Phytophthora Species in Rivers and Streams of the Southwestern United States

Rio A. Stamler,a Soumalia Sanogo,a Natalie P. Goldberg,b Jennifer J. Randallab

Department of Entomology, Plant Pathology, and Weed Science, New Mexico State University, Las Cruces, New Mexico, USA; Department of Extension Plant Sciences, New Mexico State University, Las Cruces, New Mexico, USA

ABSTRACT

Phytophthora species were isolated from rivers and streams in the southwestern United States by leaf baiting and identified by sequence analysis of internal transcribed spacer (ITS) ribosomal DNA (rDNA). The major waterways examined included the Rio Grande River, Gila River, Colorado River, and San Juan River. The most prevalent species identified in rivers and streams were Phytophthora lacustris and P. riparia, both members of Phytophthora ITS clade 6. P. gonapodyides, P. cinnamomi, and an uncharacterized Phytophthora species in clade 9 were also recovered. In addition, six isolates recovered from the Rio Grande River were shown to be hybrids of P. lacustris × P. riparia. Pathogenicity assays using P. riparia and P. lacustris failed to produce any disease symptoms on commonly grown crops in the southwestern United States. Inoculation of Capsicum annuum with P. riparia was shown to inhibit disease symptom development when subsequently challenged with P. capsici, a pathogenic Phytophthora species.

IMPORTANCE

Many Phytophthora species are significant plant pathogens causing disease on a large variety of crops worldwide. Closer examinations of streams, rivers, and forest soils have also identified numerous Phytophthora species that do not appear to be phytopathogens and likely act as early saprophytes in aquatic and saturated environments. To date, the Phytophthora species composition in rivers and streams of the southwestern United States has not been evaluated. This article details a study to determine the identity and prevalence of Phytophthora species in rivers and streams located in New Mexico, Arizona, Colorado, Utah, and Texas. Isolated species were evaluated for pathogenicity on crop plants and for their potential to act as biological control agents.

The number of characterized Phytophthora species is rapidly expanding as new habitats are investigated and existing collections are reexamined using sequence-based diagnostic procedures (1). Multilocus phylogenetic analysis of 234 isolates from 82 Phytophthora species confirmed the presence of 10 well-defined clades within the genus (2). Comparative analysis of the cytochrome c oxidase subunit 1 (COX1) locus and the internal transcribed spacer (ITS) locus for 1,204 isolates representing 23 oomycete genera indicated that both loci are effective as DNA barcodes for species identification (3). Genus-wide diagnostic keys confirm that the ITS sequence is a valid set of characters for identification of Phytophthora spp. that closely match and often exceed the resolution of classically utilized morphological characters (4). However, certain closely related species such as P. rubi and P. fragariae have identical ITS sequences, and multilocus sequence analysis is required for species-level identification (5).

Phytophthora species were traditionally considered to be primary plant pathogens with little to no saprophytic ability. However, this assumption is now being cast in doubt due to the widespread identification of Phytophthora spp. in streams and forests from across the globe (6–13). Currently, these species are hypothesized to be waterborne opportunistic pathogens, foliar pathogens, and fine-root or canker pathogens (14). The majority of species found in streams and forests belong to ITS clade 6 based on phylogenetic analysis of the 5.8S ribosomal sequence and the flanking internal transcribed spacers I and II (15). Careful sequence characterization of ITS clade 6 isolates identified multiple well-defined species that can be organized into three distinct subclades (8). These species are usually thermophilic and sexually sterile, although exceptions have been noted (6).

A survey of rivers and streams in Oregon and Alaska identified P. gonapodyides, Phytophthora taxon Salixsoil, and two uncharacterized species as the predominant Phytophthora species in water-sheds in the northwestern United States (9). Phytophthora taxon Salixsoil, originally isolated from Salix roots in the United Kingdom, has subsequently been formally described and renamed P. lacustris, while the two unknown species have been formally described and named P. borealis and P. riparia for their prevalence in forest and riparian ecosystems, respectively (16, 17). Surveys of waterways in Eastern United States identified a wide diversity of Phytophthora spp. including multiple taxa with unique sequence signatures (7, 11). Many of the recovered species were known woody plant pathogens, including members of the P. citricola complex. A promising new approach for identifying Phytophthora species involves genus-specific amplification and next-generation sequencing of environmental DNA (eDNA). This technique was...
used to identify 35 species of *Phytophthora* from Northern Spain, including 13 putative novel species (13).

