DETERMINING POTENTIAL SOURCES OF RACE NON-SPECIFIC RESISTANCE TO THE LATE BLIGHT PATHOGEN, *Phytophthora infestans* (Mont.) de Bary, IN ADVANCED BREEDING MATERIALS OF CULTIVATED POTATO, *Solanum tuberosum* L.

By

Brett William Johnson

Bachelor of Science in Horticulture

Oklahoma State University

Stillwater, Oklahoma U.S.A.

2015

Submitted to the Faculty of the Honors College of Oklahoma State University in partial fulfillment of the Degree of Bachelor of Science in Horticulture with Honors, December, 2015.

ACKNOWLEDGMENTS

For the extensive training and mentorship I have received both in the class-room as well as throughout this project, I extend my sincerest gratitude to the director of my thesis, Dr. Bruce Dunn. For working in so many ways to make this research possible and in no small way helping me to become a more confident scientist I express my deepest appreciation to the second reader of my thesis, Dr. Carla Garzon. For the extensive service put forth in generating the statistical analysis that contributed greatly to the present work, I would like to express my thankfulness and appreciation to Dr. Mark Payton.

I extend a very special thank you to those individuals at the International Potato Center (CIP), namely, Dr. Merideth Bonierbale, Manuel Gastelo, Wilmer Perez, and Dr. Hanelle Lindquist-Kreuze, whom over the course of my time at CIP provided me with instruction, insight, and direction, which contributed most profoundly to the present work. Additionally, I would like to recognize the extensive contributions of Manuel Gastelo in working at length to educate an aspiring plant breeder, such as myself. His willingness to select materials for evaluation and to direct the primary research that is the subject of the present work represents a major contribution to both my education as well as my thesis project. For these reasons and more I am very grateful.

To the many individuals in Peru that made my time there so enriching, rewarding, and productive, I am fortunate to call you friends. To Juan Concha, in particular, I am thankful for your friendship, assistance, and interest in the present work.

Last, but not least, I extend my greatest appreciation to my wife, Sarah, whose encouragement I have to thank for my success, and whose companionship has meant that every accomplishment in my life is both a shared and joyous event.

Name: Brett William Johnson

Date of Degree: December, 2015

Title of Study: Determining potential sources of race non-specific resistance to the late blight pathogen, *Phytophthora infestans* (Mont.) de Bary, in advanced breeding materials of cultivated potato, *Solanum tuberosum* L.

Major Field: Horticulture and Landscape Architecture

Abstract: *Phytophthora infestans* (Mont.) de Bary, the causal agent of late blight disease of potato, is widely considered to be the most important plant disease of potato. The presence of major genes (R-genes) in breeding materials under improvement for resistance to this oomycete plant pathogen has created difficulties in the identification of minor genes (polygenes), and has hindered breeding efforts focused on concentrating sources of race-nonspecific resistance into the cultivated potato (*Solanum tuberosum* L.). The International Potato Center (CIP) responded to this challenge in the 1990's by developing a methodology for R-gene extraction by use of detached leaf assay experimentation. The present study analyzes 31 advanced clones of the LBHT population, under improvement at CIP for race-nonspecific resistance to late blight disease and performance under elevated temperatures (>18℃), to determine potential sources of race-nonspecific resistance in the absence of known, *Solanum demissum* Lindl., derived *R*-genes. As a second objective we compared the disease response of the 31 advanced clones challenged with isolates; PE8006, Pox109, Pox155, and Pox110, in detached leaf assay experiments, with disease responses observed in previous field trials, where materials were challenged with the prevalent field inoculum found in Oxapampa, Peru, in order to validate methodology.

The nine advanced clones; 398180.292, 398190.89, 398208.219, 398208.704, 302531.43, 302533.49, 302534.43, 302542.62, and 302551.26, are reported to be either free of *Solanum demissum* derived R-genes or containing partially incompatible R-genes. From within these nine clones, a single clone (302551.26) demonstrated a response to inoculation by both a race 0 (Isolate PE8006) as well as the three complex races tested, which exceeded 50% mean lesion area in detached leaf assays. This response was confirmed by field evaluations. These results indicate that clone 302551.26 has the greatest potential to be free of known *S. demissum* derived R-genes according to established methodology. Of the nine advanced clones of interest, three clones (398180.292, 398190.89, and 398208.704) have demonstrated levels of resistance lower than 50% across both lab and field evaluations. These materials may contain either, partially defeated R-genes or high levels of polygenic resistance to *P. infestans*. The horizontal character of their resistance could be used to the benefit of breeding programs working towards the objectives of improving race-nonspecific resistance to *P. infestans*.
Using the Pearson correlation coefficient it was determined that a correlation at $P \leq 0.001$ exists between the evaluation factors of detached leaf assay sporulation intensity (Lab PI) and detached leaf assay lesion area (Lab LA) within each individual isolate of *P. infestans*. However, a correlation between the factors of evaluation, (Lab LA) and field evaluation of lesion area (Field LA) were found in only a single complex isolate, Pox109 at $P \leq 0.05$. These results validate the use of Lab PI and Lab LA together to increase certainty in resistance responses of materials tested, as well as, demonstrate that the use of known lab isolates in screening resistant materials is largely unrepresentative of virulence found in the seasonal field inoculum in Oxapampa, Peru.

Introduction

Within the family Solanaceae there exist several genera of tremendous economic importance, which include *Capsicum* L., *Solanum* L., *Nicotiana* L., and *Petunia* Juss. (Olmstead *et al.*, 2008). The genus *Solanum* contains approximately 1,400 species (Olmstead *et al.*, 2008), with potato (*S. tuberosum* L.), tomato (*S. lycopersicum* L.) and eggplant (*S. melongena* L.) being highly significant food crops (Ames and Spooner, 2008) of tremendous economic importance. Potato has a large global distribution with over 100 countries engaged in production (He *et al.*, 2012). In 2009, the world produced over 329 million metric tonnes of potato with over 50% being produced in developing countries (FAO, 2010). The current level of potato production worldwide is largely a result of the substantial increase in potato production occurring in developing countries where production has risen consistently over the previous half century, and is up from only 11 percent of the global production in the 1960's (FAO, 2010). Production in India nearly doubled in the first decade of the 21st century, while output from growers in developed countries, such as the United States and Russia, has remained fairly constant over the same time period (NPC, 2013). Today, the potato remains the world's fourth most important crop, led only by wheat, rice, and maize (He *et al.*, 2012).

Late blight disease of potato, caused by *Phytophthora infestans* (Mont.) de Bary, is possibly the most devastating contemporary disease of potato worldwide and is considered the most important threat to world potato production (Mahadour *et al.*, 2013) . *Phytophthora infestans* has great historical significance due to its causal role in the Irish Potato Famine of the 19th century (Goss *et al.*, 2014). Species in the genus *Phytophthora* are oomycetes and are members of the order Peronosporales, an order that is shared by *Pythium* sp., which are known for the seed rot and damping-off diseases that they cause (Agrios, 2005). The symptoms caused by *P. infestans* in potato manifest as small necrotic lesions on leaf and stem tissue that expand rapidly until entire leaves become necrotic and stems become structurally compromised (Schumann and D'Arcy, 2000). Potato tubers are infected later in disease development

when events such as rain or overhead irrigation wash sporangia from the infected leaf tissue into the soil, where they contact the tubers (Schumann and D'Arcy, 2000).

The pathogen presents two sexual mating types, denoted as A1 and A2, both of which are required for the pathogen to complete its sexual cycle and produce diploid resting spores, known as oospores. Before the 1980's the A2 mating type was restricted to several geographic regions of Mexico, which include the Toluca valley. These regions of Mexico are now widely considered to be the "center of origin" of *P. infestans* (Goss *et al.,* 2014). The number of oospores produced can be variety dependent with susceptible potato varieties being capable of producing copious amounts of leaf-borne oospores (Agrios, 2005). The greater distribution of the A2 mating type raises concerns that the pace of evolution of *P. infestans* may quicken and more regular advances in virulence may be produced. Another concern is the appearance of *P. infestans* in potato growing regions not previously conducive to disease development as a result of changing climate conditions characterized by increased temperature or precipitation (Gastelo *et al.,* 2014). As resistance to widely used chemical control agents, such as Metalaxyl, becomes increasingly common in populations of *P. infestans* the need to employ resistant varieties will become increasingly necessary (Solomon-Blackburn *et al.,* 2007).

