Chapter 3.

Pathogenicity to *Salvia officinalis* and *Gerbera jamesonii* of *Phytophthora* Species Recovered from Recycled Irrigation Water

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ABSTRACT

Members of the genus *Phytophthora* are generally considered pathogenic on plants and individual species of *Phytophthora* may be parasitic on quite narrow or very broad ranges of host species. However, research on the pathogenicity of *Phytophthora* species that have been recovered from recycled irrigation water to ornamental crops is lacking. Yet *Phytophthora* species are serious plant pathogens, capable of inciting disease epidemics in nursery production. *P. cactorum, P. capsici*, *P. citrophthora, P. cryptogea*, *P. drechsleri*, and *P. nicotianae,* recovered from recycled irrigation water at a container nursery in Virginia in 2000, were tested for pathogenicity to *Salvia officinalis* and *Gerbera jamesonii* in the greenhouse. Test plants were inoculated with three concentrations of zoospore suspension (10,000; 5,000; 2,500 zoospores/plant). *P. cactorum, P. capsici, P. citrophthora,* and *P. nicotianae* caused significant mortality of *S. officinalis*, while *P. drechsleri* and *P. cryptogea* caused insignificant or no disease, respectively. Only *P. cactorum* showed limited pathogenicity on *G. jamesonii*. Many sage plants, while exhibiting no aboveground symptoms, harbored inoculum long after *Phytophthora*-inoculation. Fresh root and shoot weight analyses of asymptomatic plants remaining at termination of pathogenicity experiments demonstrated that *Phytophthora* inoculation may either reduce or stimulate plant shoot growth, but little effect is apparent on roots. This is the first report on the pathogenicity of *P. cactorum, P. capsici,* and *P. citrophthora* to *S. officinalis* and *P. cactorum* to *G. jamesonii.* The results of this research indicate that

disinfestation of recycled irrigation water in nursery production is warranted if any of these species are detected.

INTRODUCTION

A variety of species of *Phytophthora* de Bary (Ali-Shtayeh and MacDonald, 1991; Bewley and Buddin, 1921; Lauderdale and Jones; 1997; Klotz et al., 1959; MacDonald et al., 1994; McIntosh, 1966; Oudemans, 1999; Pittis and Colhoun, 1984; Taylor, 1977; Thomson and Allen, 1974; von Broembsen, 1984; Whiteside and Oswalt, 1973; Wilson et al., 1998) have been recovered from recycled irrigation water. Members of the genus *Phytophthora* are generally considered pathogenic on plants and individual species of *Phytophthora* may be parasitic on quite narrow or very broad ranges of host species (Erwin and Ribeiro, 1996); however, reports of pathogenicity tests for species recovered from irrigation or effluent water are limited. Examples include pathogenicity tests of *P. cryptogea* Pethybridge and Lafferty, *P. megasperma* Drechsler, and *Phytophthora* sp. to *Prunus persica* L. [Batsch.] (Taylor, 1977); *P. citrophthora* (R. E. Smith and E. H. Smith) Leonian, *P. parasitica* Dastur (synonym, *P. nicotianae* Breda de Haan), and *P.* spp. to *Citrus jambhiri* Lush. (Thomson and Allen, 1974); *P. cactorum* (Lebert and Cohn) J. Schröeter, *P. cambivora* (Petri) Buisman, and *P. citricola* Sawada to *Malus* sp. Mill. (McIntosh, 1966); and *Phytophthora* spp. to cabbage, cotton, pea, and tomato (Shokes and McCarter, 1979). No pathogenicity tests of individual *Phytophthora* spp. have been performed on ornamental crops with two exceptions: *P. gonapodyides* (Petersen) Buisman was tested on *Antirrhinum majus* L., and *P. cinnamomi* Rands and *P. gonapodyides* on *Chamaecyparis lawsoniana* [Andr. Murray] Parl. (Pittis and Colhoun, 1984).

