

# Resistance to *Phytophthora erythroseptica* and *Pythium ultimum* in a Potato Clone Derived from *S. berthaultii* and *S. etuberosum*

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## ABSTRACT

Tubers of several potato clones and cultivars were screened for susceptibility to infection by zoospores of *Phytophthora erythroseptica* (causal agent of pink rot) and mycelia of *Pythium ultimum* (causal agent of leak) over a three-year period, from 2003-2005. Incidence of infected tubers (%) and penetration of rot (mm) were the parameters used to determine the susceptibility of each potato clone. Responses of each potato clone were compared to cultivars with known resistance or susceptibility to these pathogens. Tubers of cultivars Atlantic and Snowden have moderate resistance to infection and colonization by *P. erythroseptica* and *P. ultimum*, respectively, and were used as the resistant checks. Cultivars Russet Norkotah and Red Norland are susceptible to infection by both pathogens. A number of potato clones demonstrated resistance to pink rot equal to or greater than the control cultivar Atlantic, including Etb 6-5-2, ND5822C-7, ND6956b-13, ND7443Ab-44, ND7443Ab-181, ND7818-1Y and J101K6A22. In addition to demonstrating the highest resistance to pink rot, Etb 6-5-2 was the only clone that demonstrated resistance to leak greater than or equivalent to the resistant cultivar Snowden. Etb 6-5-2 is a backcross derivative from a somatic hybrid of *Solanum etuberosum* and *Solanum berthaultii* and will be investigated further as a potential source of resistance to these two storage rot diseases.

## RESUMEN

Los tubérculos de varios clones y cultivares de papa fueron tamizados para susceptibilidad con zoosporas de *Phytophthora erythroseptica* (agente causal de pudrición rosada) y con micelio *Pythium ultimum* (agente causal de pudrición acuosa o gotera) en un periodo de tres años, del 2003 al 2005. La incidencia de tubérculos infectados (%) y la profundidad de la pudrición (mm) fueron los parámetros usados para determinar la susceptibilidad en cada clon de papa. Las respuestas de cada clon fueron comparadas con las de cultivares de conocida resistencia o susceptibilidad a estos patógenos. Tubérculos de los cultivares Atlantic y Snowden que tienen resistencia moderada a la infección y colonización por *P. erythroseptica* y *P. ultimum* respectivamente, fueron usados como testigo resistente. Los cultivares de Russet Norkotah y Red Norland son susceptibles a la infección de ambos patógenos. Varios clones de papa mostraron resistencia a la pudrición rosada, igual o mayor que el testigo Atlantic, incluyendo, Etb 6-5-2, ND5822C-7, ND6956b-13, ND7443Ab-44, ND7443Ab-181, ND7818-1Y y J101K6A22. Además de demostrar muy alta resistencia, Etb 6-5-2 fue el único clon que demostró resistencia a la gotera en mayor proporción o equivalente al cultivar resistente Snowden. Etb 6-5-2 es producto de una retrocruza derivada de un híbrido somático de *Solanum etuberosum* y *S. berthaultii* y será investigada adicionalmente como fuente potencial de resistencia a estas dos pudriciones de tubérculos almacenados.

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Additional Keywords: *Solanum tuberosum*, pink rot, leak, *S. bulbocastanum*, partial resistance

## INTRODUCTION

Pink rot of potato (*Solanum tuberosum* L.) caused by *Phytophthora erythroseptica* Pethyb. and leak caused by *Pythium ultimum* Trow are two important soil-borne diseases of potato (Secor and Gudmestad 1999). The name 'pink rot' describes the diagnostic pink color that appears in infected tuber tissue when cut and exposed to air for 20-30 minutes (Cairns and Muskett 1933; Goss 1949; Jones 1945). The common disease name of 'leak' is descriptive of the exudation of water droplets from the blackish, soft, watery breakdown of tissue of infected tubers (Blodgett and Ray 1945; Jones 1935). These diseases can cause severe yield losses in fields and in storage facilities (Secor and Gudmestad 1999).

Infection of tubers by *P. erythroseptica* generally occurs via stolons (Lonsdale et al. 1980). However, tubers can also be infected by zoospores through tuber eyes (Salas et al. 1997) and tuber wounds (Salas et al. 2000). *P. ultimum* is incapable of penetrating the undamaged skin of the tuber (Hawkins and Harvey 1919; Taylor et al. 2004). Thus, infections by the leak pathogen predominantly originate from cuts and wounds, and only occasionally occur through the stem end (Blodgett 1945).

Studies on cultivar susceptibility to *P. erythroseptica* and *P. ultimum* are scarce and generally outdated, involving potato cultivars no longer in production or not grown in the United States. Furthermore, most studies on cultivar susceptibility to pink rot and leak involve one pathogen or the other and do not evaluate susceptibility to both diseases. In studies involving pink rot, Cairns and Musket (1939) evaluated 51 cultivars and found all were susceptible. Similarly, Jones (1945) determined that all eight cultivars tested were susceptible to pink rot. Other studies have identified that cultivars exhibited various levels of susceptibility. Goss (1949) reported that cvs Irish Cobbler and Kasota had a lower incidence of pink rot infection than cvs Warba and Pawne. Fernandez-Northcote et al. (1972) reported that tubers of only four clones were found to be resistant to pink rot after screening 13 cultivars and 242 native clones from Peru. Lennard (1980) also identified differing levels of susceptibility to pink rot among cultivars. More recently, Peters and Sturz (2001) reported that plantlets of cvs Butte and Russet Burbank were the least susceptible to pink rot, and those of cvs Goldrush and Yukon Gold were the most susceptible. In a follow-up study, Peters et al. (2004) determined that tubers of cvs Norland and Shepody were significantly more susceptible to pink rot than those of Goldrush, Russet Burbank and Butte.