The "Gene-for-Gene" concept demonstrated by Flor (1942) first provided the empirical evidence that in some host-pathogen systems the state of compatibility was conditioned by the presence of a specific resistance gene, now called a major or R-gene, which reside in the host and correspond with a specific gene for virulence within the pathogen. In a gene-for-gene system host susceptibility is specific meaning that R-genes impart resistance to all pathogenic races except those which carry the virulence genes corresponding to each R-gene present in the host (Vanderplank, 1984). The knowledge that the Mexican wild species, *Solanum demissum* Lindl., contained major genes that functioned in immunity to late blight disease was first determined by Salaman (1911). Black *et al*. (1953) identified four *S.demissum* derived R genes (*R1, R2, R3, R4*) and determined nomenclature appropriately describing this phenomenon within the Potato-*P. infestans* system. The introgression of and selection for these *S. demissum* derived major-

genes has characterized breeding efforts for resistance to late blight disease in the cultivated potato for several decades between the 1920's and 1960's (Landeo, 1993a).

Major genes (R genes) function by recognizing the pathogen and eliciting a hypersensitive response, which results in the rapid production of necrotic lesions and callose deposition around the site of infection, halting the spread of the pathogen (Landeo, 2002). The continued efficacy of vertical resistance relies on host avoidance of pathogenic races that contain R-gene corresponding virulence factors (Vanderplank, 1984). Recently, the hypersensitive response mechanism in the cultivated potato and its relatives; *Solanum sucrense* Hawkes, *Solanum berthaultii* Hawkes, *Solanum microdontum* Bitter, and *Solanum stoloniferum* Schltdl & Bouche, have become understood (Vivianne *et al.,* 2000). The hypersensitive response has been observed in the case of both major gene as well as quantitative resistance (polygene) mediated responses, which gives strength to the notion of the existence of "weak" R-genes (Vivianne *et al.,* 2000). To confound analysis further, some R-genes, such as *R*10 and *R*11, have been reported to behave in much the same way as horizontal resistance, imparting reduced foliar lesion infection until in the same manner as other R-genes the materials experience complete breakdown when challenged with the appropriate pathogenic race (Landeo, 1993c).

The breakdown or rapid loss of host-resistance due to major genes has necessitated the need to identify and concentrate polygenes, which provide race-nonspecific resistance against *P. infestans*, into improved potato varieties (Landeo, 2002). The ubiquitous presence of R-genes in the advanced materials of many breeding programs, and the mixing of major genes (R-genes) with minor genes (polygenes) in resistant materials, has the consequence of masking the contributions of minor genes to resistance responses across many races of *P. infestans* (Landeo *et al.,* 1993). Advanced materials developed by the International Potato Center (CIP) share in this issue, which has necessitated the removal or "extraction" of materials containing known R-genes of *S. demissum* origin.

In 1993, a methodology to extract "R-genes" responsible for race-specific resistance to *P.infestans* from potato germplasm was developed by researchers at CIP, and this methodology was documented in Landeo *et al*., (1993). This process of R-gene extraction has been integral to the establishment of what CIP has denoted as Population B, which is a population that is theoretically free of R-genes derived from *S. demissum*. At CIP, Population B remains under continuous improvement for the elevation of levels of horizontal or durable resistance to *P.infestans*. Race specific R-gene mediated resistance is general and susceptibility is specific, being conditioned by the presence of specific and corresponding virulence factors. Therefore, a pathogen race 0 is defined as bearing no virulence factors and therefore being unable to infect a host with a single R gene (Vanderplank, 1984; Vivianne *et al.*, 2000). However, Landeo *et al.*, (1993) further defines partially incompatible R-genes as being present in materials that are compatible with a race 0 but show a leaf area infection of less than 50% when challenged. Materials that exhibit infection levels higher than 50% in both the simple (race 0) and complex race(s) can be said to have the least possibility of containing R-genes (Landeo *et al.*, 1993).

Since 2004, in response to projections of future difficulties in potato production as a result of climate change, CIP has been developing a generation derived from Population B, denoted LBHT, that is being adapted to warm environments, resistant to late blight and displays mid-season maturity under short day lengths (Gastelo *et al*., 2014). Temperature is a particularly crucial factor in yield potential of cultivated potato with the optimum temperature for tuberization falling within the range of 15°C to 18°C. Tolerance to higher temperatures (greater than 18°C) is known to be under genetic control (Levy and Veilleux, 2007). Concerning the LBHT subpopulation, breeding for tolerance to the most yield limiting biotic and abiotic factors of potato allows CIP to position these materials to be applied broadly to the most important agricultural climates and ecologies of the developing world (Gastelo *et al.*, 2014).

Objective

Determine potential sources of race non-specific resistance to *Phytophthora infestans* in 31 advanced clones of the LBHT population, under improvement by the International Potato Center, using a combination of detached leaf assay experiments and analysis of previously conducted field experiments.

Materials and Methods

Plant materials

In June of 2015, 31 clones of advanced materials developed at the International Potato Center (CIP) were planted in a screen-house at the La Molina research station, CIP Headquarters, Lima, Peru. These advanced clones had been previously selected for resistance to Late Blight (LB) disease as well as tolerance to high temperature stress (HT) and are members of a subpopulation of population B denoted LBHT. Further information on these clones relating to resistance traits, tuber characteristics, and agronomic performance, can be found in the searchable database maintained by CIP at the web address: cipotato.org/catalogue. Four varieties, Amarillis, Serranita, Canchan, and Yungay, which are commercially grown in Peru, were selected as controls due to their range in susceptibility to late blight disease. The varieties Canchan and Yungay are known to be susceptible varieties, while the varieties Amarillis and Serranita are moderately and highly resistant, respectively (Forbes *et al.*, 2014). All materials were planted on the 12th of June 2015. Each clone was represented by five plants grown in pots with one tuber per pot. At planting, tubers were fertilized with 20N-20P-20K at a rate of up to five grams per planting. The sources of fertilization used were ammonium nitrate (33N-3P), diammonium phosphate (18N-46P) and potassium sulfate (50K-18S). Twenty days after planting the pots were 'hilled' by adding an additional 3 inches of soil to each pot. At hilling each plant was fertilized with one additional gram of urea (46N). Developing shoots were fastened to stakes as each plant grew in height. Materials were monitored at 10, 26, 29, 34, and 39 days post planting for shoot emergence, general vigor, and incidence

of disease. At 39 days post planting, leaflets from each clone were collected and separated into four groups with 105 samples taken per group. Each group was inoculated independently with either race "0" or one of three complex races. Each clone or control was evaluated using three replicates and a total of 420 samples were taken for evaluation.

Phytophthora infestans isolates

Three complex and one simple race of *P. infestans* isolates were used in each experiment. Isolate PE84006 has been characterized as race "0" (absence of R-gene corresponding virulence genes) and was used here as the singular simple race. Each of the three complex isolates belong to the EC-1 lineage, which is the dominant lineage of *P. infestans* found in Peru (Perez *et al*., 2001). The complex isolates used were Pox109, Pox110, and Pox155 due to the variability in virulence factors. The complex isolates Pox109, Pox110, and Pox155 have reported virulence factors of *R1, R2, R3, R4,* (possibly) *R5, R6, R7, R8,* (possibly) *R9, R10* and *R11*; *R1, R2, R3, R4, R6, R7, R8, R9, R10*, and *R11*; *R1, R2, R3, R4, R6, R7, R8, R10, R11,* respectively. This data was obtained from the International Potato Center's corporate database.

Propagation of Phytophthora infestans isolates

Isolates were recovered from liquid nitrogen 11 days before inoculation of experimental materials. Recovered isolates of *P. infestans* were used directly to propagate the oomycete on fresh slices of potato-tuber. Inoculated potato slices were kept in a humid chamber with conditions maintained at >80% RH, 18°C and 12 hr light/dark cycles. After one week, the samples were removed and the infected tuber slices washed with distilled water. The resulting solution was passed through vacuum filtration. A 30µm filter was used to separate the mycelium from sporangia, and a 10µm filter was used to capture sporangia. Sporangia were removed from the second filter by rinsing with distilled water into a small beaker. This solution was then incubated at 6°C for 2 hr to promote zoospore release. The solution was once again passed through a 10µm filter and the filtrate containing the zoospores was collected. A second

propagation was made onto fresh, tuber slices, and the samples were incubated in the aforementioned conditions for 7 days. The zoospores collected after the second propagation were used as inoculum for the detached leaf assay experiments.

