Tuber Rot of Potato Caused by *Phytophthora nicotianae*: Isolate Aggressiveness and Cultivar Susceptibility

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Abstract

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A study was undertaken in 2008 and 2009 to examine potato (*Solanum tuberosum*) cultivar susceptibility, the potential of other host species to act as sources of inoculum for potato infections, and other aspects of potato–*Phytophthora nicotianae* interactions. Twelve isolates of *P. nicotianae* collected from five leaf, one petiole, and six tuber infections of potato from five states, as well as isolates from a variety of other host species, were evaluated for ability to cause tuber rot of potato via inoculation studies. Additionally, the susceptibility of 27 potato cultivars commonly grown in the United States to tuber infection by *P. nicotianae* was determined. Eighty-three percent of the isolates recovered from potato were highly aggressive, infecting tubers at nearly four times greater incidences than isolates originating from nonpotato hosts. With the exception of two tobacco isolates, zoospores of all isolates recovered from nonpotato hosts were able to infect potato tubers. Russet cultivars were significantly less susceptible to *P. nicotianae* than red and white cultivars in 2008, and red cultivars in 2009. Umatilla Russet was the most resistant cultivar in both years, whereas Red Norland and Dakota Rose were the most susceptible in both years. Results of a survey for *P. nicotianae* conducted in four states from 2008 through 2010 confirmed previous observations of naturally occurring infections of potato in Missouri, Nebraska, and Texas, as well as infections of potato in Michigan (documented for the first time). All isolates recovered in the survey were sensitive to mefenoxam (EC_{50} < 1.0 µg/ml).

The potato (*Solanum tuberosum* L.) has become a major food crop, ranking fourth behind wheat, maize, and rice in importance as a staple (47). As the popularity and range of production and consumption has increased, so has the realization that the potato plant is susceptible to a myriad of diseases that affect virtually every tissue and organ. Although damage to aboveground plant tissues by diseases can be substantial, pathogens that attack the belowground starch storage organ may have the most profound effect upon yield, and ultimately, storability and quality of the crop (13,30,36). Several species of fungi and oomycetes, representing a broad group of genera, are known to cause tuber rots of the cultivated potato (6,40). In North America, dry rot (*Fusarium solani* (Mart.) Sacc. and *F. sambucinum* Fuckel) (37), gray mold (*Botrytis cinerea* Pers.:Fr.) (29), leak (*Pythium ultimum* Trow) (32), late blight (*Phytophthora infestans* (Mont.) de Bary) (9), and pink rot (*Phytophthora erythroseptica* Pethybr.) (16) are among the most common of these diseases, and each can have an economic impact in a growing season. Of these, the latter two are very destructive and, therefore, could be considered the most important tuber rot diseases in nearly all potato production areas (9,13,16,30,36). Late blight tuber infections can be devastating, but generally are acute and do not cause economic losses on an annual basis (13). On the other hand, *P. erythroseptica* is ubiquitous and endemic in virtually every potato growing region; therefore, pink rot can be a chronic problem for producers, who often experience economic losses from this disease (13,16,30,36).

Pink rot first was described as a potato tuber disease in Ireland in 1913 (27) and has been known to occur in North America since 1938 (1). It has become one of the most important soilborne tuber diseases of potato in the United States in recent years (13,36). Although *P. erythroseptica* generally is considered to be the principal cause of the disease (1,3,11,16,27), several other *Phytophthora* species have been shown, via inoculation studies, to infect potato tubers and produce pink rot–like symptoms (6). Other *Phytophthora* spp., including *P. drechsleri* Tucker (4), *P. megasperma* Drechs. (3), *P. cryptogea* Pethybr. & Lafferty (12,19,31), and *P. nicotianae* van Breda de Haan (synonym = *P. parasitica*) (12,22,41), have been isolated from naturally occurring tuber infections, but none has been considered a significant potato pathogen.

P. nicotianae is capable of infecting a large range of hosts, including over 300 plant species (6). Among the most notable diseases attributed to this pathogen are brown rot, foot rot, and black shank of tobacco, as well as gummosis and root rot of citrus species. The pathogen is a recurring problem in greenhouse and nursery settings, commonly resulting in root and crown rots of annual herbaceous and perennial woody plant species. *P. nicotianae* has been reported to cause foliar blight and pink tuber rot of potato (4,6,12,22,26) but previously was not considered to be a major threat to the potato crop. The number of recently confirmed cases of foliar and tuber infections of potato caused by *P. nicotianae* (41) suggests that this species is becoming increasingly prevalent, posing a significant threat to potato production, particularly in warmer, southern growing regions of the United States. Thirty isolates of *P. nicotianae* were recovered from naturally occurring potato infections from Florida, Nebraska, Missouri, and Texas between 2005 and 2007 (41). Such outbreaks previously were rare, with only sporadic appearances recorded in Kentucky and Oklahoma in 1929 (4), North Carolina in 1969 (26), Texas in 1983 (12), and Delaware and New Jersey in 1994 (22).

Symptoms of tuber rot caused by *P. nicotianae* are similar to those of pink tuber rot commonly caused by *P. erythroseptica*, with development of a soft, spongy, watery texture in the infected tissue (12,41). However, the infected tissue often lacks the typical pink color that develops and intensifies after exposure of the cut, infected tuber surface to air, which is diagnostic of *P. erythroseptica* infections (11,16). Tuber tissue infected with *P. nicotianae* usually develops a less intense, pale pink discoloration, but generally the

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color varies from a light, creamy tan to pale brown, depending upon the cultivar (4,12,41). In some instances, infected tuber tissue turns dark brown to black after exposure of the cut tuber surface to air for an hour or more.

Zoospores of *P. erythroseptica* typically infect potato tubers through stolons but also may infect through eyes or lenticels to cause pink rot (16,17,27,36). Pink rot also may be initiated by zoospores or mycelium penetrating tubers through cuts, cracks, or other damage to the periderm made during harvest or handling operations (16,34). Little is known about the etiology and epidemiology of *P. nicotianae* tuber rot; however, it appears that the mode of infection and disease development are similar to that of common pink rot. Field observations and inoculation studies suggest that *P. nicotianae* infections are initiated through the stolons, eyes, and lenticels, similar to infections caused by *P. erythroseptica* (12,41). Laboratory studies also have demonstrated that wounds may be important infection courts (41). Tuber infections were reported to nearly double following inoculation of freshly wounded tissue with zoospores of *P. nicotianae* when compared to inoculations of nonwounded eye tissue. Isolates of *P. nicotianae* obtained from natural potato tuber or foliar infections were capable of infecting both leaf and tuber tissue, regardless of the tissue from which isolates were originally recovered (26,41). However, all isolates of *P. nicotianae* appeared to be less aggressive than *P. erythroseptica* isolates at infecting tuber tissue (41). As *P. nicotianae* infections of potato have become more prevalent, it is important to examine aggressiveness of isolates recovered from other host species, especially considering the wide host range of this pathogen (6).

Conditions of high moisture favor infection by *P. erythroseptica* and *P. nicotianae* (41). In the field, foliar infections by *P. nicotianae* commonly are concentrated in low-lying areas and along irrigator wheel tracks where compacted soil promotes the accumulation of standing water (41). Tubers produced under furrow irrigation in heavier, clay soils also are more prone to infection (12), and the pathogens readily move within the field as motile zoospores carried in irrigation water. Development of *P. nicotianae* tuber rot appears to be favored in potato-growing areas that have warmer growing seasons and where tubers are harvested when air and soil temperatures are high (12,16,41). Intermittent but severe losses can occur under such conditions (12; *unpublished data*). Since *P. erythroseptica* and *P. nicotianae* are likely to infect potato plants in a similar manner, cultural practices and applications of mefenoxam used to manage *P. erythroseptica* (13,23,24,30,36, 44,49,51,52) should help control *P. nicotianae*.

Susceptibility to *P. erythroseptica* has been assessed in potato genotypes (33) and breeding lines (46), but with the exceptions of Red Norland, Russet Norkotah, Snowden, Russet Burbank, and Atlantic examined in 2008 (41), and three cultivars and four breeding lines screened in an earlier study (12), the susceptibility of potato cultivars to *P. nicotianae* generally is unknown. Potato genotypes demonstrate considerable variability in susceptibility to *P. erythroseptica* (33,42,43,46). Therefore, susceptibility to another species of *Phytophthora* is expected to range widely. Cultivar resistance could be an important component of a management strategy, particularly if mefenoxam resistance appears in the *P. nicotianae* populations responsible for potato infections. Mefenoxam (and metalaxyl) resistant strains of *P. nicotianae* have been recovered from other plant species (7,8,14,15) and soil (48). Since *P. nicotianae* has an extensive host range, it is likely that strains from other plant species are pathogenic to potato. The probability of identifying mefenoxam resistance in *P. nicotianae* potato populations remains low. Strains from those populations are not likely to have experienced extensive exposure to the fungicide since mefenoxam is not used commonly on crops typically included in potato rotations.

