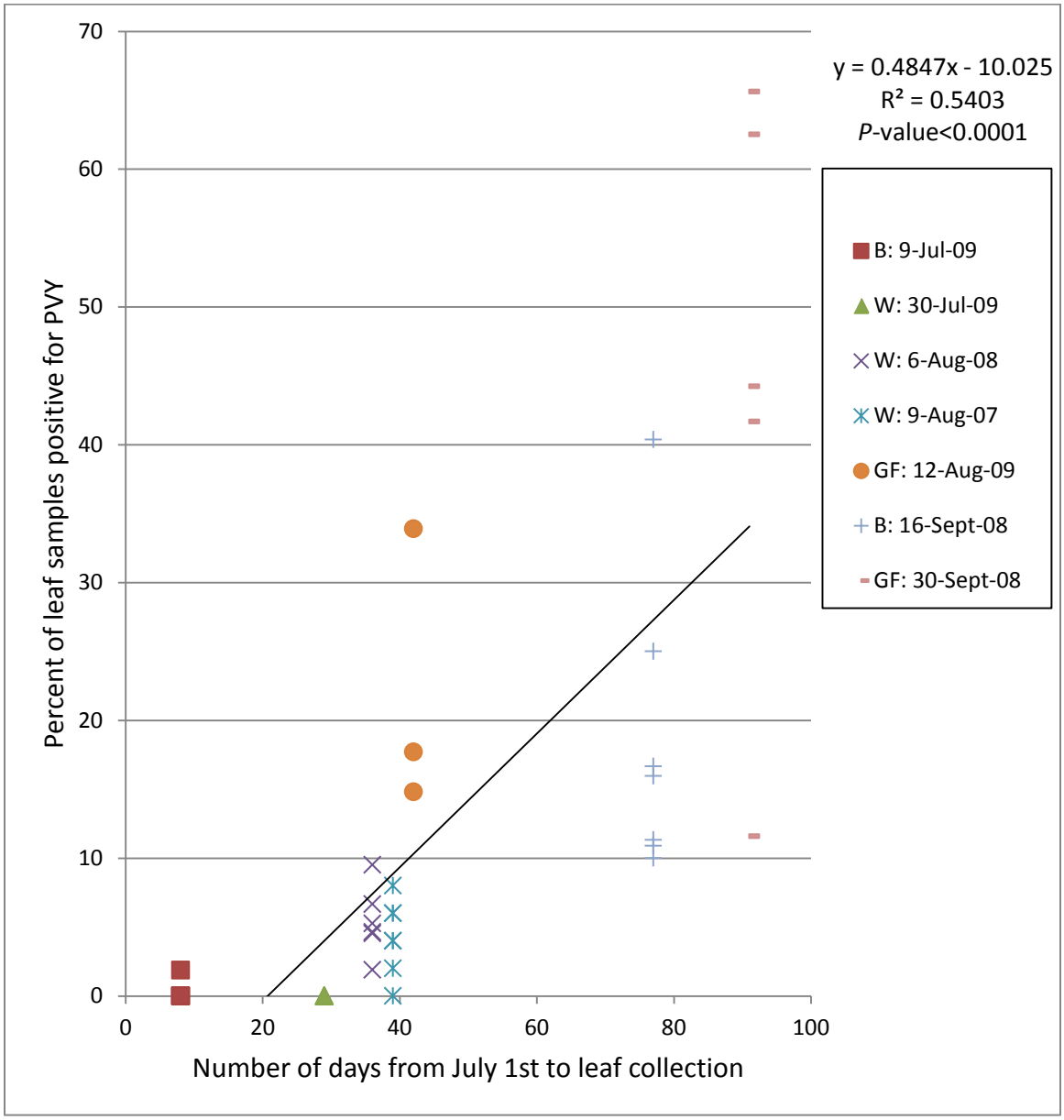

Figure 9. Percent PVY positive in population sample per timepoint/location vs. number of days from July 1st to leaf collection. This regression fit the percent PVY in each population to the number of days from July 1st to leaf collection. Legend abbreviations: B= Becker, MN; GF= Grand Forks, ND; W= Williston, ND. The dates in the legend are leaf collection dates.

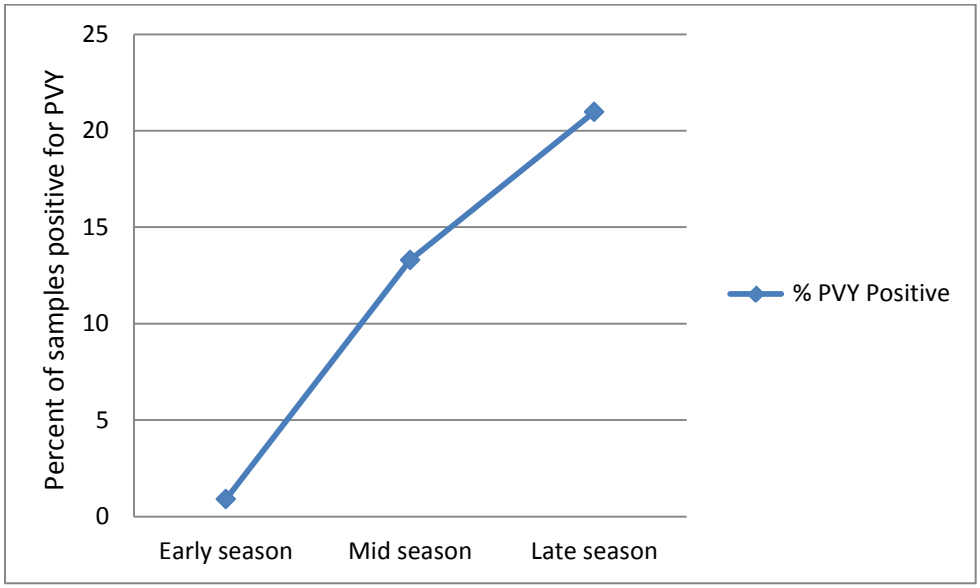

Figure 10. Leaf collection season (Early, Mid, and Late) vs. Percent PVY. Early season leaf collection time points ranged from 8 to 29 days past July 1st. Mid season leaf collection time points ranged from 36 to 42 days past July 1st. Late season leaf collection time points ranged from 77 to 91 days past July 1st.