Regarding the susceptibility of potato tubers to the leak pathogen, Jones (1935) reported that none of the 15 potato cultivars grown in British Columbia, Canada were resistant to leak. Hawkins and Harvey (1919) found that incidence of leak in cultivars can range from 0 to 91%. Priou et al. (1997) concluded that some cultivars were more susceptible than others to infection by *P. aphanidermatum*, the leak pathogen in tropical areas.

Only one study has examined the susceptibility of potato cultivars to both pink rot and leak. Salas et al. (2003) screened 34 cultivars for their susceptibility to both pink rot and leak. They found no cultivars resistant to both pathogens. However, cvs Atlantic and Snowden were found to be moderately resistant to pink rot and leak, respectively.

Since pink rot and leak storage rots generally occur together in the same production area, the identification and development of resistance in potato germplasm to both diseases would be a valuable genetic resource for potato breeding programs to exploit. The primary objective of this study was to assess the reaction of tubers of potato clones to infection by zoospores of *P. erythroseptica* and mycelia of *P. ultimum*, and to compare infection and penetration rates of both pathogens to a set of control cultivars. Emphasis was placed on evaluating germplasm from the North Dakota State University (NDSU) potato breeding program that demonstrated some level of resistance to late blight, caused by *Phytophthora infestans*, as well as potato germplasm from other breeding programs with known resistance to other pests and pathogens used extensively by the NDSU potato breeding program as parental material (Novy et al. 2002, 2004).

## MATERIALS AND METHODS

### *Potato Germplasm, Cultivation, Harvest and Pre-inoculation Handling*

Seed tubers of the four control cultivars — Atlantic, Snowden, Red Norland and Russet Norkotah — with known resistance or susceptibility to pink rot and leak (Salas et al. 2003) were obtained from seed potato producers in North Dakota (ND), Minnesota (MN), or from private companies. Atlantic was used as the pink rot resistant check and Snowden was used as the leak resistant check. Seed tubers of all selections evaluated for resistance to these two diseases were grown by the potato breeding program at North Dakota State University. A total of 11 potato clones were tested in multiple

years since they had demonstrated resistance to one or both pathogens. An additional 59 potato genotypes were evaluated in only one year over the course of these studies. Whole or cut seed tubers were used for planting. All check cultivars and potato clones were produced in single rows (30 m) in field plots with overhead irrigation near Dawson, ND, in 2003-2005. Cultural practices during the growing season were those recommended for potato production in North Dakota. Tubers were harvested at maturity, visually inspected for pink rot and leak symptoms, and placed in a room at 15 C and 90% RH for two weeks to promote wound healing. Thereafter, tubers of all cultivars and potato clones were stored at 10 C for two to three months before inoculations. Disease-free tubers (140 to 190 g) were acclimated to room temperature (20 to 24 C) two to three days prior to inoculations. No natural infections of pink rot or leak were observed in any year of the study. Preliminary studies indicated that the frequency of infection by *P. erythroseptica* in surface sterilized tubers with 0.5% NaOCl and non-surface sterilized tubers were similar (Salas et al. 2003). Therefore, tubers used for inoculations were not surface sterilized or washed prior to inoculation. Tubers used for inoculation with *P. erythroseptica* had the apical and at least one lateral eye free of soil, and those used for inoculation with *P. ultimum* had intact periderm prior to inoculation.

### **Pathogen Isolates**

Previous studies demonstrated that cultivar responses to *P. erythroseptica* and *P. ultimum* were not affected by the use of multiple isolates of each pathogen. Thus, only one isolate of each pathogen was used in the studies reported here (Peters et al. 2004; Salas et al. 2003). *P. erythroseptica* isolate PR-266-2 from Washington, obtained in 2000, was used to evaluate levels of resistance to pink rot. Isolate 153-7 *P. ultimum*, obtained in 1997 from an infected tuber from Idaho, was used to evaluate resistance to leak. Isolates were identified based on described morphological characteristics (Plaats-Niterink 1981; Stamps et al. 1990), and were used in previous studies (Salas et al. 2003; Taylor et al. 2004). Pathogenicity of both pathogens was maintained by inoculating freshly wounded Russet Burbank tubers with colonized agar plugs of the isolates. These preliminary studies showed that both isolates were highly pathogenic. Isolate pathogenicity was maintained every year through inoculation to Russet Burbank tubers and re-isolation on water agar culture plates.

### **Inoculations with *P. erythroseptica***

A method reported by Vujicic and Colhoun (1966) and modified slightly by our research group (Taylor et al. 2004) was followed to obtain zoospores of *P. erythroseptica*. Clarified V8 (CV8) juice agar and CV8 juice broth were used instead of pea extract. CV8 juice agar contained 100 ml of CV8 juice, 15 g agar, and 900 ml of deionized distilled water. A stock of CV8 juice broth contained 100 ml of V8 juice filtered through four layers of cheesecloth, and 900 ml deionized water. To obtain mycelial mats of *P. erythroseptica*, three mycelial disks (3 mm diam.) of the pathogen grown on CV8 juice agar for three days were placed on each plastic petri dish (8.5 cm), flooded with 10 ml of autoclaved CV8 broth, and incubated in darkness at room temperature (20 to 24 C) for three days. To induce sporangia formation, the CV8 juice broth was discarded, and replaced with 10 ml/petri plate of a filtered and autoclaved soil extract (100 g soil from potato field in 900 ml deionized water), after rinsing the mycelial mats two to three times with sterile deionized water. These cultures were further incubated for 36 to 48 h under continuous light in an incubator (20 C±1). Finally, to induce the release of zoospores, cultures were chilled at 10 C±1 for 1 h, and re-warmed at room temperature (20 to 24 C). Abundant zoospore release occurred within 15 to 25 minutes. A hemacytometer was used to obtain an inoculum concentration of  $2 \times 10^4$  zoospores ml<sup>-1</sup>. The zoospore suspension was chilled (8 to 10 C) until inoculations were made within 10 to 60 min. Before inoculations, tubers were placed in plastic moist chambers (33 cm long x 24 cm wide x 12 cm high) lined at bottom with plastic canvas mesh 3. Each tuber was inoculated by placing a single drop of inoculum (10 µl ~200 zoospores) on each of the three apical eyes of tubers (one apical plus two next laterals) (Taylor et al. 2004). Control tubers were inoculated only with sterile distilled water. After inoculations, tubers were covered with two layers of wet paper towels before closing the moist chambers to maintain high humidity, thereby promoting infection. All inoculated tubers in moist chambers were incubated in darkness at room temperature (20 to 22 C) for 10 to 12 days.

