Risk assessment analysis of potato genotype susceptibility to water rot-causing oomycetes

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ABSTRACT

Water rots are a group of important potato tuber rot diseases such as pink rot, Phytophthora tuber rot, and leak caused by the oomycete pathogens Phytophthora erythroseptica, P. nicotianae, and Pythium ultimum, respectively. If not managed, these diseases either alone or in combination, can cause severe yield loss and substantial reductions in quality. Growers continue to rely on fungicides for water rot management in the field and during post-harvest storage. Previous and ongoing breeding attempts have failed to identify and develop commercially acceptable potato cultivars resistant to all three diseases. This is mainly due to the complex, expensive, and time-consuming methodologies required to screen for susceptibility to water rot pathogens. Currently, potato genotypes are assessed for susceptibility to individual water rot pathogens which is labor intensive. Considerable savings in time and effort would be realized if potato genotypes could be evaluated for susceptibility to one water rot pathogen and then statistical analysis applied to determine the probability of the reaction of a genotype to the other rot pathogens. A proportional odds model was fitted to examine the risk of genotype screening outcome (ordinal) to understand the relationships among water rot causing oomycetes in potato. Compared to P. erythroseptica, P. ultimum infected genotypes having susceptibility risk was high (2.6) versus other cultivar susceptibility categories. Potato genotypes screened for P. nicotianae have a significant susceptibility risk decreased by 38% when compared to P. erythroseptica.

1. Introduction

Potato (Solanum tuberosum L.) is an extensively grown and consumed annual tuber crop in many regions of the world. The potato agro-ecosystem provides a conducive habitat for many foliar and soilborne pathogens. Of these, a number of soilborne oomycetes affect the potato crop causing potential yield, storability and tuber quality loss (Taylor et al., 2012). Several oomycetes, such as Phytophthora erythroseptica Pethybr, P. nicotianae van Breda de Haan, and Pythium ultimum Trow, are known to infect potato tubers, causing pink rot, Phytophthora tuber rot and Pythium leak, respectively (Erwin and Ribeiro, 1996; Johnson et al., 2004; Salas et al., 2003; Taylor et al., 2004). These oomycetes are most commonly found in potato production areas under high soil moisture conditions and in regions with prolonged rains during the later stages of the growing season (Goss, 1949; Jones, 1935; Taylor et al., 2004). However, in the U.S. the P. nicotianae caused tuber rot is found only in warm season production areas generally below 42°latitude (Panabieres et al., 2016). Collectively, these storage rots are col- orationally referred to as ‘water rots’ by the U.S. potato industry.

Under favorable conditions for disease development, asexually reproduced zoospores infect the tubers in field and/or during post-harvest storage. During adverse environmental conditions or absence of host, oomycetes can remain dormant in infested soils for extended periods primarily as chlamydomospores (P. nicotianae) and/or as non-motile, thick walled oospores. Under field conditions, typical tuber infections are initiated upon contact with pathogen inoculum and/or when the pathogen gains entry through wounds (Salas et al., 2000). The common outcome of these infections is a watery rot disease with a few physiological differences in tuber symptom expression with respect to color and texture (Taylor et al., 2004). Phytophthora spp. and Pythium spp. differ in mode of infection, where the former is capable of infecting the tuber via stolons, eyes, or wounds, the later can only gain entry into the tuber through damaged periderm tissue (Salas et al., 2000; Taylor et al., 2004). Tuber injuries are common during harvest and storage activities and the injury extent may range from 15 to 87% depending on cultivar and prevailing soil conditions (Hudson and Orr, 1977; Plissey, 1993; Salas et al., 2000). If left unchecked, water rot pathogens may cause significant tuber yield and quality loss extending from field to storage and storage to transit (Yellareddygari et al., 2016).