Numerous natural and artificial *Phytophthora* species hybrids have been characterized to date, typically with closely related parental genotypes (18–26). Species such as *P. alni*, a serious pathogen on Alnus trees in Europe, appear to be recently formed stable hybrid lineages with increased host specificity and virulence (25, 26). Within clade 6, the hybrid species *P. × stagnant* nothosp. was frequently isolated from irrigation reservoirs and ornamental plant nurseries in Eastern Virginia (24). Multilocus sequence analysis revealed a hybrid isolate of *P. lacustris × P. riparia* in the northwestern United States, and multiple hybrid populations were identified in Australia and South Africa among *P. amnicola, P. thermophila*, and *Phytophthora* taxon PgChlamydo (9, 23). Molecular characterization indicated that natural hybridization through sexual crossing is common among closely related clade 6 *Phytophthora* spp. as all hybrid isolates examined had uniparentally inherited mitochondrial DNA (mtDNA) but had up to three distinct nuclear signatures from different *Phytophthora* species (23). It is currently unclear if closely related *Phytophthora* spp. within clade 6 are merging through introgression as populations are moved around through human activity. Alternatively, certain clade 6 hybrid populations may not represent stable evolutionary lines as evidenced by their lack of sexual reproduction and difficulty to maintain in culture (23).

Induced, resistance to specific microbes and chemical elicitors is a well-characterized plant response, leading to augmented basal defenses (27–30). Recent research in our lab characterized the induced resistance response in *Capsicum annuum* to nonhost *P. nicotianae* inoculation (30). Nonhost *Phytophthora*-inoculated *C. annuum* plants had significant changes in their primary metabolism and displayed an increased hypersensitive response during subsequent *P. capsici* infection. However, the broad host range of *P. nicotianae* makes it an unsuitable candidate for applied research into improving crop yield. The ecological role of clade 6 *Phytophthora* spp. remains largely unstudied; however, growing evidence suggests that they may act as first colonizers of plant debris, enabling nutrients to move upward through trophic levels via zoospore grazing and by creating suitable environments for detritivores (31). *P. lacustris* has been isolated from root lesions of multiple species, and pathogenicity assays demonstrated that it is a mild pathogen on *Salix* and *Prunus* spp. (16). However, the ubiquitous nature of clade 6 species in riparian and forest ecosystems and the absence of diseased plants or trees suggest that these species do not pose a pathogenic threat to native flora.

To date, the *Phytophthora* species composition in rivers and streams of the southwestern United States has not been previously evaluated. Therefore, a limited examination was conducted to determine the identity and prevalence of *Phytophthora* spp. in rivers and streams located in New Mexico, Arizona, Colorado, Utah, and Texas. Selected clade 6 species were evaluated for pathogenicity on common agricultural crops in the Southwest and for their ability to elicit an induced resistance response in chile pepper (*Capsicum annuum*).

**MATERIALS AND METHODS**

**Isolation of *Phytophthora* species.** During summer and fall of 2012, we examined seven rivers and streams in the southwestern United States using cottonwood (*Populus* sp.) and willow (*Salix* sp.) leaves as bait to isolate *Phytophthora* species (Table 1). *Populus* and *Salix* species are widely distributed in the ecosystems examined and were both utilized as bait in all rivers and streams analyzed. Five leaves of each species were placed in cheesecloth bags, which were immersed in flowing water for 3 days. The bags were secured in place through a string tied to a branch or rock on the riverbank. A second assay was used to estimate *Phytophthora* CFU in the Rio Grande River over three consecutive weeks as follows. Water was collected from the Rio Grande River and separated into four 1-liter aliquots, and each liter was incubated with five *Salix* species or *Populus* species leaves for 3 days. Finally, cottonwood and willow leaves found suspended or freely floating in the Gila River were also recovered for analysis.