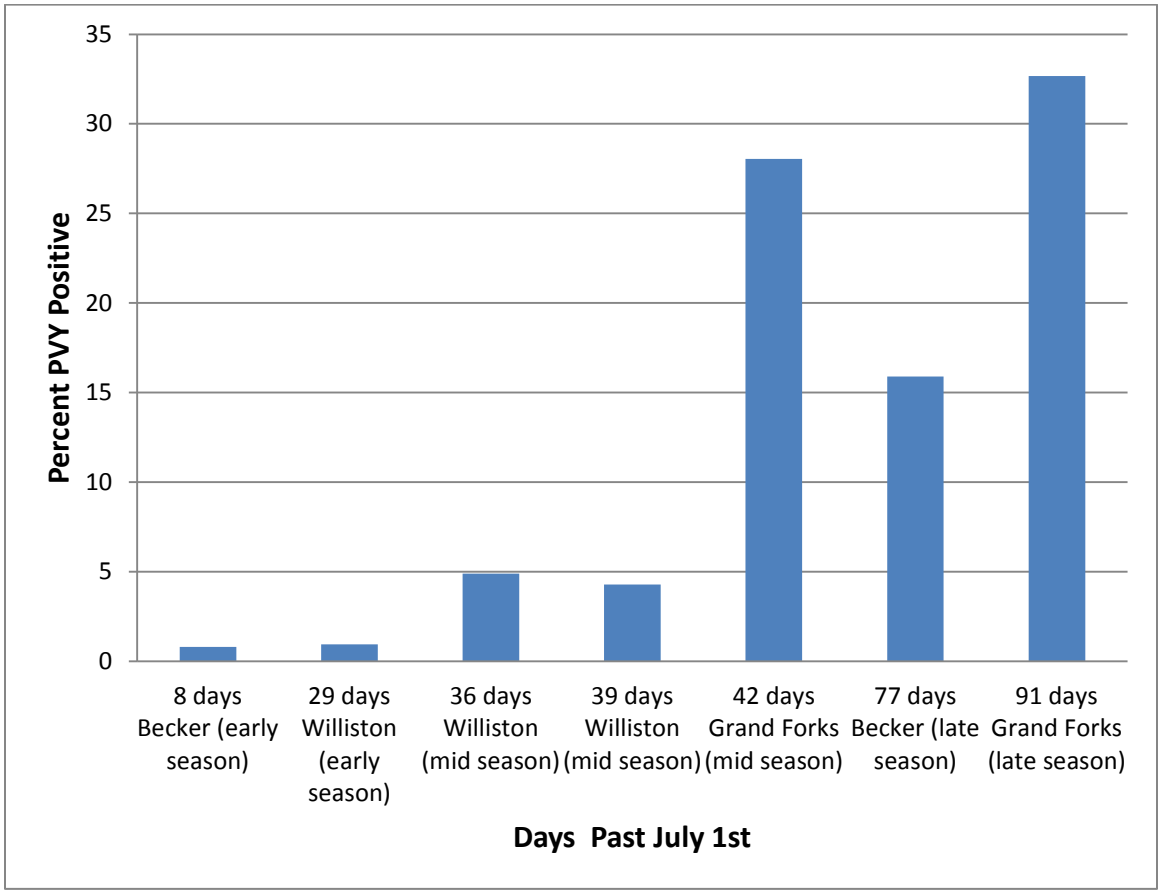

Figure 11. Percent PVY positive samples per timepoint/location and leaf collection season (Early, Mid, and Late). Early season leaf collection time points ranged from 8 to 29 days past July 1st. Mid season leaf collection time points ranged from 36 to 42 days past July 1st. Late season leaf collection time points ranged from 77 to 91 days past July 1st.