This study was undertaken to assess these suppositions and provide additional details regarding potato–*P. nicotianae* interactions. The specific objectives of this study were to: (i) assess the ability of *P. nicotianae* isolates from a variety of hosts to cause tuber rot in potato, (ii) determine the susceptibility of commonly grown potato cultivars to tuber infection by *P. nicotianae*, and (iii) characterize isolates of the pathogen collected during a survey in 2008, 2009, and 2010 that were not part of a previous survey (41). To achieve these objectives, pathogenicity and aggressiveness of *P. nicotianae* isolates collected from a range of hosts, host tissues, and locations were evaluated; and multiple potato cultivars were screened for susceptibility to tuber rot via postharvest tuber inoculations. Isolates obtained in the survey were characterized by determining mating type, sensitivity to mefenoxam, and identity to species via morphological observation and polymerase chain reaction (PCR) assays. Results from these studies should help improve management practices for *P. nicotianae* in potato production.

Materials and Methods

Isolate collection. As an extension of a survey (41) to assess the geographic distribution of *P. nicotianae* infections of potato, isolates were collected from pink rot outbreaks in commercial potato fields in four states in 2008, 2009, and 2010 (Table 1). A subset of the isolates evaluated in the study was selected from a collection of *P. nicotianae* isolates at North Dakota State University (Table 2). These isolates initially were recovered from naturally occurring infections of potato by transferring small pieces (approximately 25 mm^3) of infected potato leaf, petiole, or tuber tissue to petri dishes containing water agar (1.5% agar in deionized water). Following incubation in the dark at 20°C for 3 to 5 days, colonies with mycelia resembling that of *P. nicotianae* were isolated utilizing hyphal tip transfers. In addition, an isolate from potato was obtained from the collection of Robert Mulrooney, University of Delaware; tobacco isolates were obtained from H. David Shew, North Carolina State University; and Chuanxue Hong, Virginia Polytechnic Institute and State University, provided isolates of *P. nicotianae* recovered from other plant species and effluent water collected from a recycling irrigation system at a commercial container nursery pro-

Table 1. Origin, mating type, and mefenoxam sensitivity of previously uncharacterized *Phytophthora nicotianae* isolates recovered from potato

Collection year	Location	Number of isolates		EC_{50} (mean ± SD, µg/ml)[z]	Number of isolates recovered			
		A1[y]	A2[y]		Leaf	Petiole	Stem	Tuber
2008	Arbyrd, MO	0	6	0.049 ± 0.007	6	0	0	0
	Pearsall, TX	0	10	0.051 ± 0.006	10	0	0	0
	Dalhart, TX	0	7	0.051 ± 0.006	0	0	0	7
2009	Three Rivers, MI	0	7	0.031 ± 0.012	0	0	0	7
	Pearsall, TX	1	21	0.079 ± 0.055	6	3	13	0
	Olton, TX	0	3	0.057 ± 0.007	2	0	1	0
	Dalhart, TX	0	7	0.040 ± 0.008	2	1	4	0
2010	Columbus, NE	1	0	0.100 ± 0.073	1	0	0	0
	Pearsall, TX	0	9	0.040 ± 0.004	0	3	6	0
	Olton, TX	0	5	0.074 ± 0.036	5	0	0	0

[y] Mating type.

[z] Effective mefenoxam concentration resulting in 50% reduction of mycelial growth relative to the control treatment in which the isolates were not exposed to mefenoxam. Isolates were tested in duplicate at each fungicide concentration and the experiment was repeated. SD = standard deviation.

ducing a diverse range of perennial cultivars (2) (USDA-APHIS Permit P526P-06-02040). All isolates were transferred to clarified 10% V8 juice (CV8) agar medium (100 ml V8 juice, 1 g $CaCO_3$ centrifuged at 7,000 rpm for 5 min using a Sorvall RC5C centrifuge to remove the pulp, 15 g agar, and 900 ml deionized water) and stored in the dark at 20 ± 1°C prior to testing.

Morphological characterization. Isolates of *P. nicotianae* were grown on either 5% clarified carrot agar (CA) (50 ml Hollywood carrot juice, 950 ml deionized water, and 20 g Difco Bacto agar/liter water, clarified by filtering through Celite) or 5% CV8 agar (50 ml V8 juice, 950 ml deionized water, and 20 g Difco Bacto agar, clarified by filtering through Celite), and maintained with regular transfers. Colony morphology, and vegetative and reproductive stages of growth were examined for cultures grown for 1 to 4 weeks at 20 to 25°C in light, using a Nikon TMS Inverted Phase Contrast Microscope. Isolates were scored for appearance of hyphae, size and shape of sporangia and chlamydospores, and presence or absence of oospores. The resulting observations were compared to published descriptions of *P. nicotianae* (6,10).

DNA extraction. Mycelium of each isolate was produced in sterile pea broth by incubating petri dish cultures for 7 days at 23°C in the dark. Pea broth was prepared by autoclaving 120 g frozen peas in 1 liter deionized water, and filtering the suspension through cheesecloth. The broth was sterilized by autoclaving for an additional 30 min after filtering. DNA was extracted from mycelium using the CTAB (cetyl trimethyl ammonium bromide) method described in a previous study of *P. nicotianae* on potato (41). DNA precipitate was collected and washed three times with 100 µl cold 80% EtOH. The pellets were dried and then suspended in 30 µl nuclease-free water and stored at 4°C. Extracted DNA was run in a 1% agarose gel to confirm the quality of the DNA. DNA concentrations were determined using a NanoDrop 1000 and diluted to 10 ng/µl for PCR assays.

PCR assays. Samples were amplified using species-specific primers previously described for *P. nicotianae* (5). Primer #1 (5′-CTGACGATCCAGATCCTCTGCACG-3′) was used as the forward primer, and primer #2 (5′-CTTGCGAGGCTTGACCGC TTCCTA-3′) as the reverse primer. Amplifications were done with an Eppendorf Mastercycler thermal cycler (Eppendorf Scientific, Westbury, NY). Each reaction contained 20 ng template DNA in sterile, nuclease-free water; 2.5 µM forward primer; 2.5 µM reverse primer; 10× PCR buffer; dNTPs; and *Taq* DNA polymerase (QIAGEN). The cycling parameters were 1 cycle at 94°C for 1 min; 30 cycles of 2 min annealing at 65°C, 3 min extension at 72°C, and 1 min denaturation at 94°C; followed by 1 extension cycle at 72°C for 10 min. A negative control sample (without DNA) was included, and amplicons were visualized and photographed on a 1% agarose gel. PCR products were purified using the QIAquick PCR Purification Kit (QIAquick Gel Extraction Kit, QIAGEN). Purified PCR products were sent to the DNA Sequencing and Genotyping facility in the Life Sciences Core Laboratory Center at Cornell University for sequencing.

Mating type. The mating type of each of the *P. nicotianae* isolates was determined by pairing each isolate with known A1 and A2 isolates. Two different sets of testers were used in all pairings, and all testers were obtained from tobacco fields in NC. All mating type evaluations also included pairings of testers to confirm suitable conditions for oospore formation. Isolates were paired on CA supplemented with water-soluble cholesterol at 5 ppm, and incubated in the dark for 21 to 30 days at 20 to 24°C. Each pairing was observed microscopically for the presence of oospores. If oospores formed when an isolate was paired with the known A1 isolate, the isolate was considered to be an A2 mating type, and vice versa. Isolates that failed to form oospores with either isolate were designated as the A0 mating type.

Mefenoxam sensitivity. Mefenoxam (Ridomil Gold 4EC; Syngenta Crop Protection, Greensboro, NC) sensitivity of the isolates was determined using an in vitro screening method similar to a technique described previously (41,42,44,45). Tests were conducted on 5% CV8 agar medium (50 ml V8 juice centrifuged at 7,000 rpm for 5 min using a Sorvall RC5C centrifuge to remove pulp, 15 g agar, and 950 ml deionized water) amended with mefenoxam in a 10-fold dilution series ranging from 0.01 to 100 µg/ml. Agar plates not amended with mefenoxam served as the

Table 2. Identity, origin, mating type, and mefenoxam sensitivity of a collection of *Phytophthora nicotianae* isolates from the United States

