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Phytophthora erythroseptica (Pink Rot) Development in Russet Norkotah Potato Grown in Buffered Hydroponic Solutions II. pH Effects

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Abstract Pink rot caused by the pathogen *Phytophthora erythroseptica* Pethyb. causes severe pre- and post-harvest losses in potato. Severe losses of potato from pink rot infection suggest the need for better information on cultural factors associated with disease development. Soil pH has been regarded as a strong influence on disease development as observed by previous studies and by commercial potato growers. A hydroponic growth system was established to study the effects of pH on *P. erythroseptica* infection and colonization in Russet Norkotah potato roots. Russet Norkotah plantlets were grown in hydroponic nutrient solutions buffered at pH 5, 6, 7, and 8 and evaluated for infection by means of quantitative Real Time PCR. Acidic pH solutions produced a higher proportion of pathogen to host DNA in root tissue, indicating increased infection and colonization at low pH. Thus, pH has a unique and significant influence on the levels of infection from *P. erythroseptica* and that commercially grown potatoes would likely develop reduced tuber rot if grown in soils with pH at or above 7.

Resumen La pudrición rosa, causada, por el patógeno *Phytophthora erythroseptica* Pethyb, causa severas pérdidas pre- y post-cosecha en papa. Las grandes pérdidas por la infección de la pudrición rosa sugiere la necesidad de mejor información sobre factores culturales asociados con el desarrollo de la enfermedad. Se ha asociado al pH del suelo como de una fuerte influencia en el desarrollo de la enfermedad, como se ha visto en estudios previos y por los productores comerciales de papa. Se estableció un sistema hidropónico de crecimiento para estudiar los efectos del pH en la infección y colonización por *P. erythroseptica* en raíces de papa Russet Norkotah. Plántulas de Russet Norkotah se cultivaron en soluciones nutritivas hidropónicas amortiguadas a pH de 5, 6, 7 y 8 y se evaluaron para infección mediante PCR cuantitativa de tiempo real. Las soluciones de pH ácidos produjeron una proporción mas alta de ADN del patógeno que del hospedante en tejido radical, indicando un aumento en la infección y en la colonización a pH bajo. En consecuencia, el pH tiene una influencia única y significativa en los niveles de infección por *P. erythroseptica* y es probable que las papas comerciales desarrollen infección reducida de tubérculo si se cultivan en suelos con pH a o arriba de 7.

Keywords *Solanum tuberosum* · *Phytophthora erythroseptica* · Potato disease · Quantitative RT-PCR

Introduction

Pink rot is a soil-borne disease caused by the fungal pathogen *Phytophthora erythroseptica* Pethyb. The pathogen has been characterized as a major pre and post harvest disease of potato (*Solanum tuberosum* L.) (Taylor et al. 2004). For a review of pathogen characteristics, impairment to potato, and control options please refer to our companion paper Benson et al. (2009, Calcium Nutrition Effects). However, other soil characteristics may also influence *P. erythroseptica* populations and infection (Messenger et al.

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2003). Disease outbreaks in potato growing regions of South-Eastern Idaho have suggested a potential role of soil pH (Personal Comm. Jeff Miller).

The apparent correlation of relatively greater severity of *P. erythroseptica* infection of potatoes grown west of Rexburg, Idaho, in soils that are now acidic due to relatively high rates of nitrogen fertilizer and low carbonate irrigation water, has led to the hypothesis that pH may be a causal factor (Personal comm. Bryan Hopkins; Horneck et al. 2007). The effect of pH on other plant disease development has been documented for several pathogen-host interactions such as: club root of crucifers (Brassicaceae), *Cephalosporium* stripe on wheat (*Triticum aestivum* L.), and potato common scab (*Streptomyces* spp.). The incidence of club root of crucifers was reduced when pH was raised above 7.2, *Cephalosporium* stripe was also reduced when pH was raised from 5.1 to >6,0, and contrastingly, potato scab was significantly reduced when pH was at or below 5.0–5.2 (Barnes and McAllister 1972; Murray et al. 1992; Myers and Campbell 1985). How pH affects conditions for *P. erythroseptica* infection is not clear, although it is known that the availability of nutrients to the host is confounded depending upon the pH of the environment (Tisdale et al. 1993). The effect of soil pH in the field is difficult to separate from a depletion or an excess of particular ions, but can be evaluated using nutrient solutions in a hydroponic growth system (Benson et al. 2009). Therefore, the objective of this study is to confirm an effect of pH on the level of infection of *P. erythroseptica* in Russet Norkotah potatoes grown under hydroponic conditions.