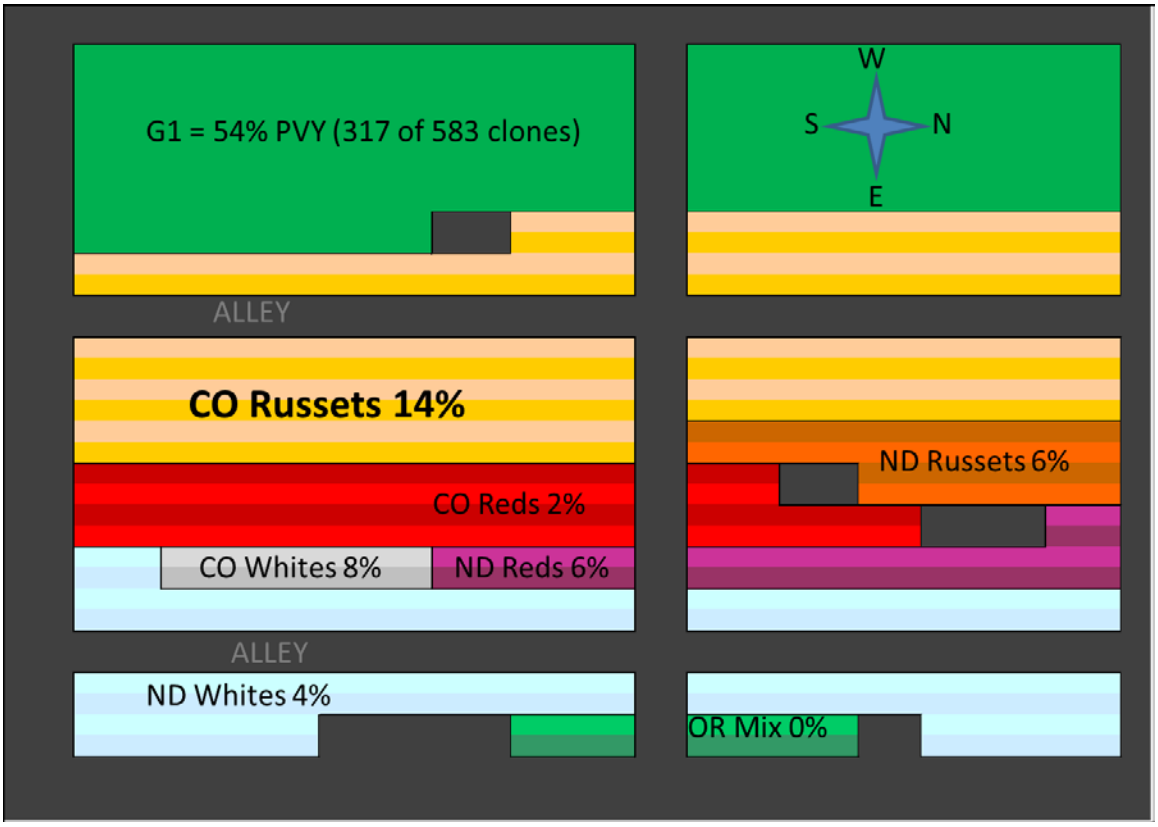

Figure 12. Single-Hill and Generation-1 (G1) plots at Becker, MN in 2007. G1 plants are potato plants that were grown in the field the previous summer and are now in their second field season. Single-hill plots are growing in the field for the first time. These single-hill plots consist of seven populations: Colorado (CO) Russets, CO reds, CO whites, North Dakota (ND) Russets, ND reds, ND whites, and Oregon (OR) mix. The percentage of PVY in the random sample from each population is plotted on this map. The black areas represent fallow ground consisting of alleys around and through the field and gaps that separate the different populations. The stripes represent rows in the single-hill field. The colors represent the following single-hill populations: CO Russets are light gold and gold, CO reds are dark red and red, CO whites are light gray and gray, ND Russets are burnt orange and orange, ND reds are violet and dark violet, ND whites are light blue and light purple, and OR mix colors are green and sea green. The G1 field is solid green. This field was rogued 18 July 2007. Leaves from single-hill plants were collected on 18 Aug. 2007 and 20 of 350 random leaf samples tested positive for PVY (5.7%).