All leaves were rinsed in tap water, surface sterilized in 70% ethanol for 1 min, and rinsed in sterile deionized water. Necrotic lesions were removed with a sterile cork borer (4 mm in diameter) and plated on clarified V8 agar (Select V8S, amended with pimaricin (0.2% [wt/vol]), rifampin (10 mg/liter), and ampicillin (250 mg/liter). Four necrotic leaf sections were placed on each V8S plate. After 2 days, colonies emerging from the plated leaf sections were transferred individually to fresh V8S plates.

**Identification of *Phytophthora* species.** All isolates were initially screened based on the mycelium growth rate, and those growing faster than 15 mm/day were assumed to be *Pythium* or other oomycete species and were excluded from further analysis (32). Careful sequence analysis with the primer sets ITS4 and ITS5 was used to identify the remaining isolates (4, 33). Hyphae from each isolate were picked using a sterile pipette tip, transferred into 100 μl sterile deionized (DI) water in a 1.5-ml Eppi tube, placed in a boiling water bath for 10 min, and then immediately placed on ice. PCRs were conducted in 20-μl final volumes using 1 μl of hyphae lysate, 1 U of platinum *Taq* polymerase, 1.5 mM MgCl2, 0.2 mM dioxynucleoside triphosphate (dNTPs) (Life Technologies, Grand Island, NY), and 0.01 mM each primer (Integrated DNA Technologies Inc., Coralville, IA). PCRs were visualized on 1% agarose gel stained with ethidium bromide. The remaining PCR product was incubated with ExoSap-It (Affymetrix, Santa Clara, CA) according to the manufacturer’s instructions and sequenced with each amplification primer using the BigDye Terminator v3.1 cycle sequencing kit (Life Technologies, Grand Island, NY). Sequencing reaction products were cleaned on Performa spin columns (Edge Biosystems, Gaithersburg, MD) and loaded onto the ABI 3100 capillary sequencing platform (Life Technologies) at the New Mexico State University (NMSU) Molecular Core Facilities.

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of samples</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>13</td>
<td>266</td>
</tr>
<tr>
<td>Rio Grande River</td>
<td>5</td>
<td>126</td>
</tr>
<tr>
<td>Dripping Springs</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Gila River</td>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td>Colorado River</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>Ruidoso River</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>San Juan River</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Soledad Canyon</td>
<td>1</td>
<td>22</td>
</tr>
</tbody>
</table>

**Table 1.** Number of leaf-bait assays performed at each location and the total number of oomycete isolates recovered on V8S agar.
using the HKY genetic distance model, bootstrapped 1,000 times with a support threshold of 70%.

**Analysis of hybrid *Phytophthora* isolates.** Isolates with mixed ITS sequence chromatograms from direct sequencing were reisolated as single-zoospore colonies. Cottonwood leaf discs (2 cm in diameter) were placed in a 24-well tissue culture plate, inoculated with mycelium plugs, and then incubated for 2 days at 28°C. One milliliter of sterile water was added to each well, and the plate was incubated under fluorescent light for 2 days at 25°C. Aliquots of 10 μL, 50 μL, and 100 μL were plated onto V8S plates and incubated 1 day at 28°C. Single zoospore colonies were identified under a dissecting microscope and transferred to V8S plates, which were incubated at 28°C for 5 days. As previously described, hyphae were collected, lysed, and used as the template for amplification with primer sets ITS4 and ITS6. Amplicons from three isolates (7, 19, and 28) were ligated into the pGEM-T Easy vector (Promega, Madison, WI) and transformed into Top10 competent cells (Life Technologies), according to each manufacturer’s instructions, and 100 μL from each transformation was plated onto LB agar containing ampicillin sodium salt (100 mg/ml), isopropyl-β-D-thiogalactopyranoside (IPTG) (0.1 mM), and X-gal (1 mg/liter) (Life Technologies) and incubated at 37°C for 1 day. Twelve white colonies derived from each isolate were picked into 100 μL sterile water in 1.5-ml microcentrifuge tubes and vortexed for 30 s, and 1 μL was used as the template for amplification with primer sets ITS4 and ITS6 as described previously. Amplicons were sequenced with each primer as described above. A global sequence alignment was constructed with representative clonal sequences and 10 GenBank sequences representing four *Phytophthora* species, and an unrooted neighbor-joining tree was constructed as above.