Materials and Methods

Due to the same materials and methods please refer to the companion paper Benson et al. (2009, Calcium Nutrition Effects) for protocols regarding Russet Norkotah growth and maintenance, plant sampling, *P. erythroseptica* acquisition and maintenance, DNA extraction and quantification, quantitative real time polymerase chain reaction, and statistical design and analysis. Statistical analysis for this research paper differed from the calcium companion study only in that infection coefficients were from pH and not calcium treatments. Solutions in which the potato plants were grown differed considerably, therefore, solutions for the pH research are listed.

Roots of four to six cm length plantlets (20 total plants per 14 L^{-1} bucket) were placed into a modified Hoagland pretreatment solution containing: mg L^{-1} concentrations were 90.1 N, 7.74 P, 93.6 K, 11.5 S, 85.9 Ca, 8.68 Mg, 1.5 Fe, 3.34 Cl, 0.37 Mn, 0.204 B, 0.029 Mo, 0.114 Zn, 0.029 Cu, 0.014 Na (Camp et al. 1987). After 14 days in the pretreatment solution, 16 uniform sized plants per bucket were randomly transferred into a modified Hoagland treatment solution containing the same mineral concentration listed above except for K that had 93.6 mg L^{-1} for pH 5.0, 98.6 mg L^{-1} for pH 6, 109.6 mg L^{-1} for pH 7, and 120.6 mg L^{-1} for pH 8.

The four pH treatments: 5.0, 6.0, 7.0, and 8.0 ($^{\pm}$0.1) are representative of the typical pH range found in soils. Each pH treatment was buffered using 390 mg L^{-1} 2-(N-morpholino) ethanesulfonic acid (MES) (Sigma-Aldrich, Inc. St. Louis, MO), which is a water soluble low salt biological buffering agent with a mid range pKa value (6.15) that is chemically and enzymatically stable. Solution pH was adjusted initially and throughout the experiment with addition of KOH. Addition of K to the nutrient solution as a function of pH adjustment was not balanced across treatments, however, it was deemed that this addition was insignificant as compared to the total K in solution. All other nutrients were identical in concentration.

The pH in the hydroponic solution was regulated by use of MES and was adjusted by addition of KOH. This base was found much more effective in raising the pH of the acidic modified Hoagland solution than either NaOH or $Ca(OH)_2$. More KOH was added as pH levels increased thus increasing the amount of K in solution; however, the minor unequal levels of K should not have influenced the plant and pathogen interaction because solutions at each pH already contained K adequate for potato growth. Potassium is often available and absorbed in excess of physiological need by many plants and extra K would not be expected to injure potato nor impact infection (Havlin et al. 2005). Therefore, KOH was the best option for adjusting the pH levels of 5.0, 6.0, 7.0 and 8.0 used in this experiment.

Results

Potatoes grown hydroponically at various pH levels had significant differences (F=0.027) in the infection coefficient level of *P. erythroseptica* as a function of solution pH. As pH became more acidic, infection coefficients were larger. The largest *P. erythroseptica* infection coefficient was observed at pH 5.0. Infection coefficients for treatments pH 5.0, 6.0, and 7.0 were significantly larger than pH 8.0, but they were not significantly different from each other (Table 1).

Samples collected at 2, 5, 8, 11, and 14 days after inoculation had significantly different infection coefficients within the potato roots. As the number of days after inoculation increased, the infection coefficients of *P. erythroseptica* increased (Fig. 1). The increase in pathogen presence as assessed by infection coefficient values over time has an approximate linear trend that increased for each treatment level. When comparisons of pH treatments were

Table 1 Effect of pH on *P. erythroseptica* infection coefficients in Russet Norkotah potato roots[a]

pH	Mean IC value	P value		
		6	7	8
5	0.8200	0.1941	0.1022	0.0051
6	0.8176		0.6579	0.0296
7	0.8142			0.0552
8	0.7971			

[a] Samples were collected 15 days after inoculation

made within each sampling time, coefficients for pH treatments differed significantly only for 14 days after inoculation. Infection coefficients for treatment pH 5.0 differed significantly from pH 7.0 and 8.0 ($P=0.0273$ and 0.005 respectively), and pH 6.0 was significantly different than 8.0 ($P=0.0448$).