### **Inoculations with *P. ultimum***

All tubers were wounded on one side before inoculations. The wounding procedure involved the removal of periderm by manually abrading a 1 cm x 1 cm area with an abrasive pad (no. 96) near the middle of one side of each tuber as previously

described (Taylor et al. 2004). The abraded area had the periderm removed with little damage to the underlying tissue. Inoculum of *P. ultimum* was prepared by growing the isolates on modified V8 juice agar (100 ml V8 juice, 1.25 g CaCO<sub>3</sub>, 900 ml water) for 48 hours. One *P. ultimum* colonized agar plug (5 mm diam.) cut from the colony margin was placed on the freshly wounded tuber tissue. Control tubers were inoculated with modified V8 juice agar plugs. The inoculated tubers were placed in plastic moist chambers as described above, and were incubated in darkness at room temperature (20 to 22 C) for 5 to 6 days.

### Disease Assessment

Inoculated tubers were removed from moist chambers and were sliced in half through the inoculation point. To evaluate pink rot, tubers were bisected longitudinally from the apical to basal ends.

Tubers were split perpendicular to the longitudinal axis to assess leak. Tuber halves were covered with moist paper towels to enhance the development of the pink discoloration diagnostic for pink rot and the watery blackish discoloration characteristic of leak. The number of tubers showing symptoms of pink rot or leak was recorded 30 min after cutting. Incidence of pink rot or leak rot was calculated as follows: (number diseased tubers/number of inoculated tubers) x 100. To determine pink rot and leak severity, the maximum width of rot (W) and the depth (D) of rot from the inoculation point were measured.

Then penetration of rot was calculated using the formula reported by Lapwood et al. (1984): Penetration =  $[W/2+(D-5)]/2$ .

### Experimental Design and Data Analysis

Each experiment involving inoculations of potato clones and check cultivars was arranged in a completely randomized design with four replicates per cultivar. A replicate consisted of a set of ten tubers per cultivar or selection. Non-inoculated tubers of each potato genotype served as controls. All control cultivars were included in each year of the study. All data were subjected by year to one-way analyses of variance utilizing the General Linear Model procedure of SAS version 9.1 (PROC GLM, SAS Institute, Inc., Cary, NC). Fischer's protected least significant difference test (LSD) ( $P = 0.05$ ) was used to determine all mean separations. Data from 11 genotypes and the

TABLE 1—Infection incidence (%) and penetration severity (mm) in 2003 of *Phytophthora erythroseptica* (pink rot) and *Pythium ultimum* (leak) on challenge inoculated tubers of control cultivars and potato clones from the North Dakota State University breeding program.

Selection <sup>a</sup>	<i>P. erythroseptica</i>		<i>P. ultimum</i>	
	Incidence (%)	Penetration (mm)	Incidence (%)	Penetration (mm)
Etb 6-5-2	0.0	—	7.5	14.2
ND 7388Ab-1	15.0	36.6	87.5	20.8
Etb 5-31-2	17.5	38.3	90.0	13.3
Etb 6-21-12	22.5	27.9	87.5	24.7
ND 7388Ab-7	25.0	31.7	92.5	23.6
ND 7388Ab-6	32.5	28.6	92.5	25.8
ND 7390Ab-13	32.5	26.5	100.0	22.8
ND 7387Ab-10R	37.5	32.1	95.0	33.0
ND 7387Ab-8R	42.5	36.6	90.0	37.2
Etb 6-21-3	45.0	32.0	82.5	23.4
Etb 6-5-5	45.0	30.5	97.5	35.2
ND 7390Ab-12	47.5	30.8	72.5	16.4
ND 7390Ab-6	52.5	28.5	80.0	20.5
ND 7393A-5Russ	52.5	34.5	92.5	32.2
ND 7390Ab-2	55.0	27.7	90.0	24.5
ND 7390Ab-10	62.5	28.2	92.5	19.2
ND 7386Ab-20	62.5	34.3	100.0	22.8
ND 7389Ab-15	65.0	33.4	100.0	31.7
ND 7389Ab-3	80.0	39.0	100.0	27.1
ND 7390Ab-16	85.0	33.0	95.0	20.2
Atlantic	27.5	31.2	72.5	24.8
Red Norland	37.5	33.3	97.5	29.9
Russet Norkotah	80.0	44.0	72.5	28.3
Snowden	62.5	35.0	82.5	27.7
Mean <sup>b</sup>	45.2	32.7	86.3	25.1
LSD <sub>P=0.05</sub>	22.0	4.9	17.9	3.5
Coefficient of Variation	34.5	10.5	14.7	9.8

<sup>a</sup>Named cultivars were added as controls for challenge inoculations; they were not grown with the clonal selections.