Fungicides continue to be the primary management tool for water...
rot diseases both in the field and in storage, although fungicides are less effective for managing leak than they are for pink rot (Johnson et al., 2004; Taylor et al., 2004). Phenylamide (metalaxyl and mefenoxam) fungicides are commonly applied to combat water rot diseases during the growing season. In many potato growing regions in U.S., identifi-
cation of Phytophthora and Pythium isolates resistant to mefenoxam and metalaxyl fungicides has hindered chemical management of the dis-
eases they cause (Johnson et al., 2004; Mulrooney, 1982; Taylor et al., 2002, 2006; Torres et al., 1985; Wicks et al., 2000). Currently, phos-
phonate (phosphoric acid) fungicides are most often used to control post-harvest storage infection of tubers caused by Phytophthora patho-
gens (Johnson et al., 2004; Miller et al., 2006; Taylor et al., 2011).

Potato cultivars have been evaluated for their susceptibility to all three water rot pathogens and clearly demonstrate that with only a few exceptions, varying levels of susceptibility exist among cultivars to all three diseases (Fitzpatrick-Peabody and Lambert, 2011; Peters and Sturz, 2001; Peters et al., 2004; Salas et al., 2003; Taylor et al., 2008b, 2012). However, the degree of susceptibility to pink rot and leak in potato cultivars, and the amount of disease control that can be achieved through the use of mefenoxam, are inter-related (Taylor et al., 2008a). Regardless, the absence of potato cultivars completely resistant to both pink rot and leak has forced growers to continue to rely on fungicide management in the field and in storage (Johnson et al., 2004; Salas et al., 2003; Taylor et al., 2011). Additionally, the increased reliance on phosphoric acid compounds may lead to fungicide selection pressure on pathogen populations resulting in pathogen insensitivity to this fungici-
cide as has been the case with mefenoxam (Taylor et al., 2002, 2006). A model for the prediction of pink rot disease development in storage has been developed to further assist potato growers in adjusting strategies to manage late season infections and infections that can occur through wounds made at harvest (Yellareddygari et al., 2016).

Breeding programs screening for cultivars resistant to water rot pathogens are sporadic (Salas et al., 2003), time-consuming, and expen-
sive. Most host screening studies have evaluated susceptibility to a single pathogen (Fitzpatrick-Peabody and Lambert, 2011; Peters and Sturz, 2001; Peters et al., 2004; Taylor et al., 2012) and only a few studies have attempted simultaneous screening of two water rot pathogens (Salas et al., 2003; Thompson et al., 2007). This is largely due to the complex and labor intensive methods needed to screen potato cultivars for three pathogens.

Risk assessment methodology provides prior notification of a risk of outcome to a grower or a researcher (Shah et al., 2013). Risk assess-
ment is commonly used in medical studies to identify and analyze po-
tential risk factors and to determine or improve the strategies for managing a risk outcome (Harrell, 2001; Prentice, 1985; Ricketson et al., 2013). For example, case-control studies usually estimate the relative risk by comparing the disease outcome in one group to that of another group (usually a placebo or reference group). Similar risk as-
essment methodologies have been applied in phytopathology. Risk levels of deoxyvinavenol toxin in Fusarium-infected wheat (Landschoot et al., 2013), Fusarium head blight epidemics risk with pre- and post-
anthesis (Shah et al., 2013), and preplanting risk assessment for gray leaf spot of maize (Paul and Munkvold, 2004) are examples of risk assessment applications. Similarly, estimating and comparing the risk differences in susceptibility of cultivars to water rot pathogens may improve the efficiency of screening process, especially when the number of genotypes to be screened is large and there are both time and resource constraints. The objective of this study was to examine geno-
type susceptibility risk levels in order to better understand the rela-
tionships among pink rot, leak, and Phytophthora tuber rot and thereby facilitate a more efficient screening process.