**Characterization of sporangial morphology and colony pattern of hybrid isolates.** To characterize the colony pattern, selected hybrid isolates were grown on potato dextrose agar (PDA) (Becton, Dickinson, and Company, Franklin Lakes, NJ) at 28°C, and the colony morphology was imaged on the sixth day with a Nikon digital camera. To induce sporangial formation, 1-cm mycelium plugs from colonies growing on V8S medium were placed on surface-sterilized cottonwood leaves and incubated in a humidity chamber at 28°C for 3 days. The mycelium plugs were removed, and necrotic leaf tissue was extracted using a 2-cm cork borer, placed in a 24-well plate with 1 ml of sterile DI water in each well, and incubated for 2 days at 25°C under fluorescent light. Structures produced by each isolate on the necrotic leaf tissue were imaged with a digital camera mounted on a compound microscope using an eyepiece adapter (Nikon, Melville, NY).

**Pathogenicity assays.** *P. lacusnris* and *P. riparia* were evaluated for pathogenicity on selected crop species: pumpkin (*Cucurbita pepo*), corn (*Zea mays*), alfalfa (*Medicago sativa*), chile pepper (*Capsicum annuum*), onion (*Allium cepa*), oats (*Avena sativa*), and barley (*Hordeum vulgare*). *P. capsici*, a known pathogen of chile and pumpkin, was also evaluated as a positive control. A single isolate from each species was grown on V8S plates for 7 days. Five plates from each species were blended with 500 ml sterile DI water, and hyphal fragments were quantified using a hemocytometer (Hauser Scientific, Horsham, PA) and adjusted to a final concentration of 10^6 hyphal fragments per ml. Crop species were grown in a greenhouse in plastic flats filled with autoclaved soilless mix (Metro-Mix 360; Sun Gro Horticulture Canada Ltd., Agawam, MA) and fertilized once with 0.5× Miracle-Gro (Scotts Company LLC, Marysville, OH). When plants reached 1 to 3 true leaf stage, flats were sprayed with blended inoculum until plants and soil were uniformly coated and placed in a humidity chamber at 28°C. After 72 h, flats were returned to the greenhouse and watered daily for 2 weeks. Plants were evaluated every 3 days for signs and symptoms of disease development. A more stringent pathogenicity test was also used to evaluate root rot disease caused by *P. riparia*. Crop species were grown as described previously and removed from soil, and root tips were trimmed with a scalpel to cause minor root damage. Root systems were dipped in a *P. riparia* solution (10^6 hyphal fragments per ml) or sterile water and repotted into 12-cm pots with autoclaved soilless mix. Plants were watered daily for 6 weeks and monitored for disease symptoms.

**Induced resistance assays.** Chile pepper plants were grown as described previously. Inoculum of *P. riparia* was prepared by first being grown in V8S broth and then being strained through cheesecloth, resuspended in sterile DI water, and blended for approximately 15 s. Each chile plant was inoculated with 500,000 hyphal fragments, kept saturated for 72 h, and then transplanted into 10-cm pots. Each pot was immediately inoculated with 3 plugs (1 cm in diameter) of *P. capsici* grown on V8S agar and placed directly against the stem at the soil line (16). Pots were placed on trays with approximately 2 cm of standing water to maintain saturated soil conditions for the duration of the experiment. Lengths of lesions developing on the stem of each plant were measured at multiple time points, starting from the soil line. For each treatment, 10 plants were inoculated with *P. capsici*, and the experiment was repeated once, yielding two runs for analysis.

**Accession number(s).** Representative ITS sequences for *Phytophthora* species isolated from all locations sampled in this study are available through the GenBank nucleotide collection under accession numbers KF750571 to KF750590 (Table 2).