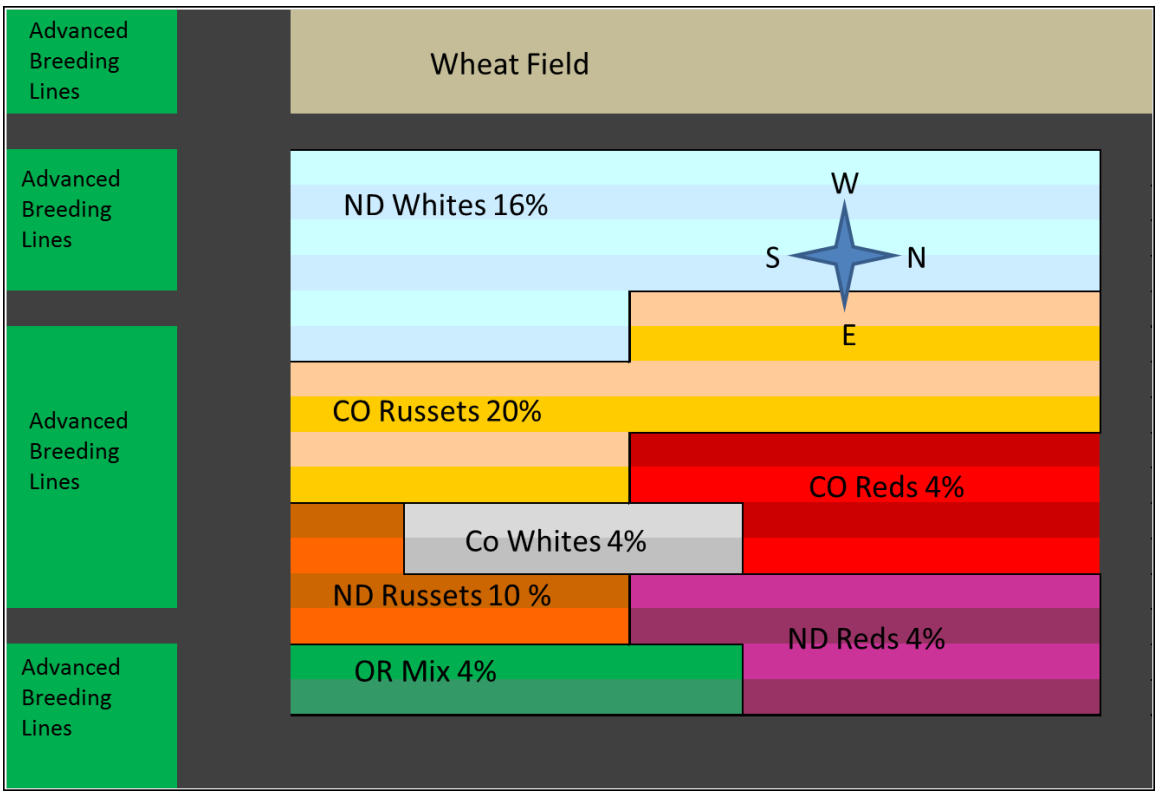

Figure 13. Single-hill and surrounding fields in 2007 at Grand Forks, MN. Advanced breeding lines are potato plants that were grown in the field for three or more field seasons. Single-hill plots are growing in the field for the first time. These single-hill plots consist of seven populations: Colorado (CO) Russets, CO reds, CO whites, North Dakota (ND) Russets, ND reds, ND whites, and Oregon (OR) mix. The percentage of PVY in the random sample from each population is plotted on this map. The black areas represent fallow ground consisting of alleys around and through the field. The stripes represent rows in the single-hill field. The colors represent the single-hill populations as follows: CO Russets are light gold and gold, CO reds are dark red and red, CO whites are light gray and gray, ND Russets are burnt orange and orange, ND reds are violet and dark violet, ND whites are light blue and light purple, and OR mix colors are green and sea green. The advanced breeding lines are solid green. The wheat is tan. The single-hill fields were rogued in early to mid-August. Leaves were on 20 Aug. 2007, and 31 of 350 samples tested positive for PVY (8.9%).

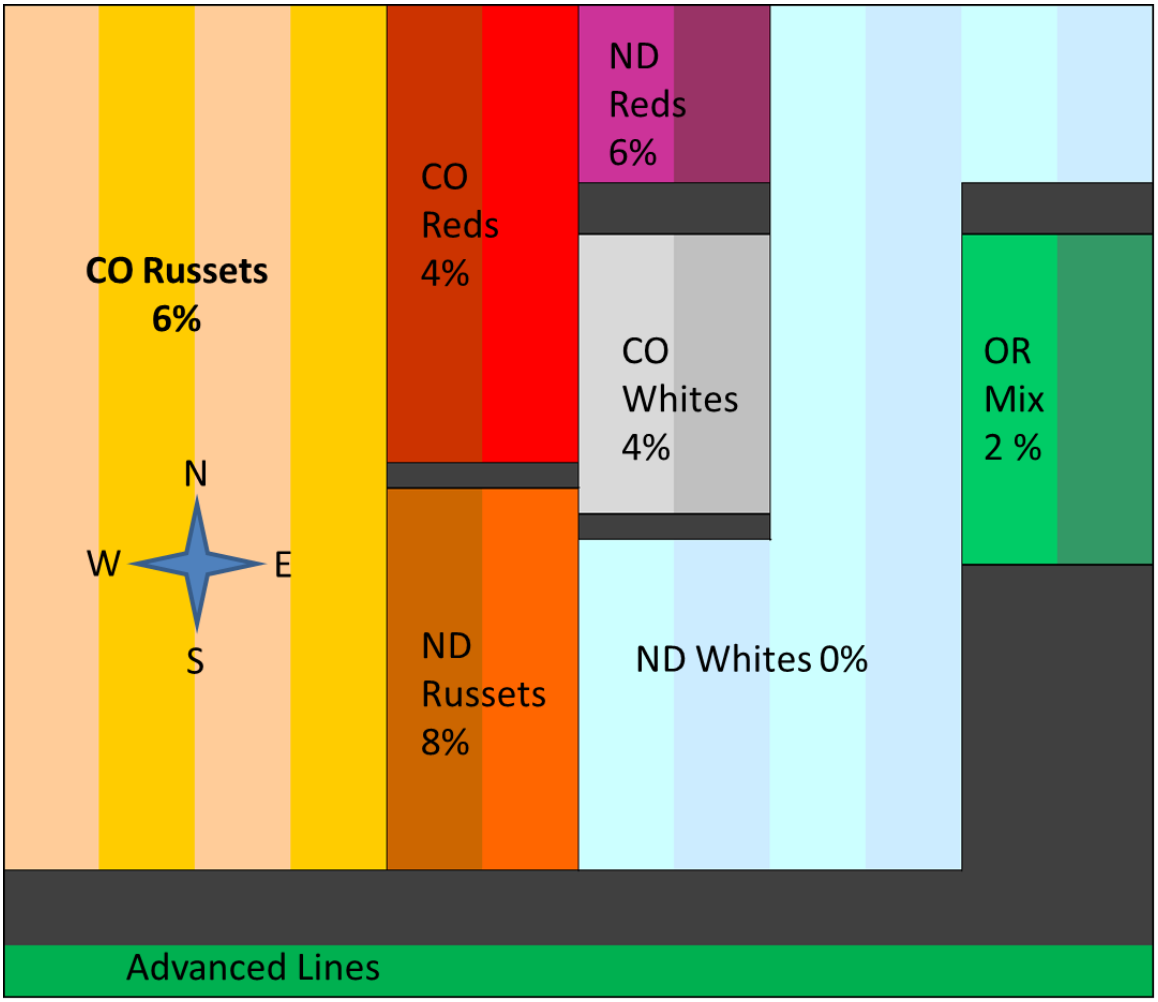

Figure 14. Single-hill field and nearby advanced lines in 2007 at Williston, ND. Advanced breeding lines are potato plants that were grown in the field for three or more field seasons. Single-hill plots are growing in the field for the first time. These single-hill plots consist of seven populations: Colorado (CO) Russets, CO reds, CO whites, North Dakota (ND) Russets, ND reds, ND whites, and Oregon (OR) mix. The percentage of PVY in the random sample from each population is plotted on this map. The black areas represent fallow ground consisting of alleys around and through the field and gaps that separate the different populations. The stripes represent rows in the single-hill field. The colors represent the single-hill populations as follows: CO Russets are light gold and gold, CO reds are dark red and red, CO whites are light gray and gray, ND Russets are burnt orange and orange, ND reds are violet and dark violet, ND whites are light blue and light purple, and OR mix colors are green and sea green. The advanced breeding lines are solid green. The leaves were collected on 9 Aug. 2007, and 15 of 350 samples tested positive for PVY (4.29%).