In monthly baiting and filtering water assays conducted at a container nursery in southwestern Virginia during 2000, *P. cactorum*, *P. capsici* Leonian*, P. citrophthora, P. cryptogea*, *P. drechsleri* Tucker, and *P. nicotianae* were recovered from an irrigation water recycling system. Pathogenicity tests with these species were performed to identify the species of most concern to plant pathologists and horticulturists involved in ornamental crop production in Virginia.

The range of crop species grown in the ornamental nursery industry is vast and pathogenicity tests were restricted to selected species due to limited human and financial resources. *Salvia officinalis* L. (sage) and *Gerbera jamesonii* H. Bolus ex J. D. Hook (gerbera) were used in these greenhouse tests. Sage was selected as a candidate for testing after the causal agent of a disease epidemic on this host was identified as *P. citrophthora* at the nursery where irrigation water assays were performed. Additionally, preliminary tests with sage demonstrated susceptibility to several *Phytophthora* spp. recovered from irrigation water sampling. Gerbera was selected due to its general susceptibility to *P. cryptogea*, which was recovered frequently from irrigation water in this study and previous water assays (Ali-Shtayeh and MacDonald, 1991; Bewley and Buddin, 1921; Lauderdale and Jones, 1997; MacDonald et al., 1994; Taylor, 1977; Wilson et al., 1998). Jee et al. (1996) reported pathogenicity of *P. cryptogea* to seven cultivars of *G. jamesonii*. Farr et al. also (1995) reported *P. cryptogea* as the cause of crown and root rot of *G. jamesonii.*

The specific objectives of this work were to assess the pathogenicity and virulence of selected isolates of *Phytophthora* spp. recovered from recycled irrigation water and to determine if inoculated isolates could be re-isolated from roots of plants exhibiting no aboveground symptoms at the termination of pathogenicity tests. Three zoospore concentrations were used in

pathogenicity tests to determine effects of these inoculum levels on mortality and plant growth. Plant pathogens spread through a recycled irrigation system must reach a susceptible crop, produce adequate inoculum levels, and encounter favorable environmental conditions for significant disease outbreaks to occur. However, what effects water-borne plant pathogens may have on plants when high rates of mortality do not result also merits investigation. To this end analyses of the fresh weight of roots and shoots of asymptomatic plants remaining at the termination of pathogenicity tests were performed to determine the effects of *Phytophthora* spp. in cases where plant mortality was not significant.

MATERIALS AND METHODS

Greenhouse conditions. Pathogenicity tests of selected *Phytophthora* isolates were performed on both sage and gerbera in a greenhouse. Temperature was maintained by the use of a heater and/or air-conditioner. Temperature and humidity were monitored with a hygrothermograph throughout the duration of the experiments (Appendix 4, Table A4.1). Lighting was natural and no supplemental lighting was provided during these tests, unless otherwise noted. Tests with *P. cactorum, P. capsici, P. citrophthora* (isolate #s: 67 and 288), *P. cryptogea, P. drechsleri* (isolate #: 179), and *P. nicotianae* were conducted during the months of May through August 2001. The first test with other isolates of *P. citrophthora* and *P. drechsleri* was conducted during September and October 2001; the second test was conducted during February and March 2002 and 1000 Watts of supplemental lighting was provided 12 h/day during this test.

Plants. Plants were started from seed (Garth Seeds and Plants, Inc., Chesterland, Ohio) in the greenhouse in 3.8 cm wide x 21.0 cm long Cone-tainers™ (Stuewe & Sons, Inc., Corvallis, Oregon) containing a composted pine bark growing medium, which is commonly used by

commercial container nurseries, amended with Metro-Mix™ and Osmocote™ fertilizer, at 0.09 m^3 and 5 kg, respectively, per 0.8 m^3 pine bark compost. Plants were inoculated from 26 to 31 days after seeding and duration of experiments ranged from 27 to 39 days (Appendix 4, Table A4.1).