Performing the R-gene test.

420 total samples were taken from the 31 clones and four controls to be tested. Samples were taken from leaves at the third or fourth most proximal node to the shoot apical meristem and were taken from only those plants found to be in either the immature or pre-flowering stages of development. Leaf tissue samples were placed in between moistened paper towels at collection, and were kept inside of a non-cooled container with a lid during transport. Within one hour after collection leaves were rinsed with tap water, and were divided with a razor into one or two leaflet sections. Petri plates containing a thin layer of 1.5% water agar were labeled, and two leaflets of the appropriate clone or control were placed onto the cover of each dish. Leaf tissue samples of each genotype were separated into 12 petri plates, and divided into four groups of three with each group inoculated with a different isolate (PE8006, Pox109, Pox155, and Pox110).

After an incubation period of 1 week the samples of each of the four isolates of *P. infestans* were obtained from the incubation chamber. A zoospore solution was obtained from this material using the method described previously. A zoospore suspension of 2000 sporangia / ml was prepared. Each of the 420 samples was inoculated with 20ul of the zoospore suspension per leaflet and two leaflets were inoculated per sample. Samples were divided into groups by isolate. Petri plates were taped closed and the number of the isolate was recorded. The petri plates were left to incubate at 18°C in the absence of light for 24 hr. Petri plates were then moved to an incubator maintained at 18°C, >80% RH, and 12 hr light / dark cycles for 6 days. Evaluations were made of each of three replications of each genotype and a visually approximated value as a percentage of total leaf area infected was assigned to each repetition. Additionally, a system of crosses (+, ++, +++) was used to denote sporulation intensity where greater intensity corresponds to more crosses assigned.

Field evaluations of advanced clones.

Materials corresponding with genotypes tested in lab experiments were evaluated in experiments over five seasons (2011-2015) at various field locations in Oxapampa, Peru. Materials were subjected to the prevalent annual field inoculum of this late blight endemic region. Evaluations were taken 7 weeks after the last fungicide application was applied, and were taken as visual approximations of lesion area as a percentage of total leaf area. Means and standard errors were generated by compiling results of all five evaluations, and results were denoted as Field LA for analysis purposes.

Statistical analysis

Statistical analyses were conducted with SAS version 9.4 (SAS Institute, Cary, NC). Effects of isolate given clone and clone given isolate were assessed with analysis of variance methods (PROC MIXED). Protected pairwise comparisons were made on the individual levels of these factors. Means and standard errors are reported. Lab and field lesion area values and PI values were compared at each isolate with Pearson correlation coefficients. Significance was set at a 0.05 level.

Results

The greatest number of highly resistant responses among all 31 LBHT-population clones were demonstrated in the leaf tissue assays challenged with the simple isolate, PE84006, where 22 clones were categorized as having 0.0 - 9.9 percent lesion coverage of leaf tissue (Figure 1). The remaining nine clones were distributed within the range between 10.0 and 59.9 percent lesion coverage (Figure 1). All clones that were found to be compatible with the simple isolate PE84006 (race 0) were also compatible with at least two complex races, and were determined, by the criteria outlined by Landeo *et al*., (1993b), to be either free of *S. demissum* derived R-genes or to contain partially incompatible R-genes (Table 1). These clones include: 398180.292, 398190.89, 398208.219, 398208.704, 302531.43, 302533.49,

302534.43, 302542.62, and 302551.26. It should be noted that in CIP's searchable database (cipotato.org/catalogue) the letters (CIP) precede the clone identification numbers used in this paper. Leaf assays challenged with Pox109 show 13 out of 31 responses in the highly resistant category (0.0 – 9.9), which was the greatest number of clones in this category for any of the complex isolates. This observation corresponds well with the mean lesion area across all clones within isolate Pox109 (Table 1) reported as 34.19 percent. The greatest number of responses within the category of highly susceptible (90.0-100.0) were found among those leaf assays challenged with the complex isolate, Pox155, where seven of the 31 clones where placed in this category (Table 1). However, the greatest mean lesion area across all clones were found in those leaf assays challenged with the complex isolate Pox110 (Table 1) reported as 61.62.

The most susceptible genotype tested was found to be the commercial variety, Yungay, used here as a control, which demonstrated a mean lesion area of 94.17 ± 2.88 percent when factored across all isolates (Table 1). The most susceptible genotype of the 31 LBHT population clones was clone 302551.26 with a reported mean lesion area of 72.06 percent when factored across all isolates (Table 1).

When analyzing the correlation between the three variables including: detached leaf assay sporulation intensity (Lab PI), detached leaf assay lesion area (Lab LA), and field evaluation of lesion area (Field LA), among each of the four individual isolates of *P. infestans* (Table 2), it was determined that in the case of all four isolates (PE84006, Pox109, Pox155, and Pox110) a correlation between Lab PI and Lab LA exists at a significance of $P \leq 0.001$. In the case of the complex isolate Pox 109, there was a correlation between Field LA and Lab LA at significance of $P \leq 0.05$. However, Pox109 was the only isolate that was found to be correlated based on these two factors. No correlation existed between Field LA and Lab PI within the clones tested (Table 2).

Discussion

According to the criteria outlined by Landeo *et al*., (1993), compatibility between a clone and a simple race or "race 0" of *P. infestans* determines either the absence of major, R-gene mediated resistance- of *S. demissum* origin, or determines partially incompatible R-genes in the clone to be tested. Under this criterion we can confirm that nine of the 31 advanced clones tested have the potential to be free of R-genes of *S. demissum* origin (Table 1). Field evaluation of disease response by leaf area lesion percentage (Field LA) confirmed either the absence of known R-genes or presence of partially incompatible R-genes by demonstrating compatible reactions or values exceeding 0.0 among all nine clones. From within the nine confirmed advanced clones a single clone (302551.26) demonstrated a disease response exceeding 50% mean lesion area after inoculation with race 0 (isolate PE8006) as well as three complex races, individually, in detached leaf assay experiments (Table 1). This disease response was validated by field evaluations, which reported a mean lesion area of 65.0% (Table 1). These results indicate that clone 302551.26 has the greatest potential to be free of *S. demissum* derived R-genes according to the methodology of Landeo *et al*., (1993).

A large majority of clones tested (>70%) demonstrated vertical resistance when challenged with the race "0" (isolate PE84006), while response to the complex races across all clones were highly variable (Table 1). These results confirm the large extent and variable identity of R-genes within the LBHT population, and validate the need for continued efforts focused on identifying race-nonspecific resistance within these materials. In all but one case, clones were compatible with at least one complex isolate of *P. infestans* (Table 1). These results demonstrate that the R-gene mediated resistance found in these materials is not durable and is subject to breakdown when challenged with *P. infestans* isolates, which present compatible virulence factors. Clone 398192.213 proved to be the one exception, demonstrating no compatibility across all four lab isolates (Table 1). However, this clone (398192.213) was tested in field trials in Oxapampa, Peru, and was found to be highly compatible (58.0 ± 9.4) with the prevailing seasonal

inoculum of this late blight endemic region, which provides an empirical demonstration of the breakdown of R-gene mediated resistance (Table 1).

The complex isolate, Pox109, demonstrated the most narrow distribution of percent leaf area infected values with the greatest contribution of responses being in the 0-9.9 category (Figure 1). This observation leads to the conclusion that many advanced clones tested may contain either *R*5 or *R*9, and that contrary to documentation noting the potential presence of corresponding virulence within isolate Pox109, these results lessen the likelihood that either of these virulence factors (*R*5 and *R*9) are present in isolate Pox109 (Table 1). These results show that many more genotypes were overcome by virulence factors in Pox110 and Pox155 than were overcome by similar factors in Pox109 (Figure 1). Researchers at CIP have confirmed the presence of *R*9 in isolate Pox110, which could explain the greater compatibility between this isolate and the materials tested to the presence of *R*9. In the case of Pox155 we can only speculate as to the mechanisms responsible for its increased compatibility. Pox155 lacks both *R*5 and *R*9 and the increased compatibility observed is contrary to what should be expected from the contributions of *S. demissum* derived R-genes alone.