### RESULTS

**Phytophthora species and prevalence.** A total of 266 isolates were recovered on selective media from seven rivers and streams in New Mexico and Utah (Fig. 1; Table 1). Whole-cell PCR amplification of ITS DNA and direct sequencing of amplicons conclusively identified 59 isolates as *Phytophthora* species. The BLAST search identified 99 to 100% identical matches in GenBank for all isolates. To avoid potentially incorrectly labeled environmental samples, sequences in the GenBank nucleotide collection from voucher specimens or species characterization publications were selected for placement of generated sequences into operational taxonomic units for final species identification (Table 2; Fig. 2). All verified *Phytophthora* species sequences grouped into well-supported monophyletic clades (>90% bootstrap support). In clade 9, all species grouped into monophyletic clades with the exception of a single *P. hydropathica* isolate from Spain. Of the isolates identified as *Phytophthora* spp. through ITS sequencing, 55 out of 59 belonged to ITS clade 6, with 28 *P. lacusnris* isolates, 18 *P. riparia* isolates, and 3 *P. gonapodydes* isolates. A single *P. cinnamomi* isolate was recovered from the Colorado River. Two isolates recovered from the Rio Grande River and a single isolate from the Gila River grouped with an uncharacterized *Phytophthora* species in clade 9, previously reported as unk3 during a survey of irrigation canals in the eastern United States (7). Samples of water collected from the Rio Grande River during three consecutive weeks yielded an average of 1.7 (±1.2) *Phytophthora* CFU per liter of water.

**Identification of hybrids of *P. riparia* × *P. lacusnris.*** High-quality sequence chromatograms were generated from the 59 isolates described above with identical sequence chromatograms for forward and reverse amplification primers. Six isolates recovered from the Rio Grande River generated low-quality chromatograms that did not align between the forward and reverse primers (Fig. 3A). The signal strengths for forward and reverse sequencing reactions dropped at the same nucleotide position (red star on Fig. 3A). There is a single nucleotide insertion/deletion event (indel) between *P. riparia* and *P. lacusnris* at this position. Close inspection of the chromatograms for low-quality regions identified discrete overlapping signal peaks (Fig. 3B). Identical sequence chromatograms were obtained from single zoospore colonies reisolated from each isolate (data not shown). To investigate the nature of

---

Stamler et al.
Hybrid isolates were morphologically indistinguishable from *P. riparia* and *P. lacustris*, with angular, petaloid colony patterns on PDA plates (Fig. 4A). The colony morphology on V8S was also petaloid with abundant aerial hyphae (not shown). Chlamydospores and hyphal swelling were absent on solid media and on cottonwood leaves. Gametangia were absent on single culture plates and on direct pairings between isolates. Sporangia were sporadically produced on cottonwood leaves when incubated with hybrid isolates and submerged in sterile water for 48 h. Consistent with *P. riparia* and *P. lacustris*, sporangia were not caducous, were angular, petaloid colony patterns (Fig. 3C).

### Sporangial morphology and colony pattern of hybrid isolates.

Hybrid isolates were morphologically indistinguishable from *P. riparia* and *P. lacustris*, with angular, petaloid colony patterns on PDA plates (Fig. 4A). The colony morphology on V8S was also petaloid with abundant aerial hyphae (not shown). Chlamydospores and hyphal swelling were absent on solid media and on cottonwood leaves. Gametangia were absent on single culture plates and on direct pairings between isolates. Sporangia were sporadically produced on cottonwood leaves when incubated with hybrid isolates and submerged in sterile water for 48 h. Consistent with *P. riparia* and *P. lacustris*, sporangia were not caducous, were ovoid to obpyriform in shape, were nonpapillate, and often displayed internal proliferation of sporangiophores (Fig. 4B).

