Further Identification of *Pythium* spp. Causing Damage to *Poa annua* L. Golf Course Greens in the Pacific Northwest

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INTRODUCTION

Pythium is frequently described as a plant pathogen, but is not limited by its host (1). In plant hosts, *Pythium* parasitism can be observed in seeds and seedlings, referred to as pre– and post-emergent damping off, respectively. Reduced growth, vigor, and even death, result as a consequence of this interaction (2). Mature plants and roots are also susceptible to *Pythium* spp., and while necrosis is less probable, host variability does influence the potential for significant loss.

In turfgrass systems, *Pythium* spp. have been described as causal agents of foliar blights, crown and root rots, and more recently, root dysfunctions (3,4). Several different species have been included in pathogenic assays. These studies support the diversity of the genus and correlate turfgrass decline to various species. This variability, with respect to causal agent, is confounded by an equally diverse temperature spectrum. Studies have indicated pathogenic activity in hot, humid regions while others appear to be problematic during wet, cool periods (5).

While it is true that *Pythium* has a history of activity in the Pacific Northwest, submissions to the WSU—Plant and Insect Diagnostic Clinic spiked during the winter of 2010 necessitating further investigation (**Fig. 1**.). The objective of this study is to provide information on *Pythium* spp. causing damage on golf course greens in the Pacific Northwest.

MATERIALS & METHODS

64 samples were collected from 25 golf courses throughout 2010 and into 2011 (**Fig. 2**.).

Species Identification

Symptomatic plant tissue was disinfected in a 10% bleach solution. The material was rinsed in deionized water and cultured onto selective CARP media. Five pieces of leaf tissue were placed onto each plate and incubated at 19º C. After 7 days, isolates were transferred to V8 media and observed for characteristic *Pythium* morphology. All isolates resembling *Pythium* spp. were analyzed by PCR amplification and sequence of the ITS rDNA region. The resulting sequences were blasted to the GenBank database, aligned using CLUSTALW2 (6), and compared to known *Pythium* isolates using MEGA5 (7).

Growth Rates

30 isolates representing 9 golf courses, and the two dominant species, were selected for a temperature growth study. A 6.5 mm agar plug was removed from the edge of a seven-day old purified V8 colony for each of the 30 isolates and centered on a 15mm x 100mm Petri dish filled with V8 media. The diameter of the plug was marked on opposing sides and then in the same fashion perpendicular to the original marking. Growth was measured in the same manner, with a digital caliper, and collected at 24, 48 and 72 hours after initial incubation. Chambers were maintained at 7, 12, 17, 22 and 25C. Two trials using three replications per isolate were completed. Data were analyzed using R (version 2.15.1). Trials were combined after testing for homogeneity of variances using the Fligner-Killeen method. Average daily growth was analyzed as millimeters per 24 hour period (mm d^{-1}) utilizing data from the 48 hour mark.

RESULTS

- From 64 samples, two species of *Pythium* are dominant: *Pythium vanterpoolii* & *Pythium torulosum.*
- **Fig. 5.** displays the proposed relationships between these isolates and known *Pythium* sequences.
- Growth rates are currently being analyzed, but initial results provide some possible distinctions between species (**Fig. 3** and **Fig. 4.**).

CONCLUSIONS

This study provides baseline information for pathogenically-active *Pythium* spp. in the Pacific Northwest. Until this time, species associated with turfgrass decline in this region were unknown. Further analysis of temperature growth data will indicate differences between species and possible differences between isolates—if those exist. Future growth chamber pathogenicity tests on turf are planned.

References:

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8) Tamura K. and Nei M. (1993) Estimation of the Number of Nucleotide Substitutions in the Control Region of Mitochondrial DNA in Humans and Chimpanzees. *Molecular Biology and Evolution* 10:512-526.