2. Materials and methods

The studies were conducted for genotype screening on P. ery-
throsporica, P. nicotianae and P. ultimum to identify resistance genetic resource material for future breeding programs. Test genotypes were planted in tuber production plots similar to those used in previous studies (Salas et al., 2003; Thompson et al., 2007), established near Inkster, North Dakota over a seven year period. A total of 13 separate post-harvest challenge inoculations were conducted on tubers har-
vested from these plots. Overall, 295 potato genotypes obtained from North Dakota State University (115) and other breeding programs (180). Each clone was screened via post-harvest challenge inoculation for susceptibility to each of the pathogens separately as previously described (Salas et al., 2003; Taylor et al., 2004, 2008b; Thompson et al., 2007). Depending on research objectives, prevailing weather conditions and availability of farm and seed resource material, planting was initiated from the first week of May to late-June. Cut seed tubers were used to establish the production plots and all cultivars were planted in replicated trials in which an experimental unit consisted of a single 30 m row. Standard agronomic and cultural practices typical of the potato crop and region (ND) were implemented during the growing season. As per crop and label recommendation, routine herbicide and pesticides were applied during the growing season for weed and pest management. As necessary the crop was irrigated using overhead sprinkler irrigation system. Two days prior to harvest, the vines were mechanically desiccated by means of a rotoblower and harvested tubers were transported to potato storage facility at NDSU for post-harvest disease screening study.

2.1. Pathogen isolates, inoculation, and disease assessment

Previously tested isolates 266-2, 06TX1-3, and 09MN10-5 of P. er-
ythrosporica, P. nicotianae, and P. ultimum, respectively, were used for challenge inoculations in all trials. Disease-free test tubers (150–200 gm) were randomly selected from the harvested production plots and inoculum preparation and post-harvest infection methodology for Phytophthora and Pythium spp. were performed as described in previous research studies (Salas et al., 2003; Taylor et al., 2004, 2006, 2008b; Thompson et al., 2007). Briefly, Phytophthora isolates were grown on plates using clarified V8 juice agar at 20–25 °C temperature. After 3 days of incubation, mycelial plugs were transferred to petri plates containing V8 broth. After plates were incubated (20–25 °C) for 3 days, V8 broth was decanted and mycelial mats are rinsed with sterile deionized water. Sporangial formation occurred after autoclaved soil water extract (10 ml) was added to each plate and incubated for 2–3 days under continuous light. Zoospores are released after cultures were subjected to chilling temperatures for 1 h followed by 30 min warming at room temperature. P. erythrosporica and P. nicotianae inoculum (at concentration of 2 × 104 zoospores ml−1) was applied on three apical eyes of each tuber by placing a single drop of inoculum. The P. ultimum isolate was grown on culture plates containing modified V8 juice agar (100 ml of V8 juice, 1.25 g of CaCO3, 15 g of agar, 900 ml of deionized water) for 2 days at 20–25 °C. (Taylor et al., 2004). For P. ultimum, inoculation was performed by wounding (using abrasive pad) the periderm of tuber and placing the pathogen colonized agar plug (5 mm) on the freshly wounded tissue.

Post inoculation, the infected tubers were counted and disease in-
cidence (I) was calculated (I = (Number of infected tubers/Number of inoculated tubers) * 100). Disease severity was measured as the rate of penetra-
tion (P) by determining the maximum depth (D mm) of the rotted tissue measured from the inoculation point over the incubation period. Typically the inoculated tubers were placed in covered plastic containers and incubated under dark and moist conditions for 3–7 days at 21 °C–24 °C for symptom development. Plastic containers, each with 10 tubers, were arranged in a randomized complete block design re-
plicated four times. The incubation period and ambient temperature varied depending upon the pathogen and genotypes used, however, for each trial P = D/T (mm/day), where D is depth of penetration and T is time in days post inoculation. Previous studies used incidence and rate of penetration following infection for characterizing cultivar
susceptibility (Taylor et al. 2008b, 2012; Thompson et al., 2007). Based on incidence and penetration results, potato clones were categorized as resistant (R < 25% incidence), moderately resistant (MR > 25% and < 50% incidence), moderately susceptible (MS > 50% incidence and low rate of penetration), and susceptible (S > 50% incidence and high rate of penetration). Low and high rate of penetration for each post-inoculation trial was calculated based on observed maximum penetration divided by two. When maximum rate of penetration was recorded as 14 mm during trial x, low and high rate of penetration were categorized as < 7 mm and > 7 mm, respectively. These differences among genotype susceptibility levels (response variables) were examined by appropriate regression analysis to understand water rot diseases (explanatory variables) in potato.