### Pathogenicity assays on crop species.

Pumpkin, corn, alfalfa, chile pepper, onion, oats, and barley were foliar, and soil was inoculated with hyphae suspensions of *P. riparia*, *P. lacustris*, and *P. capsici*. No disease symptoms were observed on any of the crop species tested with *P. riparia* or *P. lacustris* (Fig. 5). All chile pepper and pumpkin plants inoculated in the same fashion with *P. capsici* were dead within 1 week (data not shown). A more stringent assay, in which crop plants were first removed from the soil and roots were wounded and dipped directly into a *P. riparia* hyphae suspension, also failed to produce any disease symptoms in the crop species evaluated. Conversely, under the conditions of the same stringent assay, inoculation of chile pepper and pumpkin with *P. capsici* resulted in the death of all of the plants (data not shown). In chile pepper plants inoculated with *P. riparia* hyphae suspension and subsequently challenged with *P. capsici*, symptom development was delayed compared to that in untreated plants, and significant differences in lesion lengths between treatments were observed up to 24 days after inoculation with *P. capsici* (Fig. 6).

### DISCUSSION

*Phytophthora* species are classically considered to be primary plant pathogens with little to no role as saprophytes or opportunistic pathogens. However, this assumption is being challenged by the prevalence of certain *Phytophthora* species in watersheds and riparian habitats and the absence of definitive hosts. *Phytophthora* species were readily isolated by leaf baiting from seven rivers and streams examined during the summer and fall of 2012.

#### TABLE 2 Representative *Phytophthora* isolates recovered from each location

<table>
<thead>
<tr>
<th>Location</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Isolate</th>
<th>Species</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA: Colorado River, Moab, UT</td>
<td>38.33 N</td>
<td>109.59 W</td>
<td>242</td>
<td><em>P. cinamomoni</em></td>
<td>KF750569</td>
</tr>
<tr>
<td>USA: Colorado River, Moab, UT</td>
<td>38.33 N</td>
<td>109.59 W</td>
<td>277</td>
<td><em>P. riparia</em></td>
<td>KF750589</td>
</tr>
<tr>
<td>USA: Dripping Springs, Las Cruces, NM</td>
<td>32.32 N</td>
<td>106.57 W</td>
<td>245</td>
<td><em>P. lacustris</em></td>
<td>KF750579</td>
</tr>
<tr>
<td>USA: Gila River, Cliff, NM</td>
<td>33.01 N</td>
<td>108.54 W</td>
<td>118</td>
<td>*Phytophthora sp. unK3</td>
<td>KF750570</td>
</tr>
<tr>
<td>USA: Gila River, Cliff, NM</td>
<td>33.01 N</td>
<td>108.54 W</td>
<td>198</td>
<td><em>P. gonapodyides</em></td>
<td>KF750574</td>
</tr>
<tr>
<td>USA: Gila River, Cliff, NM</td>
<td>33.01 N</td>
<td>108.54 W</td>
<td>126</td>
<td><em>P. lacustris</em></td>
<td>KF750577</td>
</tr>
<tr>
<td>USA: Gila River, Cliff, NM</td>
<td>33.01 N</td>
<td>108.54 W</td>
<td>116</td>
<td><em>P. riparia</em></td>
<td>KF750588</td>
</tr>
<tr>
<td>USA: Rio Grande River, Las Cruces, NM</td>
<td>32.26 N</td>
<td>106.82 W</td>
<td>155</td>
<td>*Phytophthora sp. unK3</td>
<td>KF750571</td>
</tr>
<tr>
<td>USA: Rio Grande River, Las Cruces, NM</td>
<td>32.26 N</td>
<td>106.82 W</td>
<td>11</td>
<td><em>P. gonapodyides</em></td>
<td>KF750572</td>
</tr>
<tr>
<td>USA: Rio Grande River, Las Cruces, NM</td>
<td>32.26 N</td>
<td>106.82 W</td>
<td>217</td>
<td><em>P. lacustris</em></td>
<td>KF750578</td>
</tr>
<tr>
<td>USA: Rio Grande River, Las Cruces, NM</td>
<td>32.26 N</td>
<td>106.82 W</td>
<td>19A</td>
<td><em>P. lacustris × riparia</em></td>
<td>KF750581</td>
</tr>
<tr>
<td>USA: Rio Grande River, Las Cruces, NM</td>
<td>32.26 N</td>
<td>106.82 W</td>
<td>19B</td>
<td><em>P. lacustris × riparia</em></td>
<td>KF750586</td>
</tr>
<tr>
<td>USA: Rio Grande River, Las Cruces, NM</td>
<td>32.26 N</td>
<td>106.82 W</td>
<td>32</td>
<td><em>P. riparia</em></td>
<td>KF750587</td>
</tr>
<tr>
<td>USA: Rio Grande River, Las Cruces, NM</td>
<td>32.26 N</td>
<td>106.82 W</td>
<td>94</td>
<td><em>P. gonapodyides</em></td>
<td>KF750573</td>
</tr>
<tr>
<td>USA: Rio Grande River, Las Cruces, NM</td>
<td>32.26 N</td>
<td>106.82 W</td>
<td>93</td>
<td><em>P. lacustris</em></td>
<td>KF750576</td>
</tr>
<tr>
<td>USA: Colorado River, Moab, UT</td>
<td>38.33 N</td>
<td>109.59 W</td>
<td>11</td>
<td><em>P. lacustris</em></td>
<td>KF750580</td>
</tr>
<tr>
<td>USA: Soledad Canyon, Las Cruces, NM</td>
<td>32.31 N</td>
<td>106.57 W</td>
<td>68</td>
<td><em>P. lacustris</em></td>
<td>KF750575</td>
</tr>
</tbody>
</table>