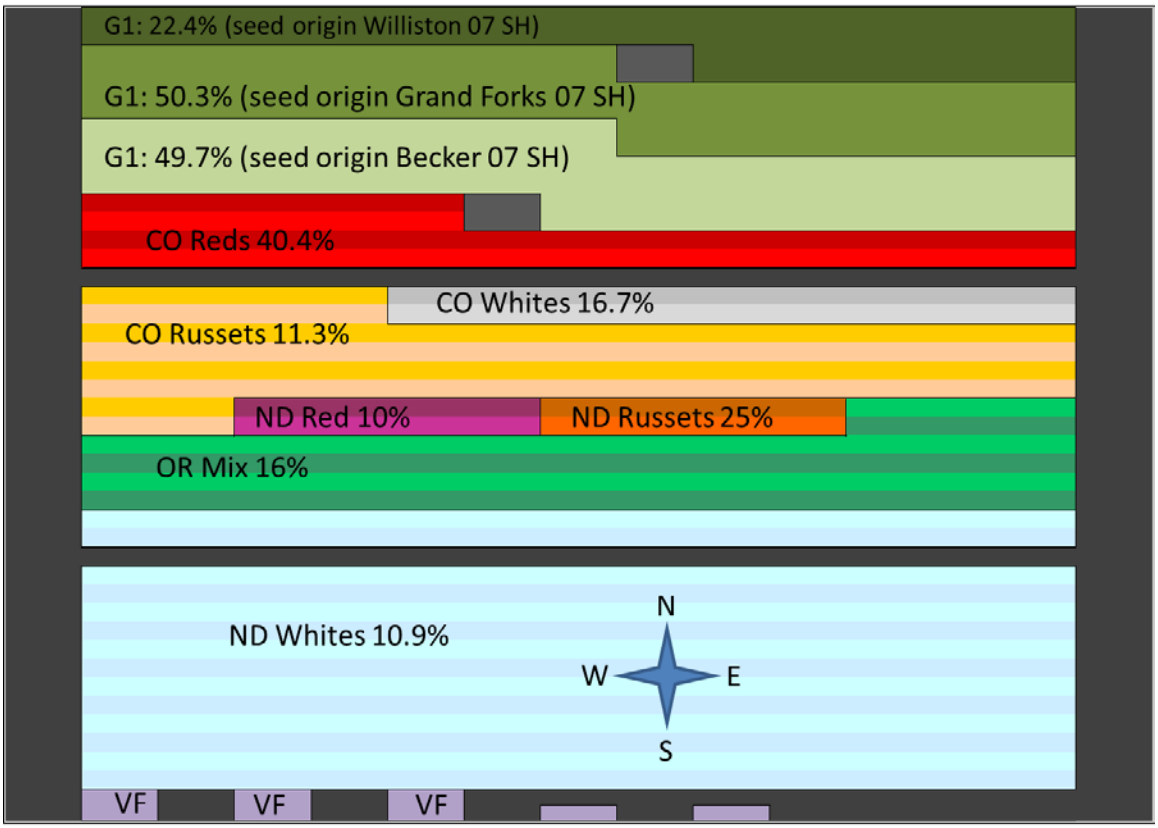

Figure 15. Single-hill and generation-1 (G1) fields in 2008 at Becker, MN. G1 plants are potato plants that were grown in the field the previous summer and are now in their second field season. Single-hill plots are growing in the field for the first time. These single-hill plots consist of seven populations: Colorado (CO) Russets, CO reds, CO whites, North Dakota (ND) Russets, ND reds, ND whites, and Oregon (OR) mix. The percentage of PVY in the random sample from each population is plotted on this map. The black areas represent fallow ground consisting of alleys around and through the field and gaps that separate the different populations. The stripes represent rows in the single-hill field. The colors represent the single-hill populations as follows: CO Russets are light gold and gold, CO reds are dark red and red, CO whites are light gray and gray, ND Russets are burnt orange and orange, ND reds are violet and dark violet, ND whites are light blue and light purple, and OR mix colors are green and sea green. The G1 field is light green, olive green, and dark green. South of the ND whites are virus free (VF) clones. Single-hill leaves were collected 16 Sept. 2008 and G1 leaves were collected between 20 Aug. and 26 Aug. 2008. Seventy-three of 459 random single-hill samples tested positive for PVY (15.9%). All clones in the G1 field were tested for PVY (857 four-hill units), and the G1 field was 43.4% positive for PVY. South of the ND whites are virus free (VF) clones.