<sup>b</sup>Mean pink rot and leak incidence and penetration across all selections.

four control cultivars evaluated from 2003-2005 were pooled after testing variance homogeneity using Levene's method and analyzed as combined experiments, as outlined by Millikin and Johnson (1992). The relationship among potato genotypes for resistance to pink rot and leak, based on incidence and severity, were determined on the combined data set by means of a one-way ANOVA and Fischer's LSD ( $P = 0.05$ ). Pearson's correlation analysis was performed on the combined data set to evaluate the relationship between infection incidence and penetration severity for each storage rot pathogen, *P. erythrosep-*

*tica* and *P. ultimum*. A Chi Square analysis was also performed to test the relationship between pink rot and leak resistance.

## RESULTS

The control cultivars used in this study reacted to challenge inoculations with *P. erythroseptica* and *P. ultimum* similarly as in previous studies (Salas et al. 2003). Atlantic demonstrated moderate resistance to pink rot, with Red Nor-

TABLE 2—*Infection incidence (%) and penetration severity (mm) in 2004 of Phytophthora erythroseptica (pink rot) and Pythium ultimum (leak) on challenge inoculated tubers of control cultivars and potato clones from the North Dakota State University breeding program.*

Selection <sup>a</sup>	<i>P. erythroseptica</i>		<i>P. ultimum</i>	
	Incidence (%)	Penetration (mm)	Incidence (%)	Penetration (mm)
ND 7818-1Y	0.0	—	66.3	20.1
J101K6A22	2.5	24.3	86.7	15.6
ND 6955b-28Y	3.8	18.9	95.0	12.5
Etb 6-5-2	10.0	17.3	48.8	12.1
ND 6956b-13	10.0	22.1	85.0	19.4
ND 5822C-7	21.3	20.3	75.0	21.4
ND 6956b-53	21.3	26.9	86.3	20.9
ND 7443Ab-181	23.8	22.1	92.5	14.4
ND 7443Ab-45	27.5	23.8	73.8	21.0
ND 7443Ab-109	31.3	20.7	96.3	18.1
ND 7443Ab-44	32.5	21.2	83.8	17.6
ND 7443Ab-103	35.0	27.1	95.0	23.2
ND 7443Ab-20	35.0	22.3	98.8	17.7
ND 7386Ab-52	41.3	23.7	100.0	24.7
ND 7443Ab-102	43.8	21.5	96.3	19.9
ND 7443Ab-184	45.0	22.4	96.3	24.0
ND 7443Ab-115	46.3	24.0	81.3	19.8
ND 7882b-7 Russ	46.3	24.7	100.0	18.2
ND 7386Ab-4	48.8	23.5	94.9	20.7
ND 2470-27	52.5	23.5	73.1	22.1
ND 7386Ab-20	52.5	23.1	96.3	24.6
ND 7443Ab-46	53.8	23.4	98.8	17.3
ND 7443Ab-72	53.8	20.1	78.8	18.0
ND 7387Ab-1R	60.0	23.9	93.8	25.3
ND 7443Ab-112	61.2	23.8	95.0	17.0
ND 8266A-2R	61.3	22.7	98.8	18.2
ND 7443Ab-161	62.5	24.0	90.0	26.4
ND 8266A-1R	78.8	18.9	72.5	9.1
Atlantic	42.5	28.8	81.3	27.1
Red Norland	67.5	29.7	96.3	29.3
Russet Norkotah	88.8	29.7	86.3	15.7
Snowden	87.5	30.6	32.5	19.6
Mean <sup>b</sup>	42.1	23.7	85.6	19.8
LSD <sub>P=0.05</sub>	15.6	4.8	11.3	3.0
Coefficient of Variation	37.6	17.7	13.2	15.2

<sup>a</sup>Named cultivars were added as controls for challenge inoculations; they were not grown with the clonal selections.

<sup>b</sup>Mean pink rot and leak incidence and penetration across all selections.

land moderately susceptible to the disease (Table 1, 2 and 3; Figure 1). Snowden and Russet Norkotah were highly susceptible to pink rot as they had been in the aforementioned studies. Snowden was moderately resistant to leak in 2004 and 2005, while the other check cultivars ranged from moderately susceptible to susceptible. Snowden appeared to be moderately susceptible to leak in 2003. Negative control inoculations, tubers inoculated with either sterile water or with modified V8 juice agar plugs, did not develop symptoms of pink rot or leak in any of the trials.

Eleven breeding selections were evaluated for resistance to pink rot and leak in two or more years. Second generation progeny (BC<sub>2</sub>) of tri-species somatic

hybrids (Novy and Helgeson 1994), Etb 5-31-2 and Etb 6-5-2, were evaluated in 2003 and demonstrated resistance to pink rot equal to or greater than the resistant control Atlantic (Table 1). Etb 6-5-2 also demonstrated excellent resistance to leak, significantly better than the control cultivar Snowden. The incidence of infection and penetration of *P. erythroseptica* and *P. ultimum* was reduced significantly in Etb 6-5-2 compared to the moderately resistant Atlantic and Snowden, respectively (Table 1). Three additional breeding clones tested in 2003 demonstrated some level of resistance to pink rot, but not to leak. However, these clones were either lost or dropped from the breeding program for other reasons and were not evaluated further.