The correlation between detached leaf assay sporulation intensity (Lab PI) and detached leaf assay lesion area (Lab LA), among each individual isolate of *P. infestans*, was expected, and assists in insuring that disease responses were not confounded by experiment factors such as, relative humidity or temperature (Table 2). The correlation at $P \leq 0.05$ between Field LA and Lab LA, within the isolate Pox109, was the only correlation made within any isolate when comparing these two factors (Table 2). These results may be explained by the prevalent field inoculum in Oxapampa, Peru displaying virulence that is most similar to this lab isolate. Previous studies have demonstrated that resistance expression between field and chamber grown *Solanum* plants is similar and that evaluation of materials by detached leaf assays is an appropriate indicator of disease response in field experiments (Vivianne *et al.*, 1999). However, our results cannot confirm those of Vivianne *et al*., (1999). This inconsistency is likely due to the previous author's use of known isolates as inoculum for both, lab and field evaluations, which contrasts with the

present study where comparisons were made between field evaluations compiled over multiple years and subject to seasonal field inoculum, and evaluations made using four known lab isolates. Results of the present study indicate that drawing correlations between defined isolates and seasonal field inoculum is tenuous. When considering the findings of Vivianne *et al.* (1999) it becomes clear that the poor correlation between lab and field analysis is likely caused by differences in virulence of the pathogen, rather than by differences in expression due to variable factors imposed by lab and field conditions.

Within the nine advanced clones found to be either without known *S. demissum* derived R-genes or containing partially incompatible R-genes, three clones have demonstrated levels of resistance lower than 50% across both lab and field evaluations (Table 1). Clones 398180.292, 398190.89 and 398208.704 have demonstrated mean lesion area percentage values of 23.75 ± 7.67, 32.50 ± 9.70, and 42.50 ± 11.22, in detached leaf assays, and 25.0 ± 12.7, 13.3 ± 4.4, and 3.8 ± 1.3, in field evaluations, respectively (Table 1). Stewart *et al*., (2003) determined that defeated R-genes are capable of conferring increased field resistance when compared with R-gene free clones. While these materials may contain partially incompatible or defeated R-genes, they may also contain high levels of polygenic resistance to *P. infestans*, and by virtue of the horizontal character of their resistance could be used to the benefit of breeding programs that are working towards the objective of improving race-nonspecific resistance to *P. infestans*.

References

1. Agrio, G. N. 2005. Plant Pathology. 5th ed. Elsevier Academic Press. London. 409-427.

2. Ames, M., and D. M. Spooner. 2008. DNA from herbarium specimens settles a controversy about origins of the European potato. *American Journal of Botany* 95(2):252-257.

3. Black, W., C. Mastenbroek, W. R. Mills, and L. C. Petersen. 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. *Euphytica* 2:173-178.

4. FAO (Food and Agriculture Organization of the United Nations) 2010. Strengthening potato value chains: technical and policy options for developing countries. Accessed on 12 October 2015 http://www.fao.org/docrep/013/i1710e/i1710e00.pdf

5. Flor, H. H. 1942. Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* 32(8):653-669.

6. Forbes, G. A., W. Perez, and J. Andrade-Piedra. 2014. Field assessment of resistance in potato to *Phytophthora infestans*. International Potato Center. Lima, Peru. Accessed on 8 December 2015 http://cipotato.org/wp-content/uploads/2014/06/006154.pdf

7. Gastelo, M., U. Kleinwechter, and M. Bonierbale. 2014. Global Potato Research for a Changing World. International Potato Research (CIP), Lima, Peru. Working Paper 2014-1. 43p.

8. Goss, E. M., J. F. Tabima, D. E. L. Cooke, S. Restrepo, W. E. Fry, G. A. Forbes, V. J. Fieland, M. Cardenas, and N. J. Grunwald. 2014. The Irish potato famine pathogen *Phytophthora infestans* originated in central Mexico rather than the Andes. *Proceedings of the National Academy of Sciences of the United States of America* 111(24):8791-8796.

9. He, Z., R. Larkin, and W. Honeycutt. 2012. Sustainable Potato Production: Global Case Studies. Springer. New York. 3-18.

10. Landeo, J. A. 1993a. Breeding for horizontal resistance to late blight in potato free of major genes. Control Integrado de las Principales Enfermedades Fungosas de la Papa. Memorias del Seminario Taller. Oct. 1993, Bellavista, Uruguay. 85-91.

11. Landeo, J. A. 1993b. A Breeding strategy to control the potato late blight disease at the International Potato Center. Control Integrado de las Principales Enfermedades Fungosas de la Papa. Memorias del Seminario Taller. Oct. 1993, Bellavista, Uruguay. 93-103.

12. Landeo, J. A. 1993c. Interference of R genes in breeding for horizontal resistance against *Phytophthora infestans*. Control Integrado de las Principales Enfermedades Fungosas de la Papa. Memorias del Seminario Taller. Oct. 1993, Bellavista, Uruguay. 111-116.

13. Landeo, J. A. 2002. Durable resistance: Quantitative/Qualitative resistance. In: Lizarraga C (ed) Proceedings of the global initiative on late blight conference, March 16-19, 1999, Quito, Ecuador.

14. Landeo, J. A., M. Gastelo, and H. Pinedo. 1993. Extraction of R-gene-free clones from contaminated gene pools. Control Integrado de las Principales Enfermedades Fungosas de la Papa. Memorias del Seminario Taller. Oct. 1993, Bellavista, Uruguay. 105-109.

15. Levy, D., and R. Veilleux. 2007. Adaptation of potato to high temperatures and salinity-A review. *American Journal of Potato Research* 84(6):487-506.

16. NPC (National Potato Council). 2013. Potato statistical yearbook: 2013. Accessed on 12 October 2015. http://nationalpotatocouncil.org/files/4613/6940/5509/2013_NPCyearbook_Web_FINAL.pdf

17. Olmstead, R. G., L. Bohs, H. A. Migid, E. Santiago-Valentin, V. F. Garcia, and S. M. Collier. 2008. A molecular phylogeny of the Solanaceae. *International Association for Plant Taxonomy* 57(4):1159-1181.

18. Salaman, R. N. 1910. The inheritance of color and other characters in the potato. *Journal of Genetics* 1:7-46.

19. Schumann, G.L., and C. J. D'Arcy. 2000. *Late blight of potato and tomato*. The Plant Health Instructor. DOI: 10.1094/PHI-I-2000-0724-01

20. Stewart, H. E., J. E. Bradshaw, and B. Pande. 2003. The effect of the presence of R-genes for resistance to late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*) on the underlying level of field resistance. *Plant pathology* 52:193-198.

21. Solomon-Blackburn, R. M., H. E. Stewart, and J. E. Bradshaw. 2007. Distinguishing major-gene from field resistance to late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*) and selecting for high levels of field resistance. *Theoretical and Applied Genetics* 115:141-149.

22. Mahadour, S., R. Pelle, J. Abiven, F. Aurousseau, H. Dubreuil, Y. L. Hingrat, and J. Chauvin. 2013. Disease progress curve parameters help to characterize the types of resistance to late blight segregating in cultivated potato. *Potato Research* 56:99-114.

23. Perez, W. G., J. S. Gamboa, Y. V. Falcon, M. Coca, R. M. Raymundo, and R. J. Nelson. 2001. Genetic structure of Peruvian populations of *Phytophthora infestans*. *Phytopathology* 91:956-965.

24. Vanderplank, J. E. 1984. Disease Resistance in Plants. 2nd ed. Academic Press, London. 5-12.

25. Vivianne, G. A., A. Vleeshouwers, W. Dooijeweert, P. L. C. Keizer, L. Sijpkes, F. Govers, and L. T. Colon. 1999. A laboratory assay for *Phytophthora infestans* resistance in various *Solanum* species reflects the field situation. *European Journal of Plant Pathology* 105:241-251.

26. Vivianne, G. A., A. Vleeshouwers, W. van Dooijeweert, F. Govers, S. Kamoun, and L. T. Colon. 2000. The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. *Planta* 210:853-864.

Table 1. Thirty-five genotypes of *Solanum tuberosum* suspected of carrying *Solanum demissum* derived R-genes were challenged with four isolates of *Phytophthora infestans* using detached leaf assays, which included a race 0 (PE84006 Isolate) as well as three complex isolates. The complex isolates Pox109, Pox110, and Pox155 are reported to carry virulence factors of; *R1, R2, R3, R4,* (possibly) *R5, R6, R7, R8,* (possibly) *R9, R10* and *R11*; *R1, R2, R3, R4, R6, R7, R8, R9, R10,* and *R11*; *R1, R2, R3, R4, R6, R7, R8, R10,* and *R11,* respectively. In detached leaf assays two leaflets were inoculated per replicate with a zoospore suspension at a concentration of 2000 sporangia / ml. Late blight severity was measured as percent lesion area covering total leaf area. Means and SE were calculated using (n=3) replicates. Field evaluations were performed in Oxapampa, Peru, over five consecutive growing seasons (2010 – 2014) and the means ± SE were compiled across all seasons. All scores are reported as mean ± SE.