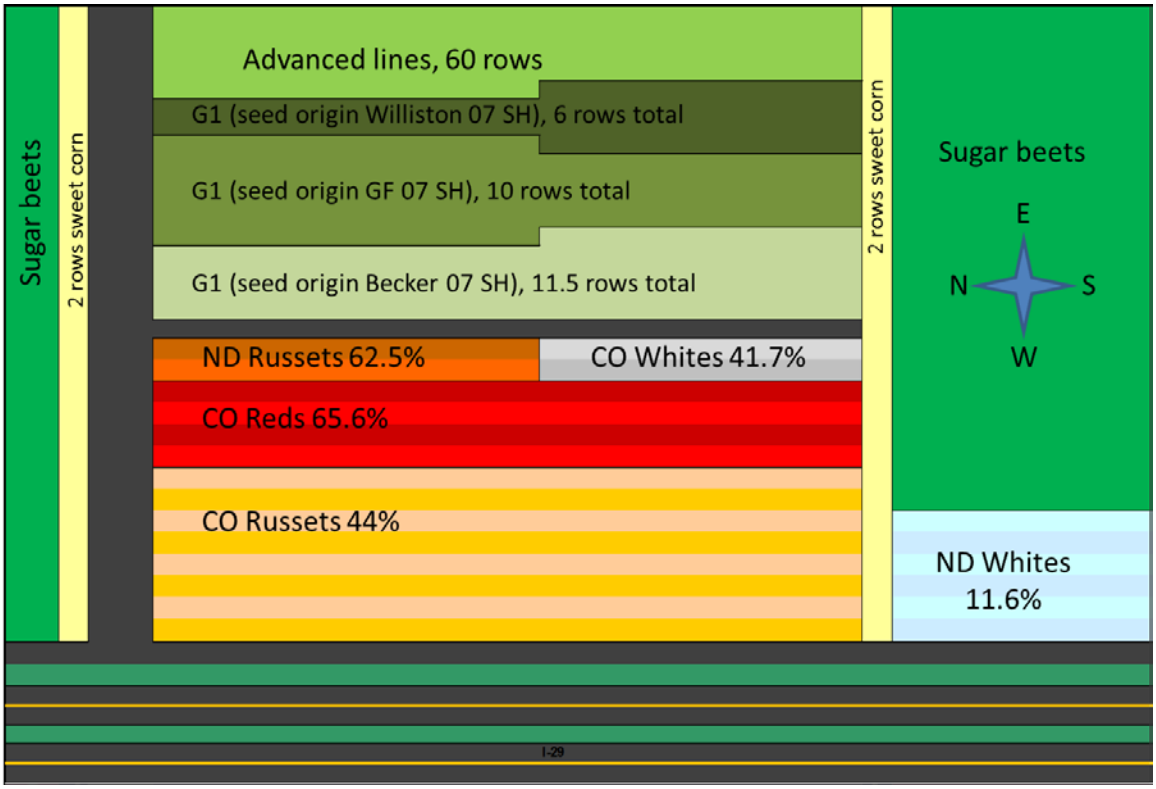

Figure 16. Single-hill plots, generation-1 (G1) plots, and surrounding features in 2008 at Grand Forks, MN. Advanced breeding lines are potato plants that were grown in the field for three or more field seasons. G1 plants are potato plants that were grown in the field the previous summer and are now in their second field season. Single-hill plots are growing in the field for the first time. These single-hill plots consist of five populations: Colorado (CO) Russets, CO reds, CO whites, North Dakota (ND) Russets, and ND whites. The percentage of PVY in the random sample from each population is plotted on this map. Interstate 29 (I-29) is represented by black with a thin gold line with road medians in sea-green. The other black areas represent fallow ground consisting of alleys around and through the field and gaps that separate the different populations. The stripes represent rows in the single-hill field. The colors represent the single-hill populations as follows: CO Russets are light gold and gold, CO reds are dark red and red, CO whites are light gray and gray, ND Russets are burnt orange and orange, and ND whites are light blue and light purple. The G1 field is light green, olive green, and dark green. The sugar beet fields are solid green. The sweet corn rows are yellow. Single-hill leaves were collected 30 Sept. 2008, and 65 of 199 samples tested positive for PVY (32.7% average).