FIG 1 Map of major rivers sampled for *Phytophthora* species during summer and fall 2012. Three additional sites were sampled in minor rivers and streams in southern New Mexico. Rivers are highlighted in blue and the Continental Divide is shown as a red line. Sampling locations for the Rio Grande River, Gila River, Colorado River, and San Juan River are indicated by red stars.

FIG 3C Phylogenetic analysis with GenBank primer sequences from all three isolates separated high-quality sequence chromatograms from both amplification primers. The consensus sequences from all three isolates were sequenced. Twenty-nine out of 36 amplicons generated colonies with interrupted hybrid isolates were T/A cloned, and 12 of these mixed ITS chromatograms, amplicons from three isolates derived from single zoospore colonies were T/A cloned, and 12 colonies with interrupted β-galactosidase activity from each isolate were sequenced. Twenty-nine out of 36 amplicons generated high-quality sequence chromatograms from both amplification primers. The consensus sequences from all three isolates separated into two distinct clades. Phylogenetic analysis with GenBank sequences grouped representative sequences from each isolate with *P. riparia* and *P. lacustris* (Fig. 3C).
previous studies utilized *Rhododendron* spp. for leaf bait assays, these species do not readily grow in the desert southwest, and thus *Salix* and *Populus* species were chosen for leaf bait due to their widespread distribution in southwestern riparian ecosystems. The predominant species identified were *P. lacustris* and *P. riparia*; however, given the limited number of sample points, this examination likely underestimates the diversity of *Phytophthora* species in the rivers and streams evaluated. Also, more sample time points would be needed to properly evaluate seasonal fluctuations in population densities. In contrast to rivers and streams in Oregon and Alaska, *P. gonapodyides* was infrequently recovered in the rivers and streams examined in this study (6). The lower optimal growth temperature of *P. gonapodyides* may limit its presence in the warmer rivers of the southwestern United States. *Phytophthora* species were found in pristine riparian habitats such as the Gila Wilderness, with no sign of plant or tree decline. Consistent with the hypothesized role of *Phytophthora* ITS clade 6 species as opportunistic or minor foliar pathogens, *P. riparia* was isolated from cottonwood leaves that were submerged in the Gila River but still attached to a growing tree. Small lesions were visible on submerged leaves, but no disease symptoms were observed on the remainder of the tree. Pathogenicity assays are needed to determine if *P. riparia* is a pathogen of cottonwood roots. However, it seems unlikely that *P. riparia* represents a threat to native ecosystems given its broad distribution and the lack of vegetative decline in the sampled ecosystems. *P. lacustris* isolates recovered from two proximal but geographically separated mountain streams (Dripping Springs and Soledad Canyon in Table 2) each differ by two
nucleotide substitution sites from the consensus sequence for *P. lacustris*, while *P. lacustris* and *P. riparia* are distinguished by six nucleotide substitution sites and a single insertion/deletion event at the ITS locus. These divergent sequences appear to represent initial speciation in these island populations. The identification of clade 6 *Phytophthora* spp. in isolated streams supports the hypothesis that these species are indigenous members of riparian habitats and likely act as first-level saprophytes rather than destructive pathogens. Six hybrid *P. riparia* × *P. lacustris* isolates were identified in the Rio Grande River based on ITS sequence analysis; however, the prevalence of hybrid isolates may be significantly underestimated by this study as only a single locus was evaluated. It appears that multiple distinct hybridization events have occurred as the previously characterized *P. riparia* × *P. lacustris* hybrid isolate had mixed chromatograms at the β-tubulin locus but a single sequence type for the ITS locus (9). Even though gamete formation is rarely seen in culture, natural hybrid isolates in clade 6 appear to be common when closely related species coexist in natural habitats. As previously reported, the hybrids isolated in this study were difficult to maintain in culture and became nonviable after repeated subculturing; however, specific isolates of *P. riparia* and *P. lacustris* have remained viable over repeated subculturing. Next-generation sequencing of eDNA will surely identify a greater diversity of *Phytophthora* spp. within riparian habitats; however, information such as interspecific hybridization will be lost through this approach.