In 2004, Etb 6-5-2 continued to display excellent resistance to both pink rot and leak (Table 2). The incidence of infection and the penetration of *P. erythroseptica* in this clone were reduced significantly compared to the moderately resistant Atlantic. Incidence of *P. ultimum* infection was significantly greater in Etb 6-5-2 than the moderately resistant Snowden; however, the penetration of the fungus was significantly less in Etb 6-5-2 than in the resistant check. Several other potato clones also

demonstrated levels of resistance to pink rot equal to or greater than Atlantic, namely ND 7443Ab-44, ND 7443Ab-181, J101K6A22, ND 6956b-13, ND 7818-1Y and ND 5822C-7 (Table 2). The penetration of *P. erythroseptica*, once the pathogen gained entry into the tubers of these clones, was reduced significantly compared to the moderately resistant check cultivar Atlantic. None of these potato clones demonstrated the level of resistance to leak as was observed in Etb 6-5-2. However,

TABLE 3—Infection incidence (%) and penetration severity (mm) in 2005 of *Phytophthora erythroseptica* (pink rot) and *Pythium ultimum* (leak) on challenge inoculated tubers of control cultivars potato clones from the North Dakota State University breeding program.

Selection <sup>a</sup>	<i>P. erythroseptica</i>		<i>P. ultimum</i>	
	Incidence (%)	Penetration (mm)	Incidence (%)	Penetration (mm)
JND 89280-6	1.3	41.5	78.8	20.0
J103-A12	5.0	25.1	97.5	22.9
J101K6A22	7.5	30.3	98.8	18.8
ND 6956b-13	8.8	35.8	96.3	29.7
Etb 6-5-2	10.0	24.3	72.4	22.0
ND 4710-10	10.0	32.9	91.3	26.0
J 103-K7	12.5	22.9	61.3	22.9
ND 4708-6PE	12.5	39.3	90.0	28.8
J 101-K6	17.5	32.8	88.8	15.9
ND 7443Ab-44	18.8	26.8	92.5	24.9
ND 7402b-185	22.5	25.0	76.3	24.2
ND 7443Ab-181	26.3	27.3	97.5	29.5
ND 7443Ab-68	27.1	24.1	96.3	18.5
ND 5822C-7	27.5	26.6	93.8	29.2
Etb 5-31-2	28.8	26.1	83.8	17.4
ND 7443Ab-48	31.3	27.7	96.3	23.0
ND 7818-1Y	41.3	26.2	97.5	26.5
ND 7443Ab-34	42.5	25.1	93.8	24.0
ND 7383Ab-11	43.8	22.7	88.8	17.1
ND 7443Ab-50	48.8	25.9	93.8	18.9
ND 7402b-135	57.5	24.5	96.3	26.5
ND 7443Ab-18	58.8	26.2	91.3	20.8
AOND95292-3 Russ	60.0	39.2	98.8	24.9
AOND 95249-1 Russ	62.5	31.9	97.1	22.2
ND 7202b-38	66.3	26.9	92.5	27.9
ND 8229-1	66.3	27.9	91.3	31.9
ND 7291b-2Y	68.8	23.5	86.3	25.0
ND 7333b-7	70.0	28.0	98.8	28.4
ND 8266A-1R	72.5	26.3	71.3	16.7
ND 8266A-2R	81.3	29.7	90.0	20.3
Atlantic	51.3	24.6	95.0	26.4
Red Norland	73.8	33.7	100.0	29.2
Russet Norkotah	76.3	33.4	91.3	27.5
Snowden	96.3	30.8	70.0	27.3
Mean <sup>b</sup>	41.7	27.7	89.8	24.0
LSD <sub>P=0.05</sub>	18.2	11.9	11.6	2.6
Coefficient of Variation	41.9	33.5	13.1	11.1

<sup>a</sup>Named cultivars were added as controls for challenge inoculations; they were not grown with the clonal selections.

<sup>b</sup>Mean pink rot and leak incidence and penetration across all selections.

potato clone ND 8266A-1R was found to be moderately susceptible to leak infection while apparently resisting penetration of the pathogen after it had gained entry into the tuber. This was deemed interesting and the clone was retained for further evaluation. As in the previous year, a number of other clones tested in 2004 were found to have moderate levels of resistance to pink rot but not to leak and were not included in further evaluations.

Ten potato clones that had been tested in previous years were evaluated again in 2005 for resistance to pink rot and leak. Of these, eight potato clones were found to have levels of pink rot resistance equal to or significantly greater than the check cultivar Atlantic: J101K6A22, Etb 6-5-2, Etb 5-31-2, ND 5822C-7, ND 6956b-13, ND 7443Ab-181, ND 7443Ab-44 and ND 7818-1Y (Table 3). A number of other potato clones tested for the first time in 2005 also demonstrated excellent resistance to pink rot and will be evaluated in future trials.

Incidence of *P. ultimum* infection in 2005 was considerably higher than in previous studies (Salas et al. 2003) (Table 3). Snowden, which has demonstrated a moderately resistant to resistant response to leak in previous studies, gave a moderately susceptible response in 2005. Etb 6-5-2 and ND 8266A-

1R were not significantly different from Snowden but had infection levels significantly lower than nearly all other potato clones and cultivars evaluated (Table 3). Interestingly, the penetration of *P. ultimum* in these two clones was significantly less than it was in the check cultivar Snowden.

Incidence of infection and depth of penetration of *P. erythroseptica* and *P. ultimum* in eleven potato genotypes and four check cultivars evaluated in 2003-2005 were tested for variance homogeneity using Levene's method. Homogeneity was found in 24 of 30 variances tested for incidence and penetration of *P. erythroseptica* and in 27 of 30 tested for *P. ultimum*. Therefore, data were combined among years for the 11 potato clones and four cultivars for further analysis (Table 4). A number of clones were found with resistance to pink rot equal to or significantly greater than the moderately resistant check cultivar Atlantic based on the incidence of infection and penetration of the pathogen: ND 7443Ab-44, ND 7443Ab-181, Etb 5-31-2, ND 5822C-7, ND 7818-1Y, ND 6956b-13, Etb 6-5-2 and J101K6A22 (Table 4; Figure 1A). Phenotypically, we regard the latter three of these genotypes to be highly resistant to pink rot since they demonstrate a combined resistance to infection and penetration of the pathogen (Figure 1A). Among these potato genotypes, only Etb

TABLE 4—Infection incidence (%) and penetration severity (mm) from 2003 to 2005 of *Phytophthora erythroseptica* (pink rot) and *Pythium ultimum* (leak) on challenge inoculated tubers of control cultivars and potato clones from the North Dakota State University breeding program.