Genotype		Lesion area					
	R-gene present[a]	PE84006 isolate	Pox109 isolate	Pox155 isolate	Pox110 isolate	Overall lab mean[b]	Field population
398098.65	yes	0.0 ± 0.0f[c]	53.3 ± 6.7defg	0.0 ± 0.0j	0.0 ± 0.0i	13.33 ± 7.11	67.5 ± 9.7
398098.203	yes	0.0 ± 0.0f	33.3 ± 24.0gh	96.7 ± 3.3ab	43.3 ± 26.0efgh	43.33 ± 12.93	18.0 ± 4.6
398098.205	yes	0.0 ± 0.0f	30.0 ± 5.8ghi	53.3 ± 27.3efgh	43.3 ± 3.3efgh	31.67 ± 8.51	28.0 ± 14.0
398098.57(0)	yes	0.0 ± 0.0f	6.7 ± 6.7ij	23.3 ± 14.5hij	20.0 ± 5.8hi	12.50 ± 4.63	16.0 ± 3.7
398180.292	no	21.7 ± 14.8de	6.7 ± 6.7ij	23.3 ± 12.0hij	43.3 ± 23.3efgh	23.75 ± 7.67	25.0 ± 12.7
398190.89	no	16.7 ± 16.7ef	60.0 ± 11.5cdef	6.7 ± 6.7j	46.7 ± 26.0efgh	32.50 ± 9.70	13.3 ± 4.4
398192.213	yes	0.0 ± 0.0f	0.0 ± 0.0j	0.0 ± 0.0j	0.0 ± 0.0i	0.0 ± 0.0	58.0 ± 9.4
398192.592	yes	0.0 ± 0.0f	0.0 ± 0.0j	0.0 ± 0.0 j	30.0 ± 5.8ghi	7.50 ± 4.11	57.0 ± 11.0
398201.51(0)	yes	0.0 ± 0.0f	6.7 ± 6.7ij	73.3 ± 3.3abcdef	20.0 ± 20.0hi	25.00 ± 9.81	36.0 ± 9.5
398203.5	yes	0.0 ± 0.0f	0.0 ± 0.0j	66.7 ± 17.6bcdef	63.3 ± 12.0abcdefg	32.50 ± 10.81	51.0 ± 14.7
398203.244	yes	0.0 ± 0.0f	0.0 ± 0.0j	96.7 ± 3.3ab	86.7 ± 3.3abcd	45.83 ± 13.90	16.3 ± 9.7
398208.29	yes	0.0 ± 0.0f	6.7 ± 6.7ij	93.3 ± 3.3abc	93.3 ± 6.7abc	48.33 ± 13.75	72.0 ± 13.5
398208.33	yes	0.0 ± 0.0f	50.0 ± 5.8efg	93.3 ± 6.7abc	86.7 ± 13.3abcd	57.50 ± 11.69	6.0 ± 1.0
398208.219	no	20.0 ± 11.5de	50.0 ± 10.0efg	0.0 ± 0.0j	36.7 ± 27.3fghi	26.67 ± 8.73	68.0 ± 12.8
398208.505	yes	0.0 ± 0.0f	0.0 ± 0.0j	20.0 ± 11.5ij	46.7 ± 13.3efgh	16.67 ± 6.89	5.0 ± 1.6
398208.62(0)	yes	0.0 ± 0.0f	33.3 ± 3.3gh	100.0 ± 0.0a	73.3 ± 8.8abcdef	51.67 ± 11.67	13.0 ± 4.6
398208.67(0)	yes	0.0 ± 0.0f	0.0 ± 0.0j	73.3 ± 17.6abcdef	80.0 ± 11.5abcde	38.33 ± 12.42	22.0 ± 10.6

398208.704	no	30.0 ± 15.3de	0.0 ± 0.0j	43.3 ± 8.8fghi	96.7 ± 3.3ab	42.50 ± 11.22	3.8 ± 1.3
302506.39	yes	0.0 ± 0.0f	0.0 ± 0.0j	56.7 ± 8.8efg	56.7 ± 8.8bcdefgh	28.33 ± 8.95	8.0 ± 4.4
302531.43	no	53.3 ± 14.5bc	20.0 ± 10.0hij	56.7 ± 16.7efg	76.7 ± 14.5abcdef	51.67 ± 8.60	11.0 ± 4.0
302533.38	yes	0.0 ± 0.0f	40.0 ± 23.1fgh	30.0 ± 15.3ghij	56.7 ± 21.9bcdefgh	31.67 ± 9.76	48.8 ± 20.7
302533.4(0)	yes	0.0 ± 0.0f	93.3 ± 6.7ab	60.0 ± 20.0defg	76.7 ± 18.6abcdef	57.50 ± 12.19	48.8 ± 16.9
302533.48	yes	0.0 ± 0.0f	76.7 ± 3.3abcd	30.0 ± 15.3ghij	93.3 ± 6.7abc	50.00 ± 11.74	N/A[d]
302533.49	no	13.3 ± 6.7ef	70.0 ± 5.8bcde	80.0 ± 20.0abcde	76.7 ± 18.6abcdef	60.00 ± 10.22	29.0 ± 11.6
302533.74	yes	0.0 ± 0.0f	20.0 ± 20.0hij	83.3 ± 6.7abcde	100.0 ± 0.0a	50.83 ± 13.40	52.0 ± 12.9
302534.1(0)	yes	0.0 ± 0.0f	0.0 ± 0.0j	90.0 ± 5.8abcd	73.3 ± 17.6abcdef	40.83 ± 13.05	41.0 ± 17.6
302534.17	yes	0.0 ± 0.0f	20.0 ± 11.5hij	63.3 ± 13.3cdef	60.0 ± 15.3abcdefgh	35.83 ± 9.49	38.0 ± 11.0
302534.43	no	25.0 ± 17.6de	60.0 ± 15.3cdef	76.7 ± 14.5abcde	53.3 ± 6.7cdefgh	53.75 ± 8.24	73.3 ± 19.6
302542.62	no	36.7 ± 12.0cd	20.0 ± 11.5hij	73.3 ± 17.6abcdef	76.7 ± 18.6abcdef	51.67 ± 9.76	13.3 ± 6.0
302551.26	no	56.7 ± 3.3b	86.7 ± 3.3ab	90.0 ± 5.8abcd	66.7 ± 17.6abcdefg	75.00 ± 5.84	65.0 ± 13.4
304079.16	yes	0.0 ± 0.0f	83.3 ± 6.7abc	70.0 ± 5.8abcdef	53.3 ± 29.0cdefgh	51.67 ± 11.54	N/A[d]
Amarilis	no	26.7 ± 3.3de	86.7 ± 6.7ab	43.3 ± 3.3fghi	46.7 ± 24.0efgh	50.83 ± 8.57	88.0 ± 4.6
Serranita	yes	0.0 ± 0.0f	0.0 ± 0.0j	3.3 ± 6.7abc	100.0 ± 0.0a	48.33 ± 14.66	35.0 ± 0.0
Canchan	yes	0.0 ± 0.0f	83.3 ± 16.7abc	76.7 ± 14.5abcde	80.0 ± 11.5abcde	60.00 ± 11.74	N/A[d]
Yungay	no	83.3 ± 8.8a	100.0 ± 0.0a	93.3 ± 3.3abc	100.0 ± 0.0a	94.17 ± 2.88	80.0 ± 9.0
Mean[e]		10.95 ± 2.16	34.19 ± 3.53	58.00 ± 3.56	61.62 ± 3.39		38.00 ± 2.69

[a] Identifies only known *Solanum demissum* Lindl. derived R-genes based on the criteria outlined by Landeo *et al*., (1993).

[b] Means + SE are calculated for each genotype across isolates: PE84006; Pox109; Pox155; Pox110, used in detached leaf assays.

[c] Means with the same letter(s) are not significantly different at $P< 0.05$ within columns.