Isolate	Origin	Collection year	Mating type	EC_{50} (mean ± SD, µg/ml)[u]	Host (source tissue)
05NE1-1[v]	Nebraska	2005	A2	0.125 ± 0.074	Potato (tuber)
06FL1-7[v]	Florida	2006	A2	0.238 ± 0.233	Potato (tuber)
06NE1-5[v]	Nebraska	2006	A1	0.094 ± 0.003	Potato (leaf)
06TX1-3[v]	Texas	2006	A2	0.137 ± 0.083	Potato (leaf)
07MO1-1[v]	Missouri	2007	A0	0.369 ± 0.131	Potato (tuber)
07MO1-3[v]	Missouri	2007	A2	0.068 ± 0.004	Potato (tuber)
07TX1-1[v]	Texas	2007	A2	0.083 ± 0.009	Potato (leaf)
07TX2-1[v]	Texas	2007	A2	0.419 ± 0.176	Potato (petiole)
08MO1-2[v]	Missouri	2008	A2	0.057 ± 0.002	Potato (leaf)
08TX1-9[v]	Texas	2008	A2	0.051 ± 0.001	Potato (leaf)
08TX2-1[v]	Texas	2008	A2	0.052 ± 0.003	Potato (tuber)
N3[w]	Delaware	1995	A2	0.322 ± 0.171	Potato (tuber)
Dav-Whi-7[x]	North Carolina	2006	A2	0.195 ± 0.099	Tobacco
Dup-Thi-1[x]	North Carolina	2006	A2	0.069 ± 0.001	Tobacco
Roc-Bak-14[x]	North Carolina	2006	A2	0.627 ± 0.386	Tobacco
Sam-Fai-6[x]	North Carolina	2006	A1	0.069 ± 0.003	Tobacco
Yad-Bro-14[x]	North Carolina	2006	A2	0.237 ± 0.198	Tobacco
17H1[y]	Virginia	2001	A2	0.077 ± 0.008	Forsythia
31A3[y]	Virginia	2004	A2	>100	Petunia
28B5[y]	Virginia	2002	A2	>100	Lavender
39D4[y]	Virginia	2006	A1	0.040 ± 0.007	Lavender
1E3[y]	Virginia	2000	A1	0.045 ± 0.007	Unknown[z]
3A4[y]	Virginia	2000	A2	>100	Unknown[z]

[u] Effective mefenoxam concentration resulting in 50% reduction of mycelial growth relative to the control treatment in which the isolates were not exposed to mefenoxam. Isolates were tested in duplicate at each fungicide concentration and the experiment was repeated. SD = standard deviation.

[v] From the collection of N. C. Gudmestad, North Dakota State University.

[w] From the collection of R. P. Mulrooney, University of Delaware.

[x] From the collection of H. D. Shew, North Carolina State University.

[y] From the collection of C. Hong, Virginia Polytechnic Institute and State University.

[z] Water was collected as effluent from a recycling irrigation system at a commercial container nursery producing a diverse range of perennial ornamental plant species.

control treatment. Disks containing mycelium and agar were excised from the margin of an actively growing colony of a 3- to 5-day-old culture of each isolate with a number 2 cork borer. A 5-mm-diameter disk of colonized agar was positioned in the center of 100 mm diameter × 15 mm deep, plastic petri dishes with the mycelium in contact with the agar medium. Testing consisted of two replications per mefenoxam concentration per isolate per trial. Plates were arranged in a randomized complete block design (RCBD) for each independent trial.

Isolate growth on mefenoxam-amended agar medium, as a measure of mefenoxam sensitivity, was assessed by measuring colony diameter in two perpendicular directions after 6 days of incubation in the dark at 20 ± 1°C. Colony diameters were averaged for the two measurements from each replicate plate, the diameter of the mycelial plug was subtracted from this average, and relative growth reduction at each fungicide concentration was calculated as follows: {100 – [(colony diameter on fungicide-amended agar medium)/(colony diameter in the nonamended control plate)] × 100}. The effective concentration (EC_{50}) resulting in 50% reduction of mycelial growth relative to the control plate was estimated for each isolate from the dose response curve. The latter was generated by plotting percent inhibition against the log-scale of fungicide concentration. EC_{50} values were determined from two independent sensitivity trials conducted for each isolate. Based upon results of previous work (42), isolates with EC_{50} values <1.0 μg/ml were considered to be sensitive to mefenoxam, and isolates with EC_{50} values >100 μg/ml were considered to be resistant to the fungicide.

Pathogenicity and aggressiveness of *P. nicotianae* isolates. Isolates of *P. nicotianae* used to evaluate pathogenicity and aggressiveness on potato were selected based on host source, geographic distribution, collection year, mating type, and mefenoxam sensitivity, in addition to traits determined for nine of the isolates in a previous characterization study (41). The 23 *P. nicotianae* isolates selected were collected in seven states from five host species or as naturally occurring isolates in irrigation water, from 1995 to 2008 (Table 2). Each isolate was grown on 10% CV8 agar medium in an environmentally controlled chamber at 20 ± 1°C in the dark. After 3 days, 3-mm-diameter disks containing mycelium and agar were cut from colony margins with a number 1 cork borer and transferred to plastic culture plates (3 disks/plate) containing 10 ml autoclaved 10% CV8 juice broth (100 ml V8 juice centrifuged at 3,000 rpm for 20 min to remove pulp, and 900 ml deionized water). The broth was removed from the plates with a sterile pipet after the liquid cultures were incubated for 72 h at 20 ± 1°C in the dark. Mycelial mats were rinsed twice with 10 ml sterile deionized water and resuspended in 10 ml autoclaved soil extract water (10%, prepared with 100 g soil from a potato field added to 900 ml deionized water). Sporangia formed within 8 to 12 h under constant illumination, and abundant zoospores were released spontaneously within 24 to 36 h. Zoospore concentration was determined using a hemacytometer and adjusted to 2.5×10^4 zoospores/ml for inoculations. To facilitate counting zoospores, zoospore motility was moderated by chilling the hemacytometer slide at 10°C for approximately 2 min before counting. Zoospore suspensions were held at 20 to 25°C until inoculations were carried out, generally within 10 to 60 min.

Tubers of Russet Norkotah (each 175 to 275 g) and Russet Burbank (each 150 to 250 g), cultivars susceptible and moderately resistant to infection by *P. erythroseptica* (33,43), respectively, were inoculated to assess pathogenicity and aggressiveness of each isolate of *P. nicotianae*. Tubers were removed from storage, inspected for damage or disease symptoms, and acclimated for 24 to 48 h at ambient temperature (24 to 27°C). Disease-free tubers with apical eyes free of soil and an intact periderm were placed in 33 cm long × 24 cm wide × 12 cm deep plastic moist chamber boxes lined with paper towels moistened with deionized water. Each tuber was inoculated at each of three apical eyes with 10 μl zoospore suspension (approximately 250 zoospores/inoculation site). Inoculated tubers were covered with four layers of paper towels moistened to saturation with deionized water. Chamber boxes were covered to establish high humidity to promote infection and incubated in the dark at an ambient temperature of 24 to 27°C for 7 days. Evaluations were conducted as two separate trials, each consisting of four replications of five tubers for a total of 40 tubers/cultivar/isolate. Plastic boxes containing inoculated tubers were arranged in a RCBD. The quantity of tubers available of the desired size and condition was limited; therefore, a water-inoculated control treatment for each cultivar was not included.

Susceptibility of potato cultivars to *P. nicotianae*. Potato germplasm used in this study included 8 red-skinned cultivars, 9 white-skinned cultivars, and 10 russet cultivars (Table 3). This material was selected to represent a broad spectrum of genetic backgrounds including a combination of established cultivars as well as recently released cultivars. Certified seed of the 26 potato cultivars was obtained from seed potato producers in North Dakota and Minnesota, and seed of Dakota Trailblazer was provided by the breeding program at North Dakota State University (courtesy of A. Thompson). Field plots were established at a nonirrigated location near Grand Forks, ND on 2 June 2008, and under overhead irrigation near Inkster, ND on 1 June 2009. Whole and cut seed tubers were planted at a 0.3-m in-row spacing in paired, 35 m long rows with rows spaced 0.9 m apart. Agronomic practices commonly used in commercial potato production in North Dakota were implemented throughout the growing season. Following harvest, tubers were inspected for damage, pink rot, and other disease symptoms, then placed into storage where they were held for 2 weeks at 15°C and 90% relative humidity to promote wound healing. Tubers subsequently were stored at 10°C until inoculation studies were conducted. Cultivar susceptibility was assessed following procedures similar to those used in previous studies evaluating susceptibility of potato germplasm to infection by *P. erythroseptica* (33,43,46) and *P. nicotianae* (41), using isolate 06TX1-3. This isolate had previously been characterized extensively and used in inoculation studies (41). Cultivar susceptibility evaluations were carried out as two separate trials each year, each trial consisting of four replications of 10 tubers/cultivar for a total of 80 tubers/cultivar screened each year.

Disease assessment. Disease development in both the pathogenicity and aggressiveness trials as well as the cultivar susceptibility trials was quantified using techniques similar to those described in previous studies (33,41–44,46). Nonwounded tubers were removed from moist chambers and cut in half through the axis of the sites of inoculation, from the apical buds to the basal stem end. Split tubers were covered with paper towels moistened with deionized water and incubated at 20 to 24°C for approximately 30 min to enhance development of tan to pale brown discoloration diagnostic of *P. nicotianae* infection. Infected tubers were counted, and disease incidence (I) was calculated as: (number of diseased tubers)/(number of inoculated tubers) × 100. Disease severity was estimated by measuring the maximum depth (D) of rotted tissue from the point of inoculation, and a penetration rate (P) was calculated as: P = D/T, where T = time (days) after inoculation.