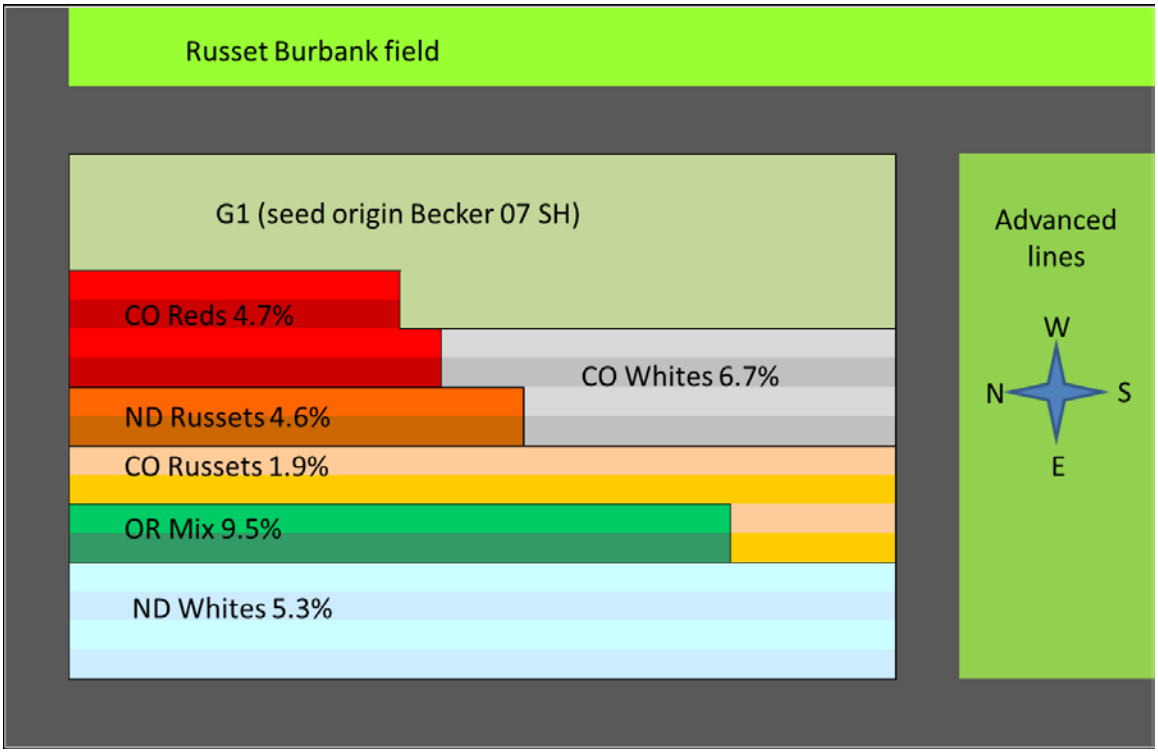

Figure 17. Single-hill plots, Generation-1 (G1) plots, and surrounding features in 2008 at Williston, ND. Advanced breeding lines are potato plants that were field-grown for three or more field seasons. G1 plants are potato plants that were grown in the field the previous summer and are now in their second field season. Single-hill plots are growing in the field for the first time. The percentage of PVY in the random sample from each population is plotted on this map. The black areas represent fallow ground consisting of alleys around the field. The stripes represent rows in the single-hill field. The colors represent the single-hill populations as follows: CO Russets are light gold and gold, CO reds are dark red and red, CO whites are light gray and gray, ND Russets are burnt orange and orange, ND whites are light blue and light purple, and OR mix colors are green and sea green. The G1 field is light green. The advanced breeding lines are solid green. The 'Russet Burbank' field is bright green. Single-hill leaves were collected 6 Aug. 2008, and 12 of 245 samples tested positive for PVY (4.9% average).

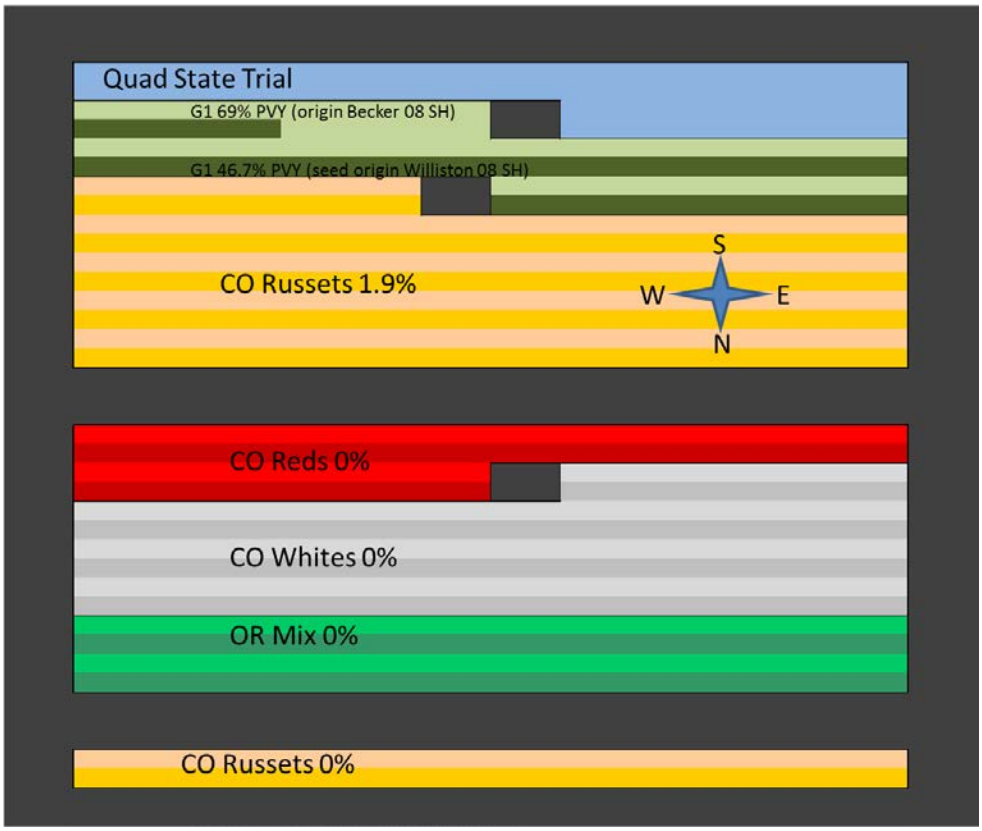

Figure 18. Single-hill and Generation-1 (G1) fields in 2009 at Becker, MN. Quad State Trials were advanced potato breeding lines that were grown in the field-grown for three or more field seasons. G1 plants are potato plants that were grown in the field the previous summer and are now in their second field season. Single-hill plots are growing in the field for the first time. These single-hill plots consist of four populations: Colorado (CO) Russets, CO reds, CO whites, and Oregon (OR) mix. The percentage of PVY in the random sample from each population is plotted on this map. The black areas represent fallow ground such as alleys around and through the field and gaps that separate the different populations. The stripes represent rows in the single-hill field. The colors represent the single-hill populations as follows: CO Russets are light gold and gold, CO reds are dark red and red, CO whites are light gray and gray, and OR mix colors are green and sea green. Three sections of potatoes were separated by about 100 meter windbreak and alley space. Leaves from single hills were collected 9 July 2009 and G1 leaves were collected 29 June 2009. Four of 493 random samples from single hills tested positive for PVY (0.8% average). All samples from single hills that were positive for PVY came from the first section that is next to the G1 field and Quad state Trial. All clones in the G1 field were tested for PVY (375 four-hill units), and the average PVY in the G1 rows was 60%. The G1 four-hill units that originated from Becker 2008 single hills were 69% infected in the 2009 G1. The G1 four-hill units that originated from Williston 2008 single hills were 46.7% infected in 2009.