2.2. Proportion odds model

The observed ordinal outcome Y with k + 1 categories was cultivar susceptibility (Y=S, MS, ..., k) and a covariate X (3 pathogens). Since there is an order among genotype susceptibility response and potential order of rating is S < MS < MR < R. Therefore, proportion odds model (POM), a class of ordinal logistic regression model was used to model the relationship between water rot pathogens and cultivar susceptibility categories. This model assumes that each explanatory variable exerts identical effect on each cumulative logit of the ordinal outcome variable (Y) regardless of the cutoff k (McCullagh, 1980). In other words, an increase or decrease due to change in explanatory variable will affect the log odds of all categories equally. POM takes the general form

\[
\log \frac{P(Y < k)}{P(Y > k)} = \alpha_i + \beta X, \quad i = 1, \ldots, k.
\]

Where, log (●) is the natural log link function, \(\frac{P(Y < k)}{P(Y > k)}\) is the cumulative odds of Y, \(\alpha_i\) is the intercept and \(\beta\) is a vector of regression coefficients. The reference category was \(P. erythroseptica\) (pink rot causal agent). Therefore, susceptibility of genotypes to \(P. nicotianae\) and leak are compared to that of reference susceptibility levels of pink rot for this study.

2.3. Statistical analysis

Welch’s t-test assuming unequal variances was performed to compare the difference between water rot pathogens for incidence and penetration. Chi-square test of independence (PROC FREQ) was performed to determine the relationship between two categorical variables. Statistical analysis of POM was performed using SAS PROC LOGISTIC. Likelihood ratio test was calculated with LOGISTIC procedure for measuring the goodness of fit of the study model. The likelihood ratio test associated P-value (< 0.05), indicates that model parameters fit significantly better than other models. Proportionality assumption that the relationship between each pair of outcome groups is the same/parallel was tested using Brant test. This test assessed the proportionality assumption based on comparing fits to the binary logistic model underlying the overall model (Brant, 1990). Furthermore, Pearson correlation (PROC CORR) among three pathogens was studied by comparing disease incidence and severity observations. All the above analysis was conducted in SAS statistical software, version 9.3.

3. Results

3.1. Descriptive analysis

In total, there were 1018 genotype susceptibility screening observations. Of these, pink rot, Pythium leak, and Phytophthora tuber rot genotype screening percentages were 35.4%, 35.3%, and 29.4%, respectively (Table 1). The highest percentage genotype screening ranks were S (37.2%), S (50.9%), and R (45.8%) for pink rot, Pythium leak, and Phytophthora tuber rot, respectively. The correlation among pathogens was studied using pathogen incidence and penetration data. When percentage tuber disease incidence was compared, significant positive correlation (r = 0.64, P = < 0.0001) was obtained between \(P. erythroseptica\) and \(P. nicotianae\). However, there was zero correlation among either of the Phytophthora pathogens and the Pythium leak pathogen with respect to percentage tuber disease incidence. The tuber incidence correlations were, \(r = -0.03, P = 0.462\) for \(P. erythroseptica\) versus \(P. ultimum\) and \(r = 0.05, P = 0.329\) for \(P. ultimum\) versus \(P. nicotianae\). There was a weak correlation between the pathogens when tuber penetration was compared. The correlations (r) for pathogen association ranged from 0.18 to 0.30.