[d] N/A identifies the clone as not being evaluated for response when challenged with field populations of *P. infestans*.

[e] Means + SE are calculated for each isolate across all clones.

Table 2. Pearson correlation coefficients (*r*) made between three variables, which include: detached leaf assay sporulation intensity (Lab PI), detached leaf assay lesion area (Lab LA), and field evaluation of lesion area (Field LA), using *n*=35, *n*=34, and *n*=32, respectively. Correlation of factors related to infection and colonization by four separate isolates (PE84006, Pox109, Pox155 and Pox110) of *Phytophthora infestans* in advanced clones of the LBHT population under continuous improvement at the International Potato Center, Lima, Peru.

	Lab PI[a]	**Lab LA[b]**
	---------- Isolate PE84006 ----------	
Field LA[c]	0.317	0.235
Lab PI[a]		0.968*** [d]
	---------- Isolate Pox109 ----------	
Field LA	0.429	0.464*
Lab PI		0.929***
	---------- Isolate Pox155 ----------	
Field LA	0.166	0.159
Lab PI		0.871***
	---------- Isolate Pox110 ----------	
Field LA	-0.04	-0.153
Lab PI		0.644***

[a] Lab PI is the measure of sporulation intensity associated with colonization by *P. infestans* within detached leaf assays.

[b] Lab LA is the measure of lesion area associated with colonization by *P. infestans* as a percentage of total leaf area within detached leaf assays.

[c] Field LA is the measure of lesion area associated with colonization by *P. infestans* as a percentage of total area and averaged across field evaluations taken over five growing seasons (2010 – 2014).

[d] Pearson correlation coefficient (r) significant at $P \leq 0.05$, ≤ 0.01, and ≤ 0.001; denoted as *, **, and ***, respectively.

Table 3. Thirty-five genotypes of *Solanum tuberosum* suspected of carrying *Solanum demissum* derived R-genes were challenged with four isolates of *Phytophthora infestans* using detached leaf assays, which included a race 0 (PE84006 isolate) as well as three complex isolates. The complex isolates Pox109, Pox110, and Pox155 are reported to carry virulence factors of; *R1, R2, R3, R4,* (possibly) *R5, R6, R7, R8,* (possibly) *R9, R10,* and *R11*; *R1, R2, R3, R4, R6, R7, R8, R9, R10,* and *R11*; *R1, R2, R3, R4, R6, R7, R8, R10,* and *R11*, respectively. In detached leaf assays two leaflets were inoculated per replicate with a zoospore suspension at a concentration of 2000 sporangia / ml. Late blight disease severity was measured by sporulation intensity given as three possible ratings; 1, 2, and 3, which corresponded to low, moderate, & heavy sporulation, respectively. Means and SE were calculated using (n=3) replicates. All values are reported as mean ± SE.

Genotype	Sporulation Intensity			
	PE84006 isolate	Pox109 isolate	Pox155 isolate	Pox110 isolate
398098.65	0.0 ± 0.0d[a]	1.0 ± 0.0b	0.0 ± 0.0e	0.0 ± 0.0e
398098.203	0.0 ± 0.0d	0.7 ± 0.3bc	2.3 ± 0.7a	1.0 ± 0.0cde
398098.205	0.0 ± 0.0d	1.0 ± 0.0b	1.3 ± 0.7b	1.0 ± 0.0cde
398098.57(0)	0.0 ± 0.0d	0.3 ± 0.3cd	0.7 ± 0.3cd	1.0 ± 0.0cde
398190.89	0.7 ± 0.7c	1.0 ± 0.0b	0.3 ± 0.3de	1.0 ± 0.6cde
398192.213	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0e	0.0 ± 0.0e
398192.592	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0e	1.0 ± 0.0cde
398201.51(0)	0.0 ± 0.0d	0.3 ± 0.3cd	1.0 ± 0.0bc	0.3 ± 0.3de
398203.5	0.0 ± 0.0d	0.0 ± 0.0d	1.0 ± 0.0bc	1.0 ± 0.0cde
398203.244	0.0 ± 0.0d	0.0 ± 0.0d	1.0 ± 0.0bc	1.0 ± 0.0cde
398208.29	0.0 ± 0.0d	0.3 ± 0.3cd	1.0 ± 0.0bc	1.0 ± 0.0cde
398208.33	0.0 ± 0.0d	1.0 ± 0.0b	1.0 ± 0.0bc	1.0 ± 0.0cde
398208.219	0.7 ± 0.3c	1.0 ± 0.0b	0.0 ± 0.0e	0.7 ± 0.3def
398208.505	0.0 ± 0.0d	0.0 ± 0.0d	0.7 ± 0.3cd	1.0 ± 0.0cde
398208.62(0)	0.0 ± 0.0d	1.0 ± 0.0b	1.3 ± 0.3b	1.0 ± 0.0cde
398208.67(0)	0.0 ± 0.0d	0.0 ± 0.0d	1.0 ± 0.0bc	1.0 ± 0.0cde
398208.704	0.7 ± 0.3c	0.0 ± 0.0d	1.0 ± 0.0bc	1.3 ± 0.3cd
302506.39	0.0 ± 0.0d	0.0 ± 0.0d	1.0 ± 0.0bc	1.3 ± 0.3cd

302531.43	1.0 ± 0.0bc	0.7 ± 0.3bc	1.3 ± 0.3b	1.0 ± 0.0cde
302533.38	0.0 ± 0.0d	0.7 ± 0.3bc	0.7 ± 0.3cd	1.0 ± 0.0cde
302533.4(0)	0.0 ± 0.0d	1.0 ± 0.0b	1.3 ± 0.3b	2.7 ± 0.3a
302533.48	0.0 ± 0.0d	1.0 ± 0.0b	0.7 ± 0.3cd	2.7 ± 0.3a
302533.49	0.7 ± 0.3c	1.0 ± 0.0b	1.0 ± 0.0bc	1.0 ± 0.0cde
302533.74	0.0 ± 0.0d	0.3 ± 0.3cd	1.0 ± 0.0bc	1.7 ± 0.3bc
302534.1(0)	0.0 ± 0.0d	0.0 ± 0.0d	1.0 ± 0.0bc	1.0 ± 0.0cde
302534.17	0.0 ± 0.0d	0.7 ± 0.3bc	1.0 ± 0.0bc	1.3 ± 0.3cd
302534.43	1.0 ± 0.0bc	1.0 ± 0.0b	1.0 ± 0.0bc	1.0 ± 0.0cde
302542.62	1.3 ± 0.3b	0.7 ± 0.3bc	1.0 ± 0.0bc	3.0 ± 0.0a
302551.26	2.0 ± 0.0a	2.0 ± 0.0a	1.0 ± 0.0bc	1.0 ± 0.0cde
304079.16	0.0 ± 0.0d	2.0 ± 0.0a	1.0 ± 0.0bc	1.3 ± 0.7cd
Amarilis	1.0 ± 0.0bc	1.7 ± 0.3a	1.0 ± 0.0bc	1.0 ± 0.6cde
Serranita	0.0 ± 0.0d	0.0 ± 0.0d	1.0 ± 0.0bc	1.7 ± 0.7cd
Canchan	0.0 ± 0.0d	1.7 ± 0.3a	1.0 ± 0.0bc	2.3 ± 0.3ab
Yungay	2.3 ± 0.3a	2.0 ± 0.0a	1.0 ± 0.0bc	3.0 ± 0.0a

[a] Means with the same letter(s) are not significantly different at P< .0001 within columns.