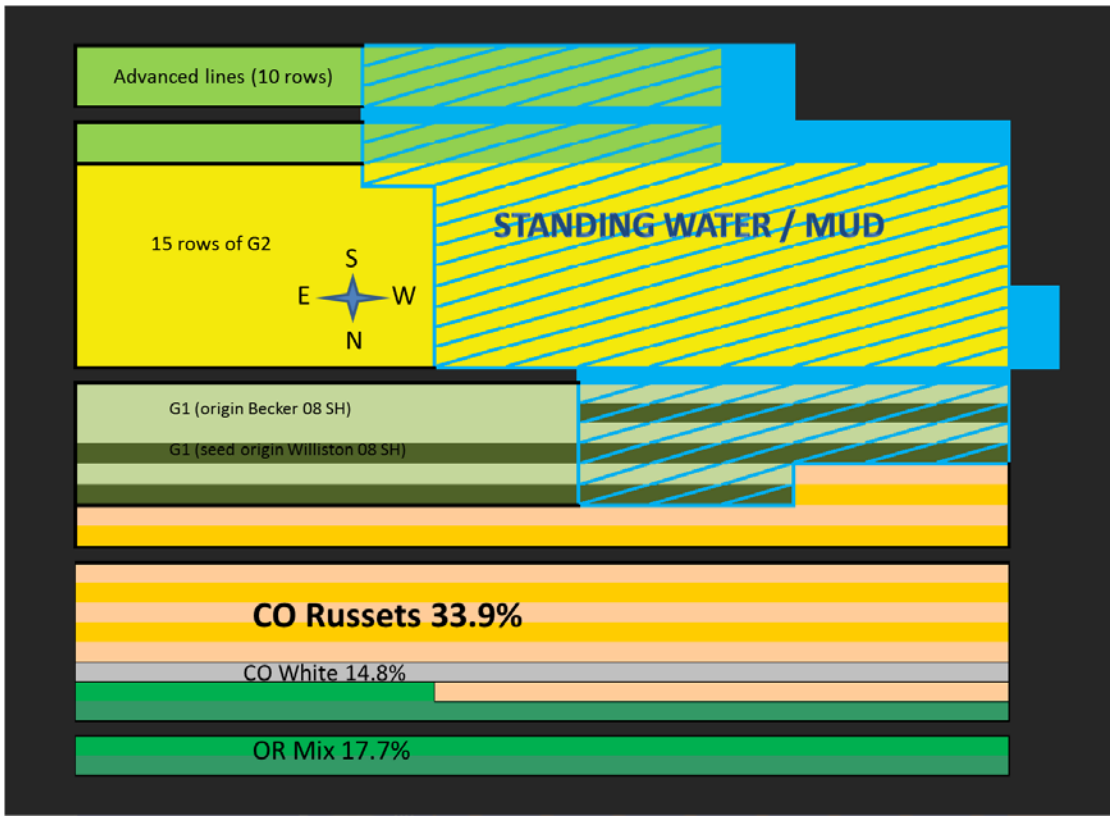

Figure 19. Single-hill, Generation-1 (G1), and surrounding features in 2009 at Grand Forks, MN. Advanced lines are potato plants that were grown in the field for more than two field seasons. G1 plants are potato plants that were grown in the field the previous summer and are now in their second field season. Generation 2 (G2) plants are potato plants that were grown in the field the previous two summers and are now in their third field season. Single-hill plots are growing in the field for the first time. These single-hill plots consist of three populations: Colorado (CO) Russets, CO whites and Oregon (OR) mix. The percentage of PVY in the random sample from each population is plotted on this map. The black areas represent fallow ground consisting of alleys around and through the field. The part of the field with diagonal blue lines was standing water or mud for much of the 2009 growing season. The horizontal stripes represent rows in the single-hill field. The colors represent the single-hill populations as follows: CO Russets are light gold and gold, CO whites are light gray and gray, and OR mix colors are green and sea green. The G1 field is light green and olive green. Generation 2 field (G2) is yellow. The advanced breeding lines are solid green. Leaves from single hills were collected 12 Aug. 2009, and 65 of 353 samples tested positive for PVY (28% average).

Figure 20. Single-hill plots and surrounding fields in 2009 at Williston, ND. U of M yield trials are advanced breeding lines field-grown for three or more years.G1 plants are potato plants that were grown in the field the previous summer and are now in their second field season. Single-hill plots are growing in the field for the first time. These single-hill plots consist of three populations: Colorado (CO) Russets, CO reds and Oregon (OR) mix. The percentage of PVY in the random sample from each population is plotted on this map. The black areas represent fallow ground such as alleys around and through the field and gaps that separate the different populations. The stripes represent rows in the single-hill field. The colors represent the single-hill populations as follows: CO Russets are light gold and gold, CO reds are dark red and red, and OR mix colors are green and sea green. The G1 field is light green and olive green. The University of Minnesota (U of M) yield trails and Montana State university trials are solid green. The Williston Experiment Station plots are solid green-yellow. Leaves from single hills were collected 30 July 2009, and 7 of 733 samples tested positive for PVY (0.95% average).