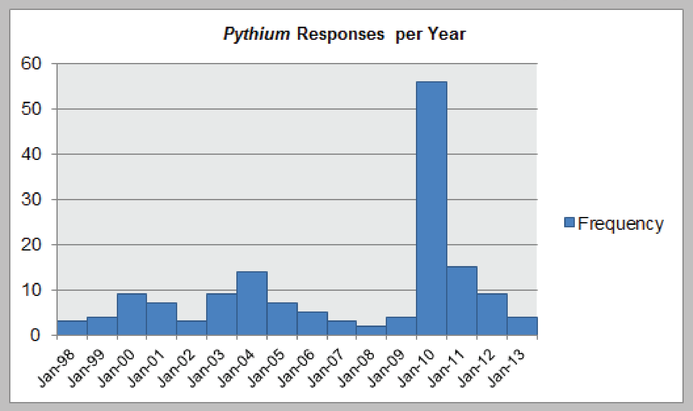

FIGURE 1. Total number of responses, by year (1998-current), of *Pythium* submissions collected at the WSU-Plant and Insect Diagnostic Clinic.

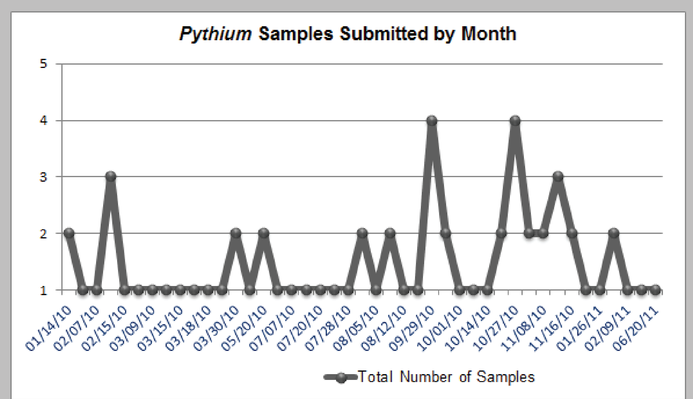

FIGURE 2. Total number of *Pythium* samples submitted to the WSU-Plant and Insect Diagnostic clinic per month during the period January 1st, 2010 through June 20th, 2011 (samples used for this study).

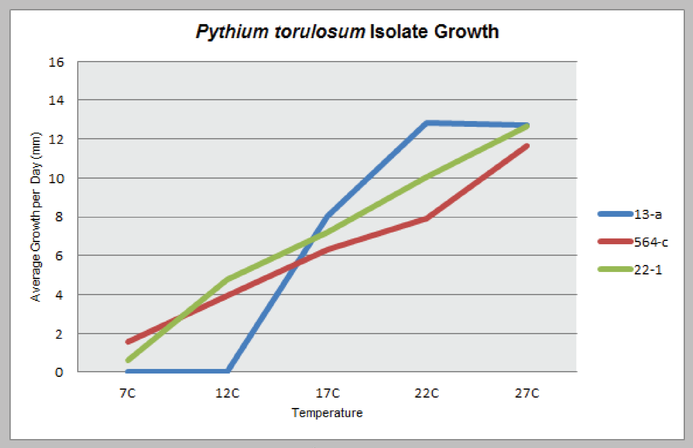

FIGURE 3. Mean growth (mm d^{-1}) of three *Pythium torulosum* isolates at five temperatures.

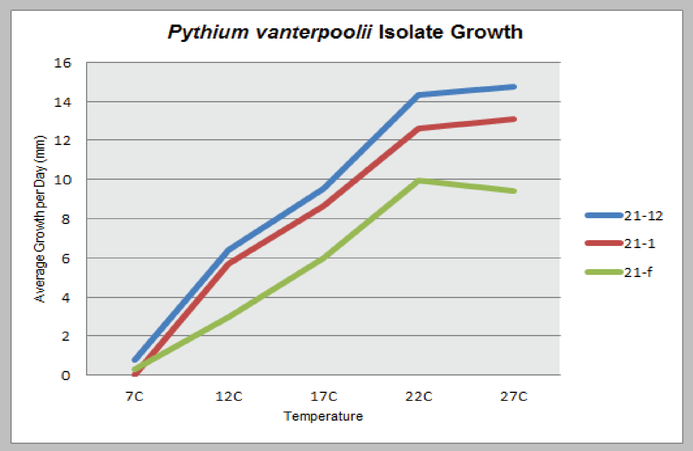

FIGURE 4. Mean growth (mm d^{-1}) of three *Pythium vanterpoolii* isolates at five temperatures.