Cultivar susceptibility also may be characterized by quantifying disease severity as the rate of tissue penetration following infection (43,46). A plot of disease incidence versus rate of tuber penetration was used to evaluate and categorize aggressiveness of the *P. nicotianae* isolates on potato tubers and susceptibility of the potato cultivars to tuber infection by *P. nicotianae*. Those isolates nearest the x-y origin displayed the lowest incidence and slowest rate of tuber penetration and, therefore, were determined to be the least aggressive on potato tubers. Similarly, those potato cultivars nearest the x-y origin displayed the lowest incidence and slowest rate of tuber penetration and, therefore, were determined to be the most resistant to tuber infection. These categorizations have been used in previous work with *P. erythroseptica* (33,42–44,46) based upon results of inoculation studies and field observations of cultivar susceptibility.

Data analyses. Levene's test for homogeneity of variance was conducted for all tuber inoculation trials. The effects of trial, as

well as trial interactions with other main effects, were included in the analyses of variance (ANOVAs) to test if data could be combined for further analysis ($\alpha = 0.05$) (20). A factorial ANOVA was performed on disease incidence (% infection) data from the isolate pathogenicity and aggressiveness trials. Trial, cultivar (Russet Burbank and Russet Norkotah), and pathogen isolate were analyzed as fixed main effects. Means were separated using Fishers protected least significant difference (LSD) test ($\alpha = 0.05$). Pearson's correlation coefficient was calculated for disease incidence (% infection) data derived from *P. nicotianae* isolate pathogenicity and aggressiveness trials to compare pink rot development in Russet Norkotah and Russet Burbank. For comparisons across types of cultivars and within each type of cultivar (red, white, or russet), a two-stage nested ANOVA was calculated with PROC GLIMMIX using potato type and cultivar nested within type as the factors for each year (trial). Type I error in these pair-wise comparisons was controlled using the Tukey adjustment (35).

Results

***P. nicotianae* distribution.** *P. nicotianae* infections of potato were verified in Nebraska, Michigan, Missouri, and Texas (Table 1). Seventy-seven isolates were collected from six locations in these states. Of these, 32 isolates were recovered from leaves, 7 from petioles, 24 from stems, and 14 from tubers across all locations and years. *P. erythroseptica* was not recovered from any infected potato samples at these sites. All isolates collected in 2008, 2009, and 2010 were sensitive to mefenoxam, with mean EC_{50} values ranging from 0.03 to 0.10 µg/ml (Table 1).

Isolate characterization. *P. nicotianae* isolate identity was confirmed by morphological characteristics and species-specific DNA amplification by PCR assay and sequencing. Colony morphology was very similar among isolates, with all isolates fitting the range in colony morphology described for the species (6). All isolates had hyphae with slight to prominent hyphal swellings, and produced a rosette to arachnoid colony type (6). All isolates produced sporangia and chlamydospores, and some isolates produced zoospores in the agar medium. Sporangia were variable in size and shape, but most were ellipsoid, ovoid, obpyriform, or spherical, and all had the prominent papilla typical of *P. nicotianae*. Chlamydospores typical of *P. nicotianae* were produced by all isolates.

P. nicotianae species-specific primers amplified DNA of the positive control isolate but not the negative control isolate. Of the 77 isolates identified as *P. nicotianae* based on morphological features characteristic for the species, only 68 had DNA amplified by the PCR assay, despite repeated efforts with the probe. Internal transcribed spacer (ITS) sequencing was completed for the 68 isolates that were amplified, and the sequences were Blasted against the available sequences of GenBank accessions through NCBI Blast. All 68 isolates were identified as *P. nicotianae* based on >99% similarity to the reported ITS sequences of *P. nicotianae* isolates. Mating-type pairings indicated that all but two of the potato isolates collected in 2008 to 2010 were the A2 mating type, with one A1 isolate collected from a potato leaf in Nebraska and one from a potato petiole in Texas (Table 1). Isolates of both mating types were used in the pathogenicity and aggressiveness trials.

Twenty of 23 *P. nicotianae* isolates used in the aggressiveness and cultivar susceptibility trials were sensitive to mefenoxam (Table 2), with EC_{50} values ranging from 0.04 to 0.63 µg/ml. However, petunia isolate 31A3 from Virginia, lavender isolate 28B5 from Virginia, and isolate 3A4 collected from irrigation water in Virginia were resistant to the fungicide (EC_{50} > 100 µg/ml). The isolates represented a diversity of mating types. Nine of the 12 potato isolates selected for this study were characterized previously (41). With the exception of 06NE1-5 (A1) and 07MO1-1 (A0), the remaining 10 isolates from potato were of the A2 mating type. Of the isolates from other hosts or irrigation water (hosts unknown), 8 were mating type A2 and 3 were mating type A1.

Isolate pathogenicity and aggressiveness. Variances across the two pathogenicity and aggressiveness trials for the isolates of *P. nicotianae* were homogeneous based on Levene's test ($P = 0.7642$); however, a significant trial-by-isolate interaction was observed ($P < 0.0001$). Inoculations of Russet Norkotah tubers

Table 3. Potato cultivars evaluated for susceptibility to tuber rot caused by *Phytophthora nicotianae*

Cultivar	Parentage[y]	Release year[z]	Maturity rating[y]
Red cultivars			
Dakota Jewel	ND2223-8R × ND649-4R	2004	Medium-late
Dakota Rose	ND1196-2R × NorDonna	2000	Early-medium
Dark Red Norland	Mutant of Red Norland	1989 (1965)	Early
Red LaSoda	Mutant of La Soda (Triumph × Katahdin)	1953 (1948)	Main-season
Red Norland	Clone from Norland (Redkote × ND626)	1965 (1957)	Early
Red Pontiac	Triumph × Katahdin	1945	Late
Sangre	Viking × A6356-9	1982	Medium
Viking	Nordak × Redskin	1963	Main-season
Russet cultivars			
Alturas	A77182-1 × A75188-3	2002	Late
Bannock Russet	A75175-1 × A75188-3	1999	Late
Dakota Trailblazer	A89163-3LS × A8914-4	2010	Full season
Goldrush	ND450-3Russ × Lemhi Russet	1992	Medium
Premier Russet	A87149-4 × A88108-7	2006	Medium-late
Ranger Russet	Butte × A6595-3	1991	Full season
Russet Norkotah	ND9526-4 Russ × ND9687-5 Russ	1987	Early-medium
Russet Burbank	Chimera of Burbank (Early Rose × ?)	1914 (1876)	Late
Silverton Russet	CalWhite × A7875-5	1999	Medium
Umatilla Russet	Butte × A77268-4	1998	Medium-late
White cultivars			
Atlantic	Wauseon × USDA B5141-6 (Lenape)	1976	Medium
Dakota Crisp	Yankee Chipper × Norchip	2005	Medium
Dakota Diamond	ND4103-2 × Dakota Pearl	2005	Medium-late
Dakota Pearl	ND1118-1 and ND944-6	1999	Medium
Kennebec	B127 × USDA 96-56	1948	Medium
Norvalley	ND860-2 × Norchip	1997	Medium
Shepody	Bake-King × F58050	1980	Medium-late
Snowden	B5141-6 (Lenape) × Wischip	1990	Full-season
Yukon Gold	W5279-4 × Norgleam	1980	Medium

[y] From online resources: http://potatoassociation.org/index.html; http://www.plantbreeding.wur.nl/potatopedigree/.
[z] Date denotes year the cultivar was released. Date in parentheses denotes release year of original cultivar from which the variant genotype was derived.

resulted in mean infection rates greater than two times that observed on Russet Burbank tubers and, therefore, a significant difference in infection incidence between cultivars ($P < 0.0001$) (Table 4). No significant interaction between the main effects of trial and *P. nicotianae* isolate was observed for Russet Burbank (*data not shown*). However, a significant interaction was detected between the main effects of trial and isolate for Russet Norkotah. This interaction resulted from isolates which caused very low tuber infection incidences and, therefore, very low variances. Therefore, infection incidence data were combined across trials within each cultivar. Significant differences in incidence of tubers infected were observed among isolates for both Russet Norkotah ($P < 0.0001$) and Russet Burbank ($P < 0.0001$) tubers (Table 4).