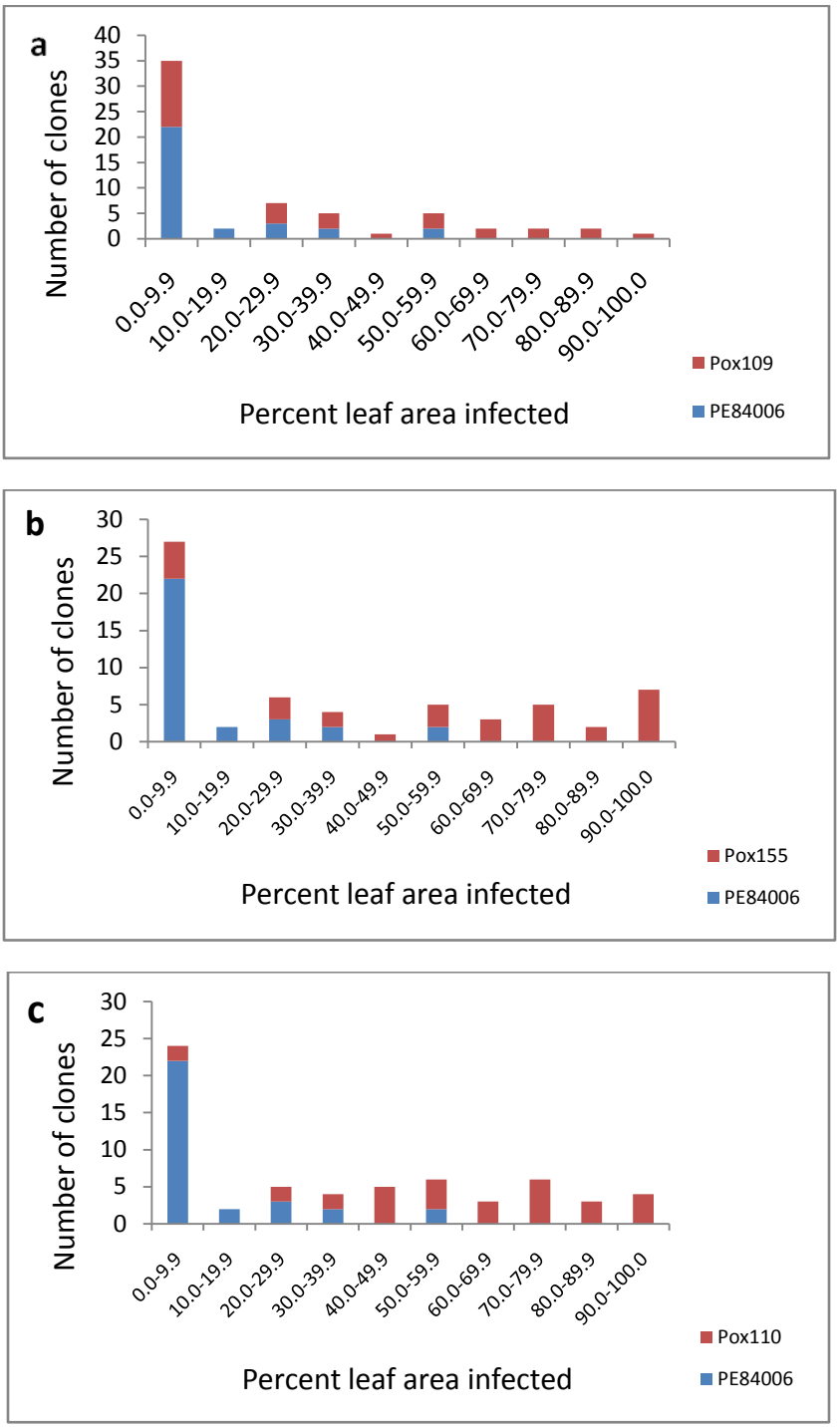

Figure 1. Thirty-one potato clones selected from the International Potato Center's LBHT population were evaluated in detached leaf assays 6 days after being challenged with one simple and three complex isolates of *Phytophthora infestans*. The leaf response to race "0"or isolate PE84006 is compared individually to the complex isolates: Pox109, Pox155, and Pox110 to assess the relative distribution of clones by percent of leaf area infected.

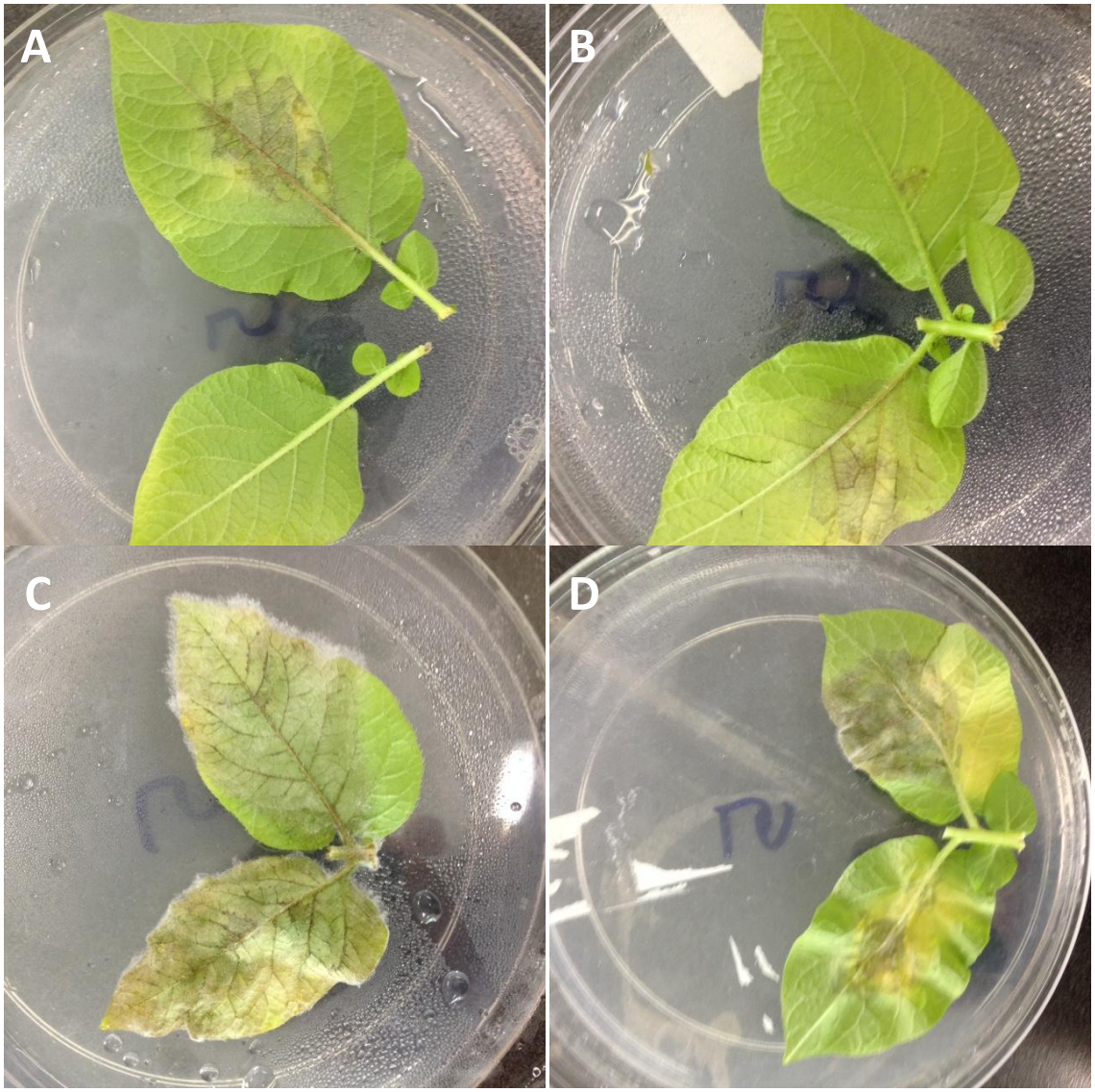

Figure 2.1. Response of advanced clone, CIP398180.292, to inoculation with four isolates of *Phytophthora infestans* in leaf tissue assays, which were applied at a concentration of 2000 sporangia / ml and 20ul of suspension per leaflet. A. Response to a simple race, isolate PE84006. B. Response to a complex isolate, Pox109. C. Response to a complex isolate, Pox110. D. Response to a complex isolate, Pox155. Advanced clone, CIP398180.292, was found to have the lowest mean percent lesion area (23.75 ± 7.67) across all four isolates of *P. infestans* among the nine clones with the potential to be free of *S. demissum* derived R-genes (Table 1).