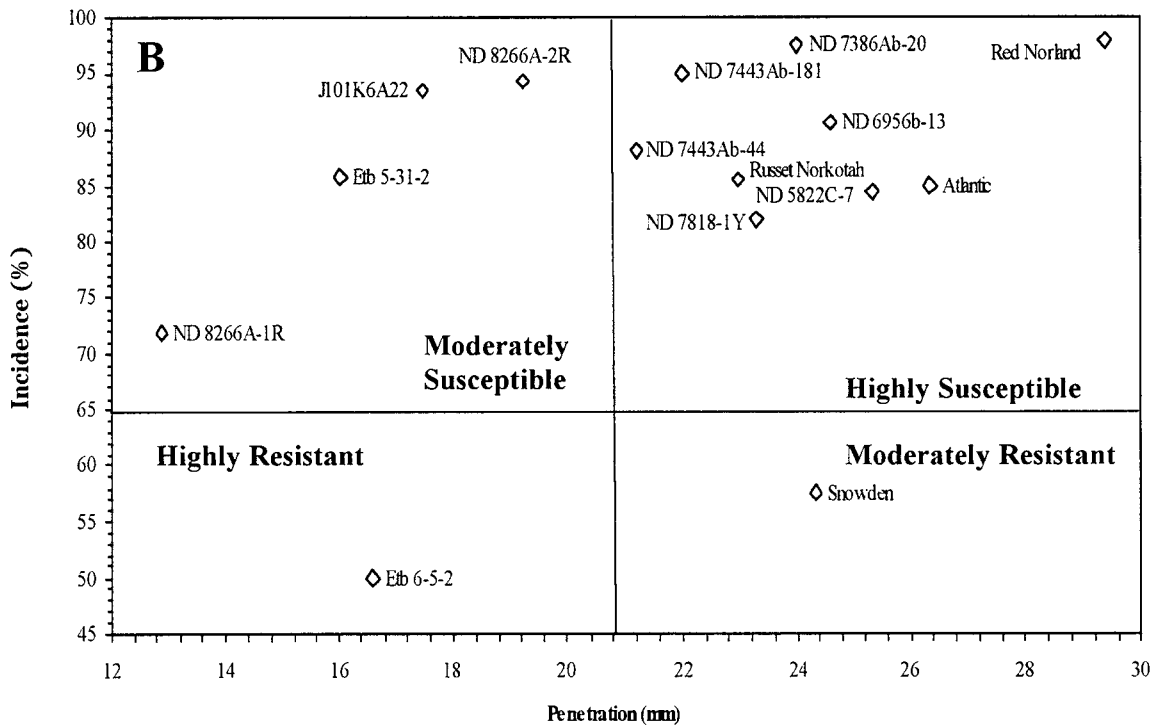
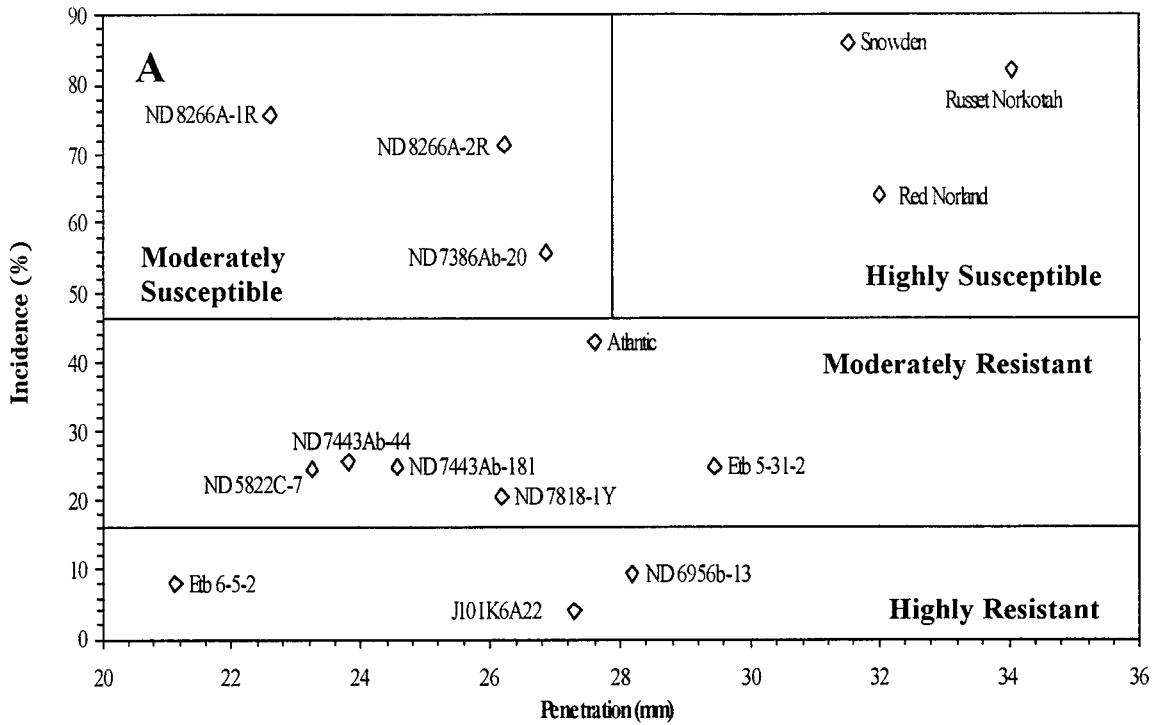
Selection <sup>a</sup>	Number of Years Evaluated	<i>P. erythroseptica</i>		<i>P. ultimum</i>	
		Incidence (%)	Penetration (mm)	Incidence (%)	Penetration (mm)
J101K6A22	2	4.2	27.3	93.6	17.5
Etb 6-5-2	3	8.0	21.1	49.9	16.6
ND 6956b-13	2	9.4	28.2	90.6	24.6
ND 7818-1Y	2	20.6	26.2	81.9	23.3
ND 5822C-7	2	24.4	23.2	84.4	25.3
Etb 5-31-2	2	25.0	29.5	85.8	16.0
ND 7443Ab-181	2	25.0	24.6	95.0	22.0
ND 7443Ab-44	2	25.6	23.8	88.1	21.2
ND 7386Ab-20	2	55.8	26.9	97.5	24.0
ND 8266A-2R	2	71.3	26.2	94.4	19.2
ND 8266A-1R	2	75.6	22.6	71.9	12.9
Atlantic	3	43.0	27.6	85.0	26.3
Red Norland	3	64.0	32.0	98.0	29.4
Russet Norkotah	3	82.0	34.0	85.5	23.0
Snowden	3	86.0	31.5	57.5	24.3
Mean <sup>b</sup>		43.2	27.4	82.9	22.0
LSD <sub>P=0.05</sub>		12.7	7.0	11.6	3.3
Coefficient of Variation		42.2	28.6	20.2	21.3