In general, isolates recovered from potato caused greater infection incidences on both cultivars compared to most isolates originating from nonpotato hosts or from irrigation water (unknown original host). Although a significant interaction between cultivar and isolate ($P < 0.0001$) was observed, this interaction was due to the magnitude of decrease in infection incidence on Russet Norkotah tubers (susceptible) versus Russet Burbank tubers (moderately resistant). Overall, all isolates of *P. nicotianae* infected tubers of cv. Russet Norkotah at a higher frequency than tubers of Russet Burbank, except when infection incidences were relatively low, as for isolates 28B5 (lavender), Yad-Bro-14 (tobacco), and 31A3 (petunia). Isolate Roc-Bak-14 from tobacco did not cause tuber infections on either cultivar (Table 4). Overall, a strong and significant correlation ($r = 0.8979$; $P < 0.0001$; $n = 23$) between disease incidence on Russet Norkotah versus Russet Burbank tubers existed across all *P. nicotianae* isolates (Fig. 1).

Tuber infection incidences for the potato isolates of *P. nicotianae* were not correlated significantly with the potato plant tissue of origin. Isolates N3 from tuber tissue and 08MO1-2 from leaf tissue resulted in the highest infection incidences on both cultivars, while inoculations with isolate 06NE1-5 obtained from a potato leaf and isolate 07MO1-1 from a tuber resulted in the lowest incidences of infection on both Russet Norkotah and Russet Burbank tubers (Table 4). Based upon infection incidence, the overall ranking of the potato isolates was consistent across both cultivars. Greater variation was observed in the rankings of isolates recovered from other host species and irrigation water than among the potato isolates. With the exception of isolates Roc-Bak-14 and Sam-Fai-6 recovered from tobacco, zoospores of all these other isolates were able to infect tubers of both Russet Norkotah and Russet Burbank following inoculation through the apical eyes (Table 4). Nearly all (83%) of the potato isolates were highly aggressive when inoculated onto tubers of Russet Norkotah and Russet Burbank (Table 4, Fig. 2). All of these isolates were of the A2 mating type. Potato isolates 06NE1-5 (A1) and 07MO1-1 (A0) exhibited moderately low aggressiveness on these cultivars. Isolate 1E3, collected from irrigation water (unknown original host), also was highly aggressive on potato tubers (Table 4, Fig. 2). Isolates 39D4 (lavender) and 3A4 (irrigation water) demonstrated moderately low aggressiveness, whereas the remaining isolates obtained from nonpotato plant hosts displayed low aggressiveness on potato (Fig. 2, Table 4).

Cultivar susceptibility. Infected tissue of potato tubers inoculated with *P. nicotianae* isolate 06TX1-3 had a spongy, rubbery, watery consistency, and the flesh color varied with cultivar from pale tan to dark pinkish brown, which is typical of *P. nicotianae* infections (4,12,41). No significant main effect of trial or significant interaction of trial with other main effects was observed for the incidence of tubers infected in 2008, when the tubers used for this experiment were produced under nonirrigated conditions (Table 5). However, in 2009, while the main effect of trial was not significant, there was a significant interaction of trial with cultivar type due to inconsistent results for two white cultivars, Dakota Crisp and Shepody. Because data were consistent for the other cultivars, and variances were homogeneous ($P = 0.2832$), data

Table 4. Infection incidence of zoospores of *Phytophthora nicotianae* isolates collected from various hosts and inoculated onto nonwounded tubers of potato cvs. Russet Norkotah and Russet Burbank

		Infection incidence (%)[u]	
Isolate	Host (source tissue)	Russet Norkotah	Russet Burbank
N3[v]	Potato (tuber)	90.0 a	62.5 a
08MO1-2[w]	Potato (leaf)	90.0 a	50.0 ab
08TX2-1[w]	Potato (tuber)	90.0 a	47.5 ab
08TX1-9[w]	Potato (leaf)	90.0 a	37.5 bcd
07TX1-1[w]	Potato (leaf)	87.5 ab	42.5 cb
07MO1-3[w]	Potato (tuber)	85.0 ab	50.0 ab
06FL1-7[w]	Potato (tuber)	85.0 ab	42.5 bc
07TX2-1[w]	Potato (petiole)	80.0 abc	45.0 bc
05NE1-1[w]	Potato (tuber)	77.5 abcd	37.5 bcd
06TX1-3[w]	Potato (leaf)	77.5 abcd	35.0 bcde
1E3[x]	Unknown[y]	75.0 bcd	30.0 cdef
06NE1-5[w]	Potato (leaf)	70.0 cde	25.0 defg
07MO1-1[w]	Potato (tuber)	65.0 de	17.5 fghi
39D4[x]	Lavender	60.0 e	5.0 hij
3A4[x]	Unknown[y]	45.0 f	12.5 ghij
Dup-Thi-1[z]	Tobacco	32.5 fg	5.0 hij
28B5[x]	Lavender	22.5 gh	20.0 efgh
Dav-Whi-7[z]	Tobacco	15.0 hi	2.5 ij
17H1[x]	Forsythia	12.5 hij	5.0 hij
Sam-Fai-6[z]	Tobacco	10.0 hij	0.0 j
Yad-Bro-14[z]	Tobacco	2.5 ij	2.5 ij
31A3[x]	Petunia	2.5 ij	2.5 ij
Roc-Bak-14[z]	Tobacco	0.0 j	0.0 j
Mean		55.0 A	25.1 B
P value		<0.0001	<0.0001

[u] Values followed by the same lowercase (column) or uppercase (row) letter are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$). The interaction of the main effects of cultivar and isolate was signifant ($P < 0.0001$). Evaluations were conducted as two separate trials, each consisting of four replications of five tubers for a total of 40 tubers/cultivar/isolate.

[v] From the collection of R. P. Mulrooney, University of Delaware.

[w] From the collection of N. C. Gudmestad, North Dakota State University.

[x] From the collection of C. Hong, Virginia Polytechnic Institute and State University.

[y] Water was collected as effluent from a recycling irrigation system at a commercial container nursery producing a diverse range of perennial ornamental plant species.

[z] From the collection of H. D. Shew, North Carolina State University.

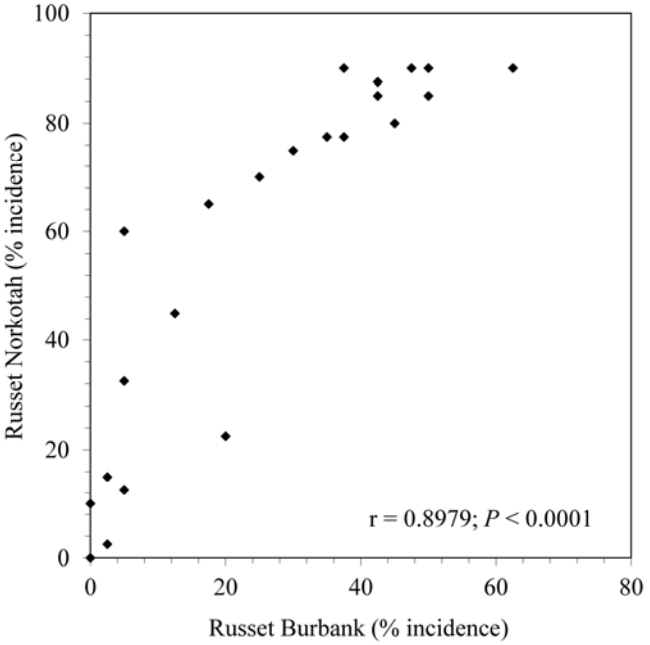

Fig. 1. Pearson's correlation coefficient comparing incidence (%) of tubers of potato cvs. Russet Burbank and Russet Norkotah with symptoms of tuber rot following inoculation with 23 isolates of *Phytophthora nicotianae*.

from the two trials within each year were combined for further analysis (Tables 5 and 6).

In 2008, when tubers were produced under nonirrigated conditions, significant differences in disease incidence were observed across all cultivars, and infection incidences ranged from 31.3% for Umatilla Russet tubers to 100% in Red Norland tubers (Table 5). A significant difference also was observed within all three phenotypic groups or types of cultivars (red, russet, and white types). Red cultivars as a whole were significantly more susceptible to *P. nicotianae* when compared to russet and white cultivars, while russet cultivars were least susceptible (Table 5). Pairwise comparisons obtained in the nested analyses indicated that significant differences were present within each cultivar type in the 2008 trials. Of the eight red cultivars, seven were statistically similar and highly susceptible. Red Pontiac had the lowest incidence of infection among the red cultivars. Six of the white cultivars were significantly more susceptible to tuber infection, with the greatest mean incidence of infection observed on Snowden and Yukon Gold tubers; while Shepody, Dakota Crisp, and Atlantic were the least susceptible of the white cultivars. Five of the 10 russet cultivars had the highest tuber incidence ratings statistically, including Gold Rush, Russet Norkotah, Alturas, Bannock Russet, and Silverton Russet. Umatilla Russet and Ranger Russet had the least number of tubers infected (although not significantly different than that of Russet Burbank, Premier Russet, and Dakota Trailblazer).