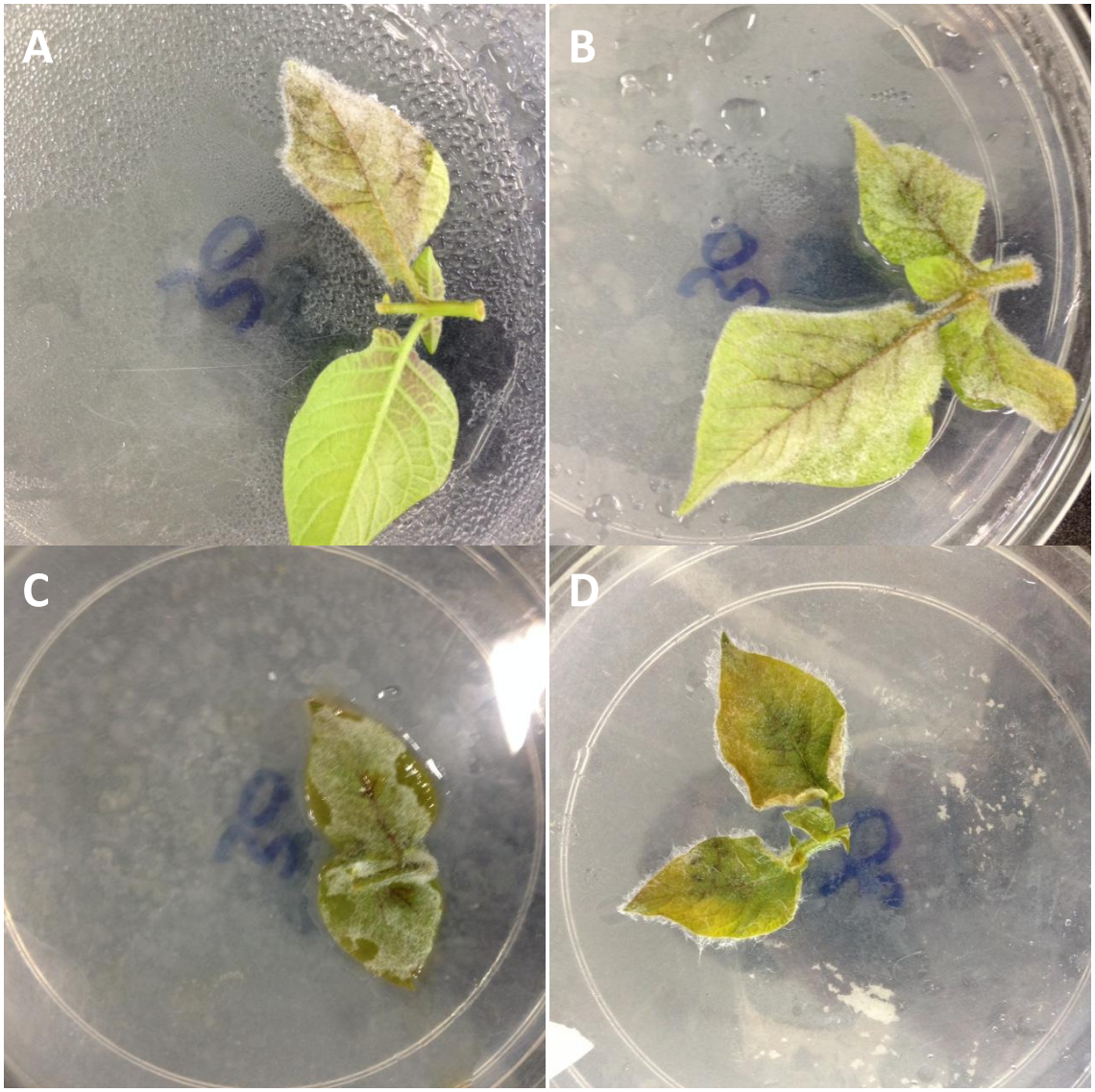

Figure 2.2 Response of advanced clone, CIP302551.26, to inoculation with four isolates of *Phytophthora infestans* in leaf tissue assays, which were applied at a concentration of 2000 sporangia / ml and 20ul of suspension per leaflet. A. Response to a simple race, isolate PE84006. B. Response to a complex isolate, Pox109. C. Response to a complex isolate, Pox110. D. Response to a complex isolate, Pox155. Advanced clone, CIP302551.26 was found to have the highest mean percent lesion area (75.00 ± 5.84) across all four isolates of *P. infestans* among the nine clones with the potential to be free of *S. demissum* derived R-genes (Table 1).

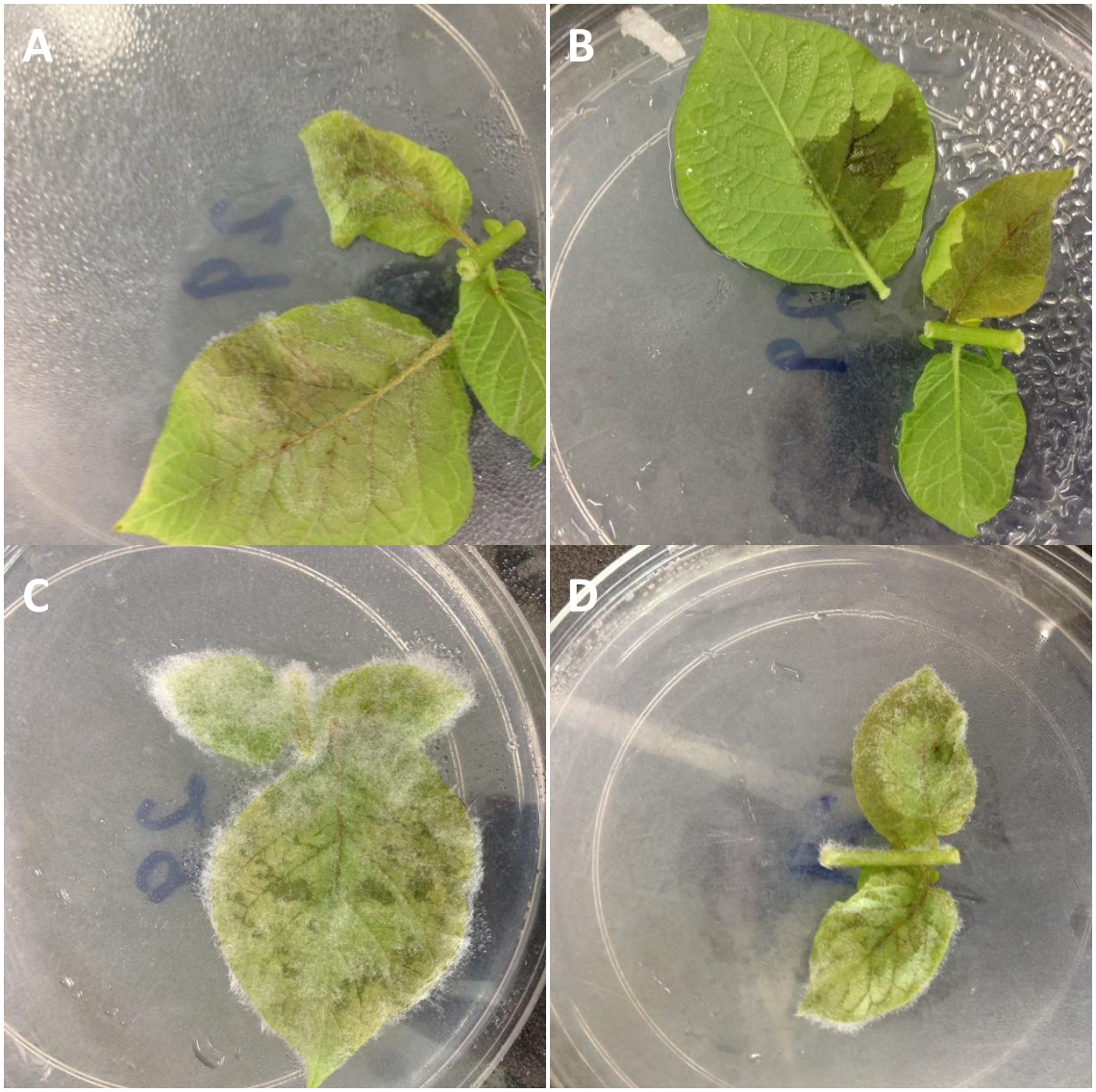

Figure 2.3 Response of advanced clone, CIP 302542.62 to inoculation with four isolates of *Phytophthora infestans* in leaf tissue assays, which were applied at a concentration of 2000 sporangia / ml and 20ul of suspension per leaflet. A. Response to a simple race, isolate PE84006. B. Response to a complex isolate, Pox109. C. Response to a complex isolate, Pox110. D. Response to a complex isolate, Pox155. Advanced clone, CIP 302542.62 was found to have a moderate mean percent lesion area (51.67 ± 9.76) across all four isolates of *P. infestans* among the nine clones with the potential to be free of *S. demissum* derived R-genes (Table 1).