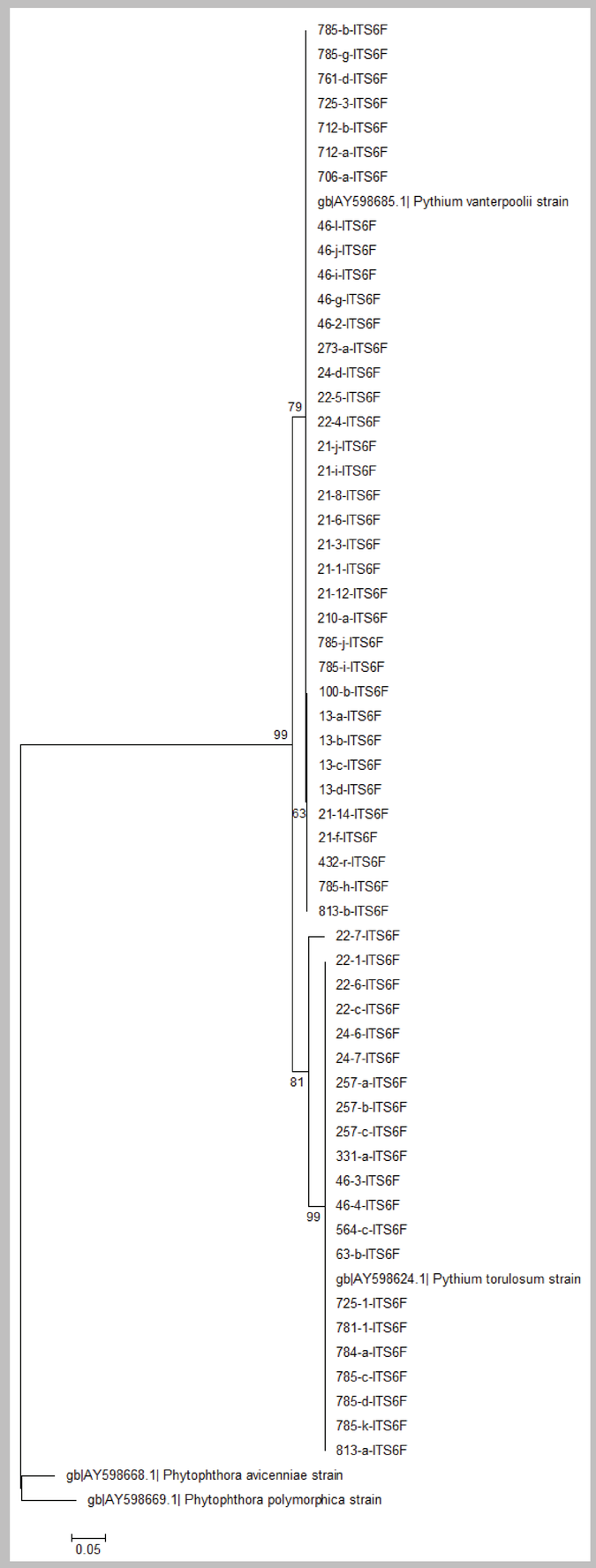

FIGURE 5. Molecular phylogeny of *Pythium* isolates inferred by using the Maximum Likelihood method based on the Tamura-Nei model (8).The tree with the highest log likelihood (-2104.4521) is shown. All positions containing gaps and missing data were eliminated. There were a total of 627 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (7).

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