The incidence and rate of penetration among pathogens varied significantly as per Welch’s t-test results. The t-test results demonstrate that mean incidence of \(P. erythroseptica\) (41.2%) was lower than \(P. ultimum\) (65.6%). The mean difference between two incidences was −24.4% and statistically significant (P < 0.0001). Similarly, mean incidence difference (9.1%) between \(P. erythroseptica\) (41.2%) and \(P. nicotianae\) (32.09%) was significant (P < 0.0001), an indication that one pathogen induces higher mean incidence in tubers than other. Mean incidence difference (33.51%) between \(P. ultimum\) and \(P. nicotianae\) was large and significant (P < 0.0001).

Furthermore, mean difference for penetration was significant between \(P. erythroseptica\) (6.9 mm) and \(P. ultimum\) (7.5 mm). Rate of penetration for \(P. erythroseptica\) (6.8 mm) was significantly (P = 0.023) higher than \(P. nicotianae\) (6.6 mm). The mean difference of penetration between \(P. ultimum\) (7.5 mm) and \(P. nicotianae\) (6.6 mm) was also statistically significant (P < 0.0001).

3.2. Proportional odds model

The null hypothesis of whether an explanatory variable (pathogen) and a response variable (genotype susceptibility) are independent of each other was tested. The null hypothesis was rejected, since the estimated chi-square test of independence P-value (0.0001) was less than the significance level (P = 0.05) and concluded that a relationship exists between pathogen and genotype screening. This model estimated that for each unit change in \(P. nicotianae\) (compared to \(P. erythroseptica\)), odds of having S versus combined MS, MR, and R categories for potato genotype screening were significantly decreased by 38%, given the other variables are held constant (Table 2). Also, potato genotypes were 2.61 times more likely to have an S reaction compared to the combined MS, MR, and R response categories for \(P. ultimum\) compared to \(P. erythroseptica\) (Table 2). The likelihood ratio test (P < 0.0001) indicated that model performed significantly well to explain the data. Proportionality odds assumption holds (not violated) based on the non-significant test statistic (> 0.05) from Brant test.

4. Discussion

Risk assessments among different pathogen exposure groups allow the researcher to plan in advance or improve the existing management approaches. This study estimated risk outcome of the genotype susceptibility level among three water rot diseases. Typically, potato tuber rot diseases caused by oomycetes can be devastating because of their ability to infect before and after harvest. Since the three pathogens used in this study belong to a common taxonomic class, share the common habitat of water-logged soils, and the diseases they cause in field and storage are similar, the odds of fungicides exerting selection pressure on a pathogen may increase by three-fold. Most commonly pink rot and leak causing pathogens have similar habitat requirements and often occur in the same potato production area (Taylor et al., 2008b). Despite being common in potato production areas, very little is known about the association among potato cultivars and their susceptibility to water rot pathogens.
Identifying and characterizing any association of disease susceptibility among oomycetes in potato cultivars is important for managing common production and storage problems confronting growers. Control practices targeting potato water rots collectively are more effective when potato cultivars have varied levels of genetic resistance for leak disease may vary depending on complex host-pathogen-environment interactions. Furthermore, additional related risk factors can be exploited of plant resistance in the development of commercially viable disease resistant cultivars and their integration in crop disease management programs is considered an important alternative to chemical-based management. Currently, most commercial potatoes grown in North America have varying levels of susceptibility to water rot diseases and are commonly susceptible to one or all three pathogens (Salas et al., 2003; Thompson et al., 2007). Although resistance to both *P. erythroseptica* and *P. ultimum* has been identified in a single potato breeding line (Thompson et al., 2007), ongoing cultivar susceptibility screening research at NDSU and elsewhere, has made little progress identifying cultivars resistant to all three water rot causing oomycetes (Salas et al., 2003; Thompson et al., 2007). Prior knowledge of screening risk outcome for pathogen of interest when compared to pathogen of non-interest can improve the efficiency of screening process. The general concept behind this study was to describe the relationship between independent and dependent variables. This existence of relationship between pathogen and genotype screening outcome was confirmed by chi-square test of independence (P value is 0.0001). Since there is a relationship, the regression model was fitted. Based on POM results for genotype screening, the risk of susceptibility to leak was significantly increased by 2.6 times when compared to that of pink rot. Previous screening studies showed that cultivars were more likely susceptible to leak than pink rot under controlled conditions (Taylor et al., 2008b; Thompson et al., 2007). In contrast, the risk of susceptibility for Phytophthora rot is significantly decreased by 37% in potato genotypes when compared to pink rot. This indicates that the same potato cultivars have varied levels of genetic resistance for leak and pink rot. Our results are in agreement with previous work based on etiological differences between the two diseases influence the genetic source for resistance (Taylor et al., 2008b; Thompson et al., 2007). Overall, the risk estimates for genotype susceptibility levels can be applied for developing effective potato genotype susceptibility screening programs against tuber rot causing oomycetes. The knowledge gained from this analysis may be beneficial from a water rot management point of view. Based on risk assessment of water rot disease, growers can make coordinated or individual disease control decisions for field and storage conditions. For example, if a potato crop (cultivar) has been infected with multiple water rot diseases, the risk estimate between two pathogen groups can be applied to take a higher risk target oriented management approach. This risk-based management method is only suggested based on potato cultivars and actual disease may vary depending on complex host-pathogen-environment interactions. Furthermore, additional related risk factors can be