In 2009, significant differences in the incidence of infected tubers also was observed for the same potato cultivars produced under irrigation (Table 6). Across all cultivars, tuber infection inci-

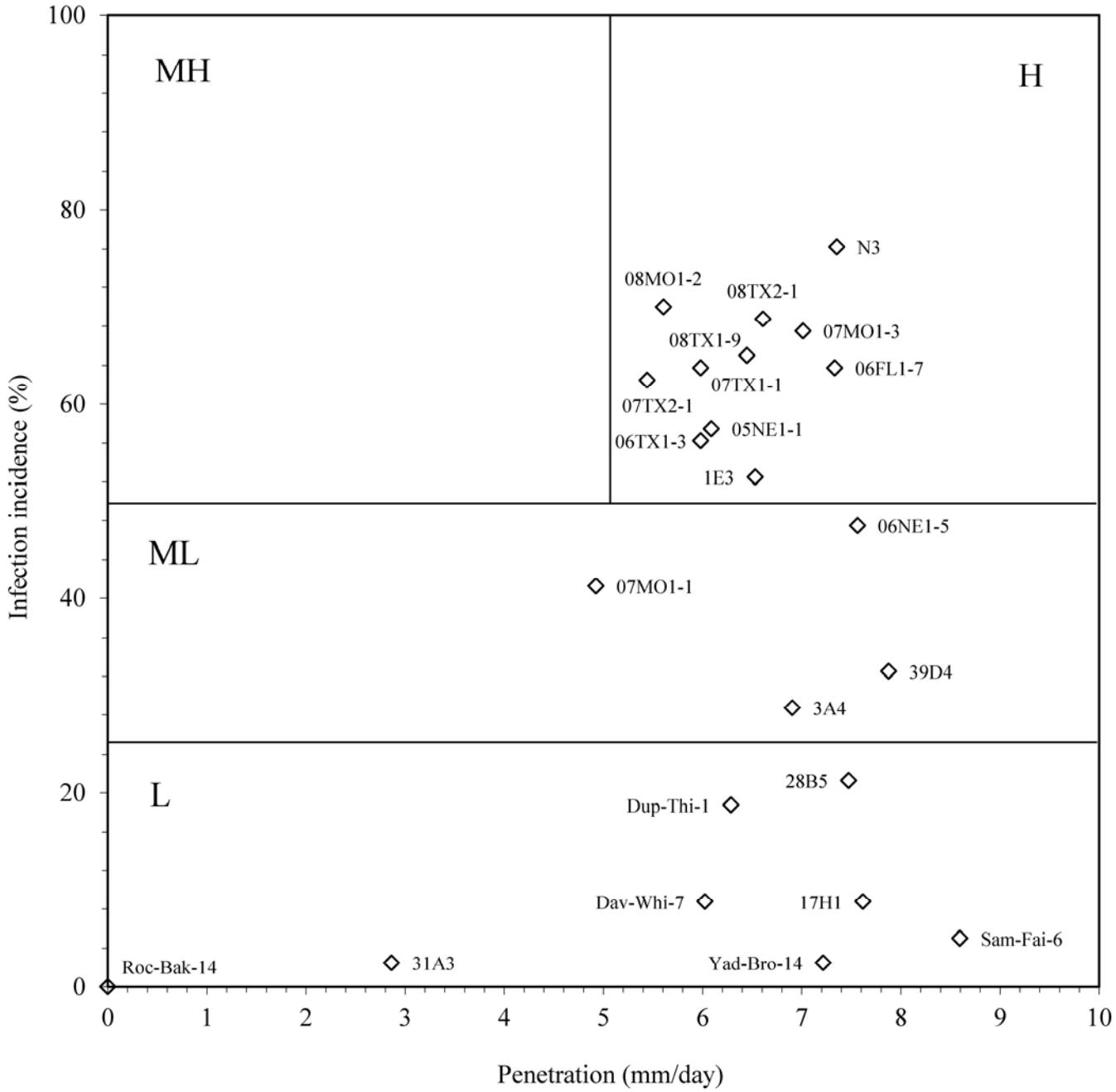

Fig. 2. Relative aggressiveness of 23 *Phytophthora nicotianae* isolates inoculated postharvest onto tubers of potato cvs. Russet Burbank and Russet Norkotah, based on paired values of incidence of tubers infected (susceptibility to tuber infection, shown on the y-axis) and rate of tuber penetration (mm/day as a measure of susceptibility of the tuber to colonization, shown on the x-axis). Symbols closer to the origin of the x-y axes denote less disease. Graph is divided to denote postulated host–pathogen interactions representing varying levels of isolate aggressiveness: high (H), moderately high (MH), moderately low (ML), and low (L). Separation of L and ML categories is based on infection incidence only. Separation of MH and H categories of infection is based on the rate of pathogen penetration into potato tubers. Refer to Table 2 for details of *P. nicotianae* isolates. Each data point is the mean of four replications in each of two trials.

dence was lower in 2009 than in 2008. In 2009, Umatilla Russet tubers again displayed the lowest incidence (7.5% tubers infected), although this was not significantly different than the incidence of tubers infected for 16 other cultivars. Tubers of Yukon Gold displayed the greatest disease incidence (76.3%), which was not significantly different than the incidence observed on tubers of nine other cultivars evaluated. As a whole, infections incidences in tubers of white and red cultivars were not significantly different. Infection incidences in russet cultivars did differ significantly from those of white cultivars but not red cultivars (Table 6). Pairwise comparisons in the nested analyses indicated that significant differences were present among russet cultivars and among white cultivars, but not among red cultivars. As in 2008, Gold Rush and Russet Norkotah had the greatest incidence of tubers infected among russet cultivars, although not significantly different from that of six other russet cultivars based on the LSD means comparison; and Umatilla Russet and Ranger Russet had the lowest incidence of tubers infected among the russet cultivars (although not significantly different from that of four other russet cultivars). Among white tuber cultivar types, Yukon Gold, NorValley, and Snowden had the greatest infection incidence, and Atlantic the least tubers infected in 2009 (later not significantly different from the tuber infection incidence of six other white cultivars).

Although considerable variation in tuber penetration rates by *P. nicotianae* was observed across all cultivars, the mean disease severity of each group was similar (red cultivars = 5.6, russet cultivars = 6.0, and white cultivars = 5.7 mm/day) (Fig. 3). Based upon the combination of tuber infection incidence and rate of tuber penetration, Yukon Gold was the cultivar most susceptible to infection by *P. nicotianae* in this study, whereas Ranger Russet and Umatilla Russet were the most resistant. Russet cultivars Dakota Trailblazer, Premier Russet, and Russet Burbank exhibited moderate levels of resistance to *P. nicotianae,* as did the white cultivars Atlantic, Dakota Crisp, and Shepody (Fig. 3).

Discussion

Research involving *P. nicotianae* infection of potato has been intermittent, and the current understanding of host–pathogen interactions primarily is based upon distributional surveys and characterization of a small group of pathogen isolates and potato cultivars (12,22,26,41). This study represents a more comprehensive assessment of potato genotype susceptibility to isolates of *P. nicotianae*, as well as pathogenicity and aggressiveness of isolates from a broad sampling of the pathogen population within four states in the United States. Previous reports have documented tuber infections caused by *P. nicotianae* isolates from potato (6,12,22,41); however, information regarding pathogenicity of isolates recovered from other host species has been scarce. In previous inoculation studies, isolates recovered from tomato failed to infect potato (3,31). However in a subsequent study, infections were observed on excised potato leaves, whole potato plants, and tubers by zoospores and mycelia of *P. nicotianae* isolates recovered from tomato and tobacco (26). Results reported in this study confirm the pathogenicity of tobacco isolates on potato, and demonstrate that zoospores of *P. nicotianae* isolates from lavender, forsythia, and petunia are capable of infecting potato tubers, albeit at a much lower frequency than isolates recovered from potato. Zoospores of all 12 *P. nicotianae* isolates in this study that were recovered from potato were able to infect potato tubers through eyes. Ten of these isolates were highly aggressive and two demonstrated moderate to low aggressiveness. The highly aggressive isolates all

Table 5. Incidence of infection of *Phytophthora nicotianae* isolate 06TX1-3 zoospores on nonwounded tubers of potato genotypes grown under nonirrigated conditions in 2008 in North Dakota

Cultivar	Infection incidence (%)[z]		Mean
Red cultivars			91.1 A
Red Norland	100.0 a	A	
Dakota Rose	96.3 ab	A	
Dark Red Norland	96.3 ab	A	
Viking	96.3 ab	A	
Sangre	91.3 ab	ABC	
Dakota Jewel	90.0 ab	ABC	
Red LaSoda	83.8 ab	ABCDE	
Red Pontiac	75.0 b	ABCDEFG	
Russet cultivars			64.6 C
Gold Rush	88.8 a	ABC	
Russet Norkotah	88.8 a	ABC	
Alturas	86.3 a	ABCD	
Bannock Russet	78.8 ab	ABCDEF	
Silverton Russet	67.5 ab	BCDEFG	
Dakota Trailblazer	58.8 b	DEFGH	
Premier Russet	57.5 bc	EFGH	
Russet Burbank	55.0 bcd	FGH	
Ranger Russet	33.8 cd	H	
Umatilla Russet	31.3 d	H	
White cultivars			78.2 B
Snowden	96.3 a	A	
Yukon Gold	95.0 a	AB	
Kennebec	93.8 a	AB	
NorValley	90.0 a	ABC	
Dakota Pearl	82.5 ab	ABCDEF	
Dakota Diamond	76.3 abc	ABCDEF	
Atlantic	65.0 bcd	CDEFG	
Dakota Crisp	57.5 cd	EFGH	
Shepody	47.5 d	GH	
P value	<0.0001		<0.0001

[z] Within each phenotypic group of cultivars, means followed by the same lowercase letter are not significantly different based on pairwise comparisons using Tukey's adjustment (α = 0.05). Among all 27 potato cultivars, and within each phenotypic group, means followed by the same uppercase letter are not significantly different.