Based on our recent findings of nonhost *P. nicotianae*-induced systemic resistance in *C. annuum*, the *Phytophthora* spp. identified...
in this study were evaluated for their potential as biological amendments in commercial cropping systems. *P. lacustris* and *P. riparia* did not cause disease symptoms on crop species commonly grown in the southwestern United States, including pumpkin (*Cucurbita* spp.), corn (*Zea mays*), alfalfa (*Medicago sativa*), chile pepper (*Capsicum annuum*), onion (*Allium cepa*), oats (*Avena sativa*), and barley (*Hordeum vulgare*). In this study, *P. riparia* successfully primed *C. annuum* defense responses as was observed by the delay in lesion development upon infection by *P. capsici*. While all plants inoculated with *P. capsici* in this study did eventually develop root rot and stem lesions, the quantitative delay in symptom development is consistent with that previously described during priming with nonhost *P. nicotianae* or β-aminobutyric acid (BABA) (30). In previous work, BABA was shown to induce resistance in plants based on abscisic acid-dependent priming for callose deposition during necrotroph infection (34). More research is needed to elucidate the molecular mechanism behind nonhost *Phytophthora* species-induced resistance; however, conserved pathogen-associated molecular patterns (PAMPs) present on all *Phytophthora* spp. can be hypothesized to elicit localized defense responses leading to systemic acquired resistance based on mobile plant signaling molecules. The *Phytophthora* spe-
Phytophthora spp. in Rivers of the Southwest

Phytophthora capsici root rot in Capsicum annuum

FIG 6 Development of stem lesions on Capsicum annuum plants inoculated with hyphal suspensions of Phytophthora riparia and subsequently challenged with P. capsici. The lesion length for each plant was measured at multiple time points starting at the soil line, and bars represent the standard errors of the mean for each treatment (n = 10).

ACKNOWLEDGMENTS

We acknowledge Lucio Sanchez, Miranda Butler-Valverde, and Esteban Molina for their technical assistance with this study.

Support was provided by USDA special grant 2010-34186-20979, the NM Chile Improvement Project (CHIP), and the New Mexico Agriculture Experiment Station.

ACKNOWLEDGMENTS

We acknowledge Lucio Sanchez, Miranda Butler-Valverde, and Esteban Molina for their technical assistance with this study.

Support was provided by USDA special grant 2010-34186-20979, the NM Chile Improvement Project (CHIP), and the New Mexico Agriculture Experiment Station.

REFERENCES


August 2016 Volume 82 Number 15 Applied and Environmental Microbiology aem.asm.org 4703

Downloaded from http://aem.asm.org/ by guest on August 29, 2017


