Crop Protection 115 (2019) 59–63

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

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

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ARTICLE INFO

Keywords:
Proportional odds
Odds ratio
Water rot
Cultivar susceptibility

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 colloquially 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 chlamydospores (*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

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https://doi.org/10.1016/j.cropro.2018.09.014
Received 24 April 2018; Received in revised form 30 July 2018; Accepted 18 September 2018

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., identification of *Phytophthora* and *Pythium* isolates resistant to mefenoxam and metalaxyl fungicides has hindered chemical management of the diseases they cause (Johnson et al., 2004; Mulrooney, 1982; Taylor et al., 2002, 2006; Torres et al., 1985; Wicks et al., 2000). Currently, phosphonate (phosphoric acid) fungicides are most often used to control post-harvest storage infection of tubers caused by *Phytopthora* pathogens (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 fungicide 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 expensive. 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 assessment is commonly used in medical studies to identify and analyze potential 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 assessment methodologies have been applied in phytopathology. Risk levels of deoxynivaenol 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 genotype susceptibility risk levels in order to better understand the relationships 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. erythroseptica, 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 harvested 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 rotobeater 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. erythroseptica*, *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. erythroseptica* and *P. nicotianae* inoculum (at concentration of 2×10^4 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 incidence (I) was calculated (I = (Number of infected tubers/Number of inoculated tubers) * 100). Disease severity was measured as the rate of penetration (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 replicated 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