Table 6. Incidence of infection of *Phytophthora nicotianae* isolate 06TX1-3 zoospores on nonwounded tubers of potato genotypes grown under irrigated conditions in 2009 in North Dakota

Cultivar	Infection incidence (%)[z]		Mean
Red cultivars			36.1 AB
Dakota Rose	48.8 a	ABC	
Red Norland	43.8 a	ABC	
Sangre	43.8 a	ABC	
Dakota Jewel	40.0 a	BCD	
Dark Red Norland	33.8 a	BCD	
Red LaSoda	27.5 a	BCD	
Red Pontiac	26.3 a	BCD	
Viking	25.0 a	BCD	
Russet cultivars			31.5 B
Gold Rush	53.8 a	AB	
Russet Norkotah	51.3 ab	ABC	
Bannock Russet	50.0 ab	ABC	
Silverton Russet	47.5 abc	ABC	
Premier Russet	35.0 abcd	BCD	
Alturas	23.8 bcd	BCD	
Russet Burbank	20.0 cd	BCD	
Dakota Trailblazer	17.5 d	CD	
Ranger Russet	8.8 d	D	
Umatilla Russet	7.5 d	D	
White cultivars			39.4 A
Yukon Gold	76.3 a	A	
NorValley	52.5 ab	AB	
Snowden	48.8 ab	ABC	
Dakota Pearl	40.0 bc	BCD	
Shepody	38.8 bc	BCD	
Dakota Diamond	32.5 bc	BCD	
Dakota Crisp	25.0 bc	BCD	
Kennebec	23.8 bc	BCD	
Atlantic	17.5 c	CD	
P value	<0.0001		<0.0001

[z] Within each phenotypic group of cultivars, means followed by the same lowercase letter are not significantly different based on pairwise comparisons using Tukey's adjustment (α = 0.05). Among all 27 potato cultivars, and within each phenotypic group, means followed by the same uppercase letter are not significantly different.

were of the A2 mating type; however, the isolates exhibiting moderately low aggressiveness were A0 and A1. This difference in aggressiveness could partially explain the preponderance of the A2 mating type recovered from potato infections.

Of the nine isolates of *P. nicotianae* from nonpotato hosts evaluated in this study, and two isolates obtained from irrigation water (unknown original host plants), only a single isolate from tobacco was nonpathogenic on tubers of potato cvs. Russet Norkotah and

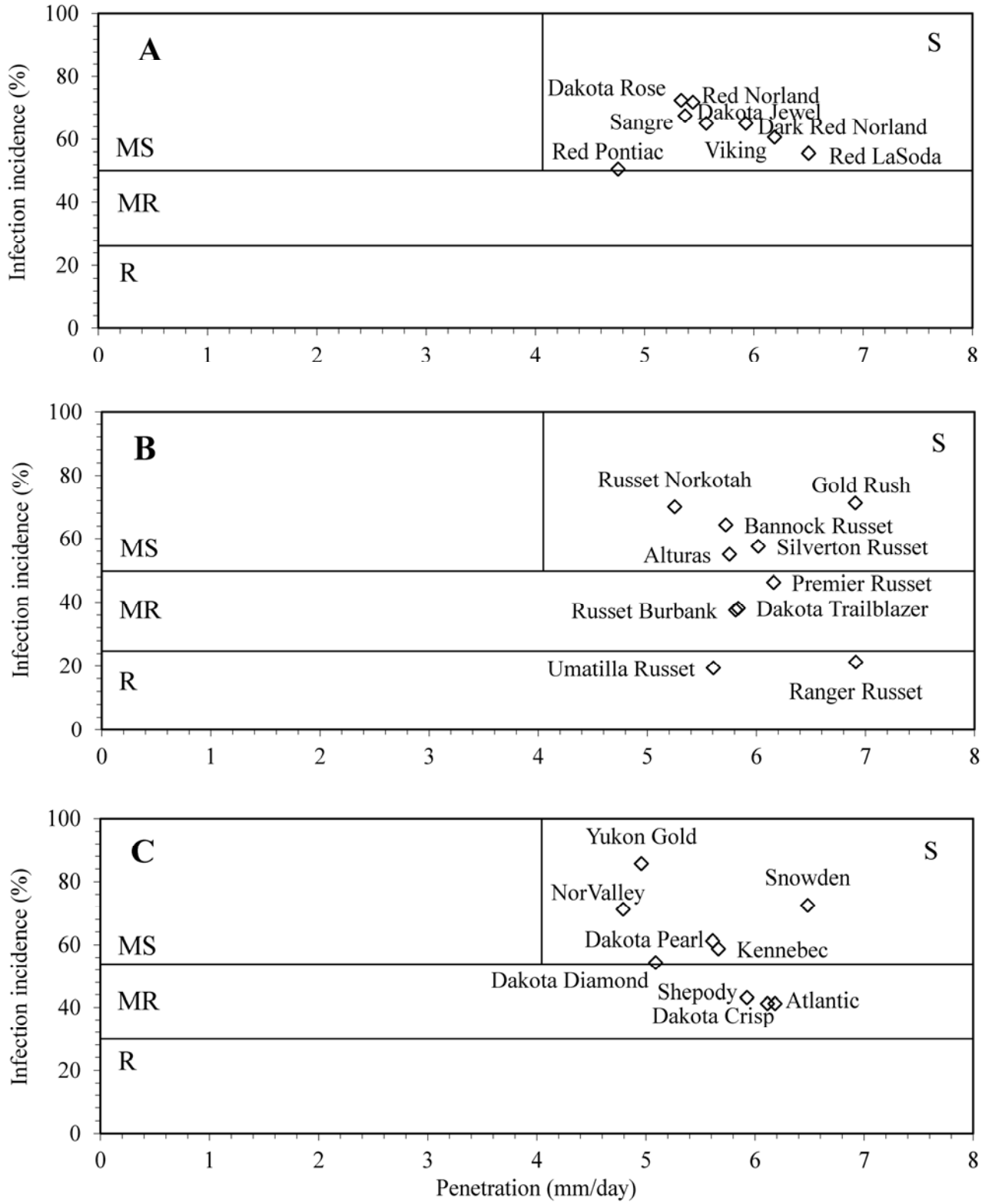

Fig. 3. Relative susceptibilities of **A,** red, **B,** russet, and **C,** white potato cultivars to infection by 23 isolates of *Phytophthora nicotianae* based on paired values of incidence of tubers infected (susceptibility to infection, shown on the y-axis) and rate of tuber penetration by the isolate (mm/day as a measure of susceptibility to colonization, shown on the x-axis). Symbols closer to the origin of the x-y axes denote less disease. Graph is divided to denote postulated host–pathogen interactions representing susceptible (S), moderately susceptible (MS), moderately resistant (MR), and resistant (R) reactions. Separation of R and MR categories is based on infection incidence only. Separation of MS and S categories is based upon the rate of pathogen penetration into potato tubers. Refer to Table 2 for details of *P. nicotianae* isolates. Each data point is the mean of four replications in each of two trials.

Russet Burbank. However, this isolate, as well as other weakly aggressive isolates, proved moderately to highly aggressive on wounded tubers when inoculated as zoospores or mycelium (*data not published*). These observations are consistent with results obtained previously (41), where the ability of *P. nicotianae* isolates recovered from potato to infect tubers of five potato cultivars was greatly enhanced by inoculating wounded versus nonwounded tuber tissue. It is possible that weakly aggressive *P. nicotianae* strains originating from nonpotato hosts could be opportunistic and more aggressive as wound pathogens than on healthy, nonwounded tubers. This could be explored in future studies. The lower level of aggressiveness observed for *P. nicotianae* strains recovered from nonpotato hosts in this study suggests that these strains may pose less of a threat to potato production than strains established as potato pathogens. Therefore, growers probably should be more concerned about preventing spread of the pathogen from fields with *P. nicotianae* infestations than from alternative host species. Since viable and pathogenic isolates of *P. nicotianae* were recovered from irrigation water in this study, this potential source of inoculum should be considered when assessing inter- and intrafield spread of the pathogen.

