General Discussion

Clearly, recycled irrigation water at the nursery investigated was infested with *Pythium* and *Phytophthora* spp. The common occurrence of *Pythium* spp. in the water warrants additional investigation into the identification of species in the culture collection and their importance as plant pathogens. A relatively large diversity of *Phytophthora* spp. was identified, but recovery of some species was quite limited. Specifically, in the two-year study, *P. cactorum , P. capsici, P. citricola,* and *P. nicotianae* were recovered only 1 to 4 times and only between the months of greatest nursery activity from May through September. Therefore, it may be that these species are transient inhabitants of the nursery, introduced by new crops, which are unable to survive and reproduce within the irrigation system. Or these species may not be easily recovered by the assay techniques employed in this work and may only be recovered when populations peak to threshold recovery levels.

Although *P. citrophthora* was recovered from baits and non-chlorinated irrigation water, it was primarily recovered in nursery effluent, which indicates either that propagules of this species are adapted to the relatively harsh conditions (i.e. rapid temperature fluctuations, ultraviolet light, and high salts) in effluent water or that populations in container plants are relatively high. The limited recovery of this species from other locations may indicate that this species is not as well adapted as *P. cryptogea* and *P. drechsleri* to a recycled irrigation system and/or that rhododendron leaf baits are not selective for this species. However, *P. citrophthora* was recovered over a relatively long period of the year (May through September), indicating at least a seasonal residence in the nursery.

P. *drechsleri* and *P. cryptogea* were the most frequently recovered species and their recovery occurred over the longest period, from early spring through fall, which suggests that these species are well adapted to the conditions and able to reproduce within the recycled irrigation system. However, recovery of these two species was primarily through rhododendron baits and recovery from effluent was extremely limited or lacking. *P. drechsleri* and *P. cryptogea* may have greater saprophytic than pathogenic abilities. This hypothesis warrants further investigation. Additionally, river water should be assayed to determine if these species are introduced into the nursery through that source.

Results of the investigation of watering with chlorinated or non-chlorinated water indicate that disinfestation of irrigation water is warranted; however, the inability to isolate *Phytophthora* or *Pythium* spp. from roots in the non-chlorinated treatment indicate that diseases other than Phytophthora or Pythium root rot are responsible. However, this does not discount the risk associated with any levels of these organisms in irrigation water, since they are associated with multicyclic disease and epidemics in favorable environments. Additionally, the majority of *Phytophthora* spp. tested for pathogenicity was found to cause significant mortality and, in some cases, asymptomatic plants harbored inoculum over relatively long periods of time. A thorough investigation should be designed to determine the cause of the deleterious effects observed when plants were irrigated with non-disinfested recycled irrigation water. This may lead to new avenues of research into waterborne plant pathogens.

Another risk associated with recycling irrigation water, which has not been investigated, is a potential for development of resistance to mefenoxam, which is used both for treatment of plants in disease outbreaks and as a prophylactic by the nursery and greenhouse industry. There is a high potential for development of resistance to mefenoxam by *Phytophthora* and *Pythium*

spp. with continued use of this fungicide and reuse of effluent water creates a situation in which these organisms are continuously exposed to mefenoxam, albeit in small doses. At the nursery where this investigation was performed, water in the irrigation reservoir was tested four times for mefenoxam and between 0.01 to 0.15 ppm mefenoxam were found in the water in three of the four tests. Therefore, investigations into development of resistance to mefenoxam by the Pythiaceae and concentrations of mefenoxam in recycled irrigation water could be critical to preservation of mefenoxam's activity against these plant pathogens.

Traditional assay methods employed in this work have limitations and certainly skew results in a negative manner. These methods are most likely preferentially selective for certain species and inhibitory to others. They are also very time consuming, labor intensive, and dependent on morphological features, which are subject to interpretation and environmental factors. However, use of these methods was necessary for conducting a preliminary survey of populations of organisms in water. Due to the limitations of hymexazol-amended media in recovery, improvements in these methods, specifically modifications of media, may lead to more accurate recovery levels in water assays. Development of reliable, rapid, and unbiased detection methods with practical application for the nursery and greenhouse industry is warranted. Further determination of the importance of species recovered from assays in Virginia, in terms of their frequency of association with recycled irrigation water and pathogenicity will help to decide which species should be prioritized for development of detection tests.