<sup>a</sup>Named cultivars were added as controls for challenge inoculations; they were not grown with the clonal selections.

<sup>b</sup>Mean pink rot and leak incidence and penetration across all selections.

6-5-2 demonstrated resistance to leak similar to the moderately resistant check cultivar Snowden (Table 4; Figure 1B). Again, based on combined infection and penetration of the pathogen, it appears that resistance to leak in Etb 6-5-2 is slightly greater than that level of resistance found in Snowden over the three years these two genotypes were evaluated together (Figure 1B).

Incidence of infection and penetration was not correlated for either pink rot ( $r = 0.43$ ;  $P = 0.146$ )



**FIGURE 1.** Responses of tubers of various potato clones (n = 11) and cultivars (n = 4) to challenge inoculations of the pink rot pathogen, *Phytophthora erythroseptica* (A) and the leak pathogen *Pythium ultimum* (B). Vertical and horizontal divisions of each graph represent postulated resistant and susceptible reactions of each potato genotype to pink rot (A) and leak (B) based on the incidence of infection (%) (y-axis) and penetration of the pathogen (mm) (x-axis).



or leak ( $r = 0.25$ ;  $P = 0.401$ ), suggesting that resistance to infection and resistance to penetration of the pathogens once they have gained entry is inherited independently. This is clearly illustrated by the fact that two clones, ND 8266A-1R and ND 8266A-2R, express a moderately susceptible to susceptible response to leak based on incidence of infection but a resistant response based on penetration (Table 4). The lack of relationship between pink rot and leak resistance among 11 clones and four cultivars was demonstrated using Chi square analysis ( $\chi^2 = 6.5$ ;  $P = 0.952$ ).

## DISCUSSION

A considerable amount of effort is being placed globally on the development of genetic resistance to both the foliar and tuber rot phase of late blight (Douches et al. 1997; Douches et al. 2002; Park et al. 2005; Platt and Tai 1998). One could argue that in the United States, tuber rots caused by *P. erythroseptica* and *P. ultimum* are economically more important on a yearly basis. Although late blight tuber rot infections tend to be acute and spectacularly devastating when they occur, in most production years few growers experience economic loss from this phase of the disease. Pink rot and leak are chronic diseases, present every year in nearly every potato production region. Storage rot surveys conducted in each of the past nine years in ND and MN indicate that pink rot and leak are of nearly equal importance, with one or the other being more important in any single year (Taylor and Gudmestad, unpublished data). Yet to our knowledge, the studies reported here represent the first attempt of a potato breeding program to evaluate and develop genetic resistance simultaneously to both of these storage rot diseases.

Pink rot and leak have historically been managed quite effectively through the use of mefenoxam-based fungicides (Taylor et al. 2004; Wicks et al. 2000). Unfortunately, resistance to mefenoxam in *P. erythroseptica* and *P. ultimum* has been reported (Taylor et al. 2002) and appears to be getting more widespread and important in many potato production areas (GA Secor and NC Gudmestad, unpublished). This has led to the development and use of other fungicide chemistries, such as phosphorous acid (Johnson et al. 2004). Phosphorous acid applied to the foliage is much more costly than mefenoxam-based fungicides and is active only against *P. erythroseptica*, providing no control of *P. ultimum* (Johnson et al. 2004). Clearly, in the absence of new fungicides for the control of

pink rot and leak, the development of genetic resistance to these two storage rots is critically important. The use of cultivars with genetic resistance to pink rot and leak has multiple advantages. Resistant cultivars would provide an additional management tool in areas where these diseases are a chronic problem and could potentially reduce the level of inoculum in the soil over time. Resistant cultivars also would reduce the need for mefenoxam applications and thus potentially limit the development of resistance to the fungicide in production areas with mefenoxam-sensitive *P. erythroseptica* populations. Additionally, such cultivars could reduce disease incidence in areas having mefenoxam-resistant populations, where applications of the fungicide are no longer effective.