Prior work (41) demonstrated significant differences in susceptibility to *P. nicotianae* among one red-, two white-, and two russet-skinned potato cultivars. Similarly, significant differences were observed in this study among 27 cultivars representing these phenotypic groups. Cultivar susceptibility to *P. erythroseptica* has been shown to vary greatly within each of these phenotypic groups (33). Tuber rot incidence of five potato cultivars inoculated with either *P. erythroseptica* or *P. nicotianae* was significantly correlated (41). Therefore, the significant differences in infection rates observed among cultivars within each phenotypic group in this study were expected. Although some potato cultivars appeared moderately resistant to *P. nicotianae* tuber infections, most genotypes were highly susceptible. Cultivar resistance currently may not play a major role as a management strategy for this pathogen, but results of this study could prove valuable to potato breeders for developing cultivars with enhanced resistance to *P. nicotianae*.

The potato cv. Atlantic initially was described as resistant to "wet rot" (50), and consistently has proven moderately to highly resistant to tuber infections by *P. erythroseptica* zoospores (33,43,46). These and other observations (41) demonstrate that Atlantic also expresses moderate resistance to *P. nicotianae* and could be a source of resistance for developing new germplasm of white-chipping cultivars. Ranger Russet and Umatilla Russet also expressed high levels of resistance to *P. nicotianae*, and in areas where infections by this pathogen have been confirmed in potato crops, particularly where this is a recurring problem, growers might consider planting these cultivars. The cv. Butte is in the lineage of both of these russet cultivars (21,25). Therefore, each of these cultivars could be assessed as parental material for developing cultivars resistant to this pathogen. Future work could explore the role of other host species in disease epidemiology and examine susceptibility of common potato genotypes to foliar blight caused by *P. nicotianae*, which was not evaluated in this study.

P. nicotianae was isolated in 2005 from potato tubers collected in Nebraska that exhibited symptoms similar to pink rot, and in 2006 from potato leaves grown in Nebraska and Texas as well as from infected tubers from Florida (41). A total of 33 isolates was collected at seven locations in four states from 2005 to 2007 from foliar and/or tuber lesions, indicating that *P. nicotianae* was becoming more prevalent as a pathogen of potato. Isolates of *P. nicotianae* collected from infected potato tissues in 2008 to 2010 were characterized in this study. This survey verified the presence of *P. nicotianae* in Texas, Missouri, and Nebraska; and isolates were obtained from additional locations in these states to those reported previously (41). Seven isolates also were collected from natural tuber infections in Michigan, representing the first report of *P. nicotianae* infection of potato in that state.

Based on the geographical range of *P. nicotianae*–infected potato samples collected prior to 2009, it was hypothesized that this pathogen may be confined to warmer potato production areas (41). However, the pathogen and associated foliar blight and tuber rot are now known to be more prevalent than earlier observations suggested. Foliar blights and tuber rots caused by *P. nicotianae* have not been documented in potato growing areas of the United States located above the 43rd parallel (3,11,22,26,41). Located at 41.96°N, the confirmation of potato infection by *P. nicotianae* in Michigan represents the northernmost documentation of the disease to date on potato, although only ~56 km north of the Nebraska site (41.42°N) in this study. It is not known whether the presence of *P. nicotianae* in potato crops in northern states can be as devastating as occurrences in southern, warmer potato growing areas of the United States.

P. erythroseptica is homothallic and does not produce chlamydospores (6). Oospores are the resting stage and serve as the primary inoculum source for this pathogen. On the other hand, *P. nicotianae* is heterothallic and capable of producing both chlamydospores and oospores, with the former considered the primary inoculum source (6). Evidence suggests that germination of chlamydospores of *P. nicotianae* is inhibited by cold temperatures and enhanced by extended warming periods of high temperatures, with maximum germination occurring after 100 to 150 degree days (expressed in °C, cumulative average temperature above a base of 10°C) have accumulated (18). Based upon that study, temperatures of 32°C for 5 days may provide optimum conditions for propagule germination. Oospores of *P. erythroseptica* require a long chilling period for germination (28). The effect of soil temperature on germination efficiency of these propagules may influence the geographic distribution of *P. erythroseptica* and *P. nicotianae*. Oospores of *P. erythroseptica* and chlamydospores of *P. nicotianae* germinate via a germ tube which is capable of growing as mycelium or as mycelium terminating in sporangia, both of which may remain viable in the soil for at least 4 years (6,28). Therefore, although survival of *P. erythroseptica* and *P. nicotianae* is dependent upon the specific propagules produced by each species, the functionality of these propagules appears comparable.

The etiologies of *P. erythroseptica* pink rot in tubers and *P. nicotianae* tuber rot generally appear to be similar; therefore, practices commonly implemented to manage pink rot should be effective against *P. nicotianae* (6,13,16,36). It is important for potato growers to avoid soil compaction, plant in well-drained soils, and avoid excessive irrigation, particularly toward the end of the growing season. Proper periderm development should be promoted by allowing sufficient time between vine killing and harvest. Harvest and tuber handling procedures should be adjusted to minimize tuber damage. Fungicides, particularly mefenoxam (metalaxyl), augment these agronomic management strategies. The phenylamide fungicide mefenoxam (Ridomil Gold EC and Ultra Flourish EC) has been the only fungicide used to manage pink rot in potato for many years (23,24,30,36,49,51,52), and remains an effective tool for controlling *P. erythroseptica* in populations sensitive to the fungicide (13,42,44). Metalaxyl also is highly toxic to *P. nicotianae* (39). However, metalaxyl-resistant strains of *P. nicotianae* have been recovered from other host species, particularly herbaceous annuals grown in nurseries where the fungicide often is used repeatedly (7,8,14,15). To our knowledge, all isolates of *P. nicotianae* recovered from potato to date have been sensitive to mefenoxam, with sensitivities similar to those reported for isolates not previously exposed to the fungicide (38). Currently, the threat of resistant strains moving from nonhost species to potato crops is limited, as the level of aggressiveness exhibited by the two metalaxyl-resistant isolates recovered from tobacco and lavender in this study was very low and similar to that of metalaxyl-sensitive isolates recovered from nonpotato hosts. Mefenoxam has been employed successfully to prevent significant economic losses in commercial potato production fields where *P. nicotianae* infections have been confirmed (*unpublished data*), and should continue to provide effective control of tuber rot by this pathogen. However, care should be taken by growers to minimize the possibility of mefenoxam resistance developing in potato *P. nicotianae* populations.

In areas where *P. nicotianae* is likely to be present, the scarcity of reports of the pathogen infecting potato may be due, in part, to low levels of infection in fields. As with other oomycete tuber rot pathogens, infections usually are detected in low-lying areas; in areas with compacted, poorly drained soil; and along irrigator wheel tracks. In addition, *P. nicotianae* infections sometimes may be misdiagnosed. Symptoms of tuber rot differ subtly from those of pink rot caused by *P. erythroseptica*, and foliar blight caused by *P. nicotianae* can be confused with symptoms of late blight caused by *P. infestans* (41). It is imperative that growers be aware of these factors, particularly since incidences of *P. nicotianae* infections can be relatively low.

Among the 110 isolates of *P. nicotianae* recovered from potato and characterized to date by the authors of this study, nearly all have been of the A2 mating type. The presence of both the A1 and A2 mating types previously was verified at a single location in Nebraska (41), and now at a single location in Texas. The presence of the A1 mating type in Nebraska was reconfirmed in 2010. Although inoculation studies demonstrated that the A1 mating type is capable of infecting potato tubers, A1 isolates only have been recovered from leaf/petiole infections in fields. While production of oospores is important for long-term survival as well as a source of soilborne inoculum of many *Phytophthora* species, the scarcity of populations comprised of mixed mating types of *P. nicotianae* suggests that chlamydospores may be important survival structures of this pathogen in most potato growing areas where the pathogen has been found. In the two locations in this study where the *P. nicotianae* population comprised both mating types, oospores could afford an additional means of survival but, more importantly, provide a means of sexual reproduction and genetic recombination. If mefenoxam routinely is used to control *P. nicotianae* infections of potato in these areas, the possibility of resistance developing to the fungicide is amplified by the presence of both mating types. The sensitivity of the pathogen populations to mefenoxam should be assessed regularly in these locations.

Information regarding potato cultivar susceptibility to *P. nicotianae*, *P. nicotianae* isolate pathogenicity or aggressiveness on potato, and mefenoxam sensitivity of *P. nicotianae* isolates, as described in this study should prove valuable to potato breeders and growers for developing strategies for managing *P. nicotianae*. Recent research has added significantly to knowledge of this pathogen, but additional work is needed, including research on the etiology and epidemiology of the disease, and how various factors impact spatial and temporal distribution of the pathogen in potato production regions.

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