### Table 1
Genotype screening characteristics against potato water rot causing pathogens.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>N (% of total)</th>
<th>Genotype rating</th>
<th>No. of genotype susceptibility rating</th>
<th>Percentage (%)</th>
<th>Example cultivar for genotype rating</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phytophthora erythroseptica</strong></td>
<td>360 (35.4)</td>
<td>Moderately resistant</td>
<td>133</td>
<td>36.9</td>
<td>Dakota Ruby</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderately susceptible</td>
<td>1</td>
<td>0.3</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resistant</td>
<td>92</td>
<td>25.6</td>
<td>Atlantic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Susceptible</td>
<td>134</td>
<td>37.2</td>
<td>Snowden</td>
</tr>
<tr>
<td><strong>Pythium ultimum</strong></td>
<td>359 (35.3)</td>
<td>Moderately resistant</td>
<td>46</td>
<td>12.8</td>
<td>Dakota Trailblazer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderately susceptible</td>
<td>85</td>
<td>23.7</td>
<td>Dakota Russet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resistant</td>
<td>45</td>
<td>12.5</td>
<td>Snowden</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Susceptible</td>
<td>183</td>
<td>50.9</td>
<td>Atlantic</td>
</tr>
<tr>
<td><strong>Phytophthora nicotianae</strong></td>
<td>299 (29.4)</td>
<td>Moderately resistant</td>
<td>110</td>
<td>36.8</td>
<td>Dakota Ruby</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderately susceptible</td>
<td>1</td>
<td>0.3</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resistant</td>
<td>137</td>
<td>45.8</td>
<td>Atlantic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Susceptible</td>
<td>51</td>
<td>17.1</td>
<td>Snowden</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1018</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* N indicates total number of genotypes screened.

### Table 2
Proportion odds model estimated odds ratios associated with genotype screening outcome in tubers inoculated with water rot pathogens.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Odds ratio</th>
<th>95% CI (L-U)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phytophthora erythroseptica</strong></td>
<td>Reference group</td>
<td></td>
</tr>
<tr>
<td><strong>Pythium ultimum</strong></td>
<td>2.606</td>
<td>1.98-3.43</td>
</tr>
<tr>
<td><strong>Phytophthora nicotianae</strong></td>
<td>0.375</td>
<td>0.28-0.5</td>
</tr>
</tbody>
</table>

CI = 95% Confidence Interval (L: Lower-U: Upper), * indicate significant difference because the CI interval does not contain one.
identified and estimated to improve the current model. For example, risk factors like method of infection, cultivar type, location where crop grown, environmental conditions such as temperature, planting and harvest days, storage conditions, storage duration etc., can be added to the existing model or used to develop a new model.

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References