Discussion

As pH levels became more acidic the infection coefficient (IC) values increased, representing higher pathogen DNA and subsequent greater disease severity. These IC values correspond to disease severity accurately in that they relate the amount of pathogen DNA to host DNA as a measurement of infection, which can be accurately quantified because the high sensitivity of quantitative RT PCR allows for discrete differences in pathogen concentrations to be distinguished (Vargas and Nielsen 1972). The relationship of increasing pathogen DNA to more acidic solutions was linear and demonstrated the affects pH can have on *P. erythroseptica* as a root pathogen. Previous studies with other plant pathogens have demonstrated similar results (Barnes and McAllister 1972; Myers and Campbell 1985). Our results provide an explanation to an observed greater incidence of pink rot decay in potato fields and storages of Southern Idaho (personal comm. Jeff Miller and Bryan Hopkins).

The infection coefficient of *P. erythroseptica* was significantly higher at pH 5 than 6, 7, or 8, but no basic or acidic limits were established in this study. Cairns and Muskett (1939) performed a series of in vitro studies and established limits for *P. erythroseptica*. It was unable to grow below pH 3.2 but no basic pH level has yet been established because of the inability to maintain media above pH 10. Optimum growth of *P. erythroseptica in vitro* was observed at pH 7.0 (Cairns and Muskett 1939). These in vitro pH growth values can be extended to field conditions, but soil pH below 4.5 or above 8.5 would be extremely unlikely in agricultural fields. The optimum pH for growth in vitro and for potato infection and colonization differs according to our results.

Soil pH influences field conditions that favor pathogen infection through nutrient availability, mineral toxicities, soil quality, and plant metabolism even when pH is not necessarily damaging to the potato. For example, low pH reduces availability of some soil minerals (promoting nutrient deficiencies such as P and Mo) and increases solubility of others (promoting aluminum and/or manganese toxicities). These and other effects on plants and microbes due to acidic pH result in subsequent plant developmental problems and weakened defenses. These acidic soils are rarely spatially uniform across a field and, as a result, large areas of the field that have acidic pH tend to have less growth and concomitant water uptake. As a result, these areas become water logged and the constant presence of water is beneficial to many plant pathogens and is especially beneficial to *P. erythroseptica* that utilizes the water for zoospore production and dissemination.

Potatoes grown in acidic soils seem to adapt to the conditions by maturing earlier and have a higher tuber to foliage ratio than those grown in basic soils (Wan et al. 1994; White 1946). Early tuber development may increase the chance of infection because tubers are exposed to *P. erythroseptica* longer resulting in storage or field losses. Sample times from this study occurred before tubers started to form but as days after inoculation increased the IC values also increased. There were significant differences among sampling times and we believe that IC values would continue to increase as days after inoculation increased, indicating that early infections will result in increased tuber rot.

Understanding that acidic conditions are associated with higher incidences and disease severity of pink rot suggests a cultural method for management. The addition of lime is frequently used to raise the soil pH of fields and should reduce *P. erythroseptica* infection. Lime can be applied to areas of fields with acidic pH via intensive soil analysis and spatial mapping to increase pH and reduce “hot spots” of pink rot incidence. This study has helped define a possible

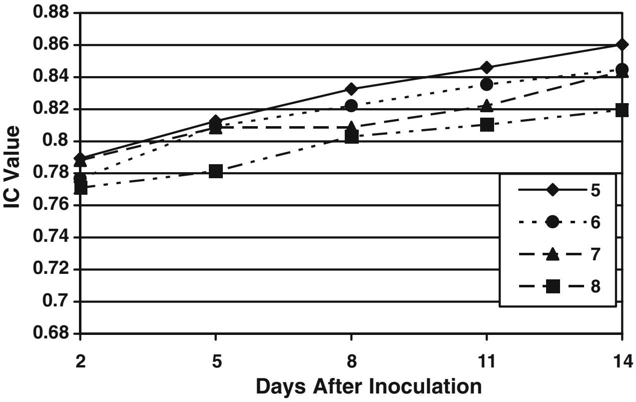

Fig. 1 Impact on *P. erythroseptica* infection coefficient (IC) in Russet Norkotah potato growing in hydroponic solutions over a 14 day time period in which pH was at 5, 6, 7, or 8

cultural method as an additional option to fungicides in managing outbreaks of pink rot. This could be increasingly important as *P. erythroseptica* becomes resistant to the few effective fungicides currently available for pink rot control (Taylor et al. 2006).

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