In previous work by our group, commercially available potato cultivars grown in the United States were evaluated for susceptibility to both pink rot and leak. Within the group of 34 cultivars evaluated, most cultivars possessed varying levels of susceptibility to these two storage rots (Salas et al. 2003). No cultivar was immune to either pink rot or leak and no cultivar possessed genetic resistance to both diseases. However, we demonstrated that Atlantic has moderate resistance to pink rot, while Snowden has moderate resistance to leak. In that work, we also clearly demonstrated that genetic resistance is expressed as resistance to infection and to penetration of each pathogen and that these traits are not necessarily linked. The studies reported here confirm those earlier findings. We acknowledge that resistance to tuber infection is probably the most important characteristic of genetic resistance to both *P. erythroseptica* and *P. ultimum*. However, the rate at which each pathogen penetrates, colonizes and decays potato tubers is also likely to be important. When potato tubers rot rapidly due to penetration by these storage rot pathogens, excessive moisture is released from the decaying tubers that induces anaerobic conditions in the healthy tubers near them, thereby inducing bacterial soft rots. Based on observations in commercial potato storages, we believe that "slow rotting," due to partial resistance to the pink rot and leak pathogens in the form of reduced penetration of potato tubers, can be the difference between effectively managing a storage rot problem and not being able to do so. Partial resistance to plant pathogens is recognized as an important component of effective disease management for a number of diseases including rusts (Dowkiw et al. 2003; Kolmer and Liu 2001; Leonard 2002) and *Phytophthora*-caused diseases (Dorrance et al. 2001; Thabuis et al. 2001; Vega-Sanchez et al. 2005). Therefore, we

feel that the evaluation of genetic resistance to *P. erythroseptica* and *P. ultimum* must be done by evaluating both the resistance to infection and the penetration of the pathogen, which represents partial resistance. We found no relationship between incidence of infection and penetration for *P. erythroseptica* and *P. ultimum* among the potato genotypes and cultivars tested over multiple years. This also was the case with the cultivars evaluated in our previous work (Salas et al. 2003). In fact, some clones such as ND 8266A-1R and ND 8266A-2R expressed a differential response to leak based on incidence of infection and penetration. This phenomenon should be investigated in more detail.

Based on the data reported here, it is apparent that there is substantial resistance to pink rot that can be exploited and used in the development of resistant cultivars. Three clones, Etb 6-5-2, J101K6A22, and ND6956b-13, were classified as highly resistant to pink rot. It is likely that wild species in the background of Etb 6-5-2 (*S. etuberosum* and *S. berthaultii*) and J101K6A22 (*S. bulbocastanum*) are contributing to their high levels of pink rot resistance. Furthermore, since J101K6A22 is a parent of ND6956b-13, it is likely that the source of pink rot resistance in this breeding selection is also derived from *S. bulbocastanum* and is indicative that resistance is highly heritable.

*S. bulbocastanum* has previously been shown to contribute a gene, RB, that confers resistance to late blight caused by *Phytophthora infestans* (Song et al. 2003). J101K6A22 is derived from the *S. bulbocastanum* accession that the RB gene was cloned from, and was previously reported as being highly resistant to late blight (Helgeson et al. 1998). *S. berthaultii* also was identified as a source of resistance to late blight, with resistance being conferred by gene  $R_{Pi-ber}$  (Rauscher et al. 2006). It is plausible that the genes for resistance to *P. infestans* identified in *S. bulbocastanum* and *S. berthaultii* may also provide resistance to closely related *P. erythroseptica*, therefore contributing to the pink rot resistance observed in J101K6A22, ND6956b-13, and Etb 6-5-2. With respect to the pink rot and leak resistance displayed by Etb 6-5-2, *S. etuberosum* cannot be directly tested as the putative source of these resistances in that it is non-tuberbearing.

While several breeding clones were identified with moderate to high resistance to pink rot, only Etb 6-5-2, tested in multiple years, had a level of resistance to leak that was equal to or greater than that found in the moderately resistant culti-

var Snowden. Etb 6-5-2 resists infection by *P. ultimum* equivalently to Snowden, but the penetration of the pathogen after entry is significantly less in Etb 6-5-2 than in this moderately resistant cultivar. In our proposed model, Etb 6-5-2 is moderately resistant to pink rot infection but also expresses partial resistance to *P. ultimum* in the form of reduced penetration after infection. Etb 6-5-2 also demonstrated significant levels of resistance to pink rot. This clone was significantly more resistant to infection than the check cultivar Atlantic; however, penetration of the fungus was similar in both genotypes.

We evaluated a number of somatic hybrid backcross derivatives during the course of the studies reported here. Somatic hybrids of *Solanum etuberosum* and *Solanum berthaultii* and their backcross derivatives have been demonstrated previously to be excellent sources of resistance to potato viruses such as potato virus Y (PVY) and potato leaf roll virus (PLRV) as well as to the green peach aphid (GPA), *Myzus persicae*. Two BC2 individuals, Etb 6-21-3 and Etb 6-21-5, were reported to possess multiple resistances to PVY, PLRV and GPA (Novy et al. 2002). Etb 6-21-3 was evaluated for resistance to pink rot and leak in 2003 and found to be moderately susceptible and susceptible, respectively, to these two diseases. Another somatic hybrid derivative, Etb 5-31-5, previously reported to possess resistance to PVY (Novy et al. 2002) was found to be resistant to pink rot and susceptible to leak. However, Etb 6-5-2 was found to be highly resistant to pink rot and to possess resistance to leak equal to the level of resistance in Snowden. We feel that Etb 6-5-2 represents an excellent source of resistance to both pink rot and leak that can be utilized by potato breeding programs throughout the United States. Etb 6-5-2 is freely available for all breeders to use. Further research should be performed to identify additional sources of resistance to these important potato tuber rot diseases.

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