Species, isolates, and inoculum. *P. cactorum* (isolate #: 65), *P. capsici* (isolate #: 279), *P. cryptogea* (isolate #: 139), *P. drechsleri* (isolate #: 179), *P. nicotianae* (isolate #: 100), and *P. citrophthora* (isolates #s: 67 and 288) were grown on 20% V8 juice agar (Appendix 1, A1.3) in 100 x 15 mm Petri dishes at 23°C in the dark until cultures nearly filled Petri dishes. The agar was then sliced into small pieces to create additional surface area for sporangia production, flooded with 20% V8 broth (Appendix 1, A1.3), and incubated at 23°C overnight in the dark. V8 broth was removed with a Pasteur pipette and the culture washed with 10 ml mineral salts solution (Appendix 1, A1.1). The wash solution was removed and 10 ml mineral salts solution added to the culture, which was allowed to rest at least 10 min at room temperature before removal of mineral salts solution. Cultures of *P. cactorum, P. capsici, P. citrophthora,* and *P. nicotianae* were flooded with another 10 ml mineral salts solution and incubated at room temperature under 40-Watt fluorescent lights overnight. Then the mineral salts solution was replaced with 10 ml fresh mineral salts solution and the culture allowed to incubate under 40-Watt fluorescent lights for 1 to 4 days until adequate numbers of sporangia were produced. Cultures of *P. cryptogea* and *P. drechsleri* typically did not produce adequate sporangia with the mineral salts regime, so they were treated with soil extract (Appendix 1, A1.4). Therefore, after removal of V8 broth and washing three times with sterile ddH_2O, cultures of *P. cryptogea* and *P. drechsleri* were flooded with soil extract and allowed to incubate at room temperature under 40-Watt fluorescent light 1 to 4 days until an adequate number of sporangia were formed.

Zoospore release was accomplished in the same manner for all *Phytophthora* spp.. When adequate numbers of sporangia had formed, cultures were washed twice with sterile ddH_2O and flooded with sterile ddH_2O before placing in the dark at 4°C for at least 45 minutes. After this cold treatment, cultures were placed under 40-Watt fluorescent lights at room temperature and monitored for zoospore release. When adequate numbers of zoospores were released, the aqueous portion was transferred with a Pasteur pipette to a 250 ml flask. This solution was gently agitated to ensure zoospore suspension and a 1-ml aliquot was transferred to a test tube. To effect zoospore encystment the test tube was vortexed for at least 1 min. While gently agitating the test tube to suspend encysted zoospores, a pipette was used to transfer suspended zoospores to a haemacytometer for zoospore quantification. Spore concentration was adjusted to 10,000 spores/ml with sterile ddH_2O for *P. cactorum, P. capsici, P. citrophthora,* and *P. nicotianae*; concentrations for *P. cryptogea* and *P. drechsleri* were adjusted with soil extract. A 1 ml aliquot of zoospore suspension was spread on each of two plates of V8 agar to check for zoospore viability. Dilutions were made with sterile ddH_2O to make additional concentrations of 5,000 and 2,500 zoospores/ml.

Inoculation. Plants were inoculated with *Phytophthora* zoospore suspensions between 26 to 31 days from seeding (Appendix 4, Table A4.1). Three zoospore concentrations (10,000, 5,000, and 2,500 zoospores/plant) were used. Approximately 24 h prior to inoculation with zoospore suspension, medium in Cone-tainers™ was watered to saturation.

Inoculation of sage plants with the three zoospores concentrations was performed immediately after transport of inoculum to the greenhouse. Plants in each treatment were inoculated with their respective treatment in 1 ml aliquots. Control plants in tests with *P. cactorum, P. capsici, P. citrophthora,* and *P. nicotianae* were inoculated with 1 ml sterile

ddH$_2$O; control plants in tests with *P. cryptogea* and *P. drechsleri* were inoculated with 1 ml soil extract. Between 9 to12 replications were used for each treatment and non-treated controls (Appendix 4, Table A4.1). All experiments were repeated. Placement of plants in the experiment was completely randomized using SAS software, version 8.0 (SAS Institute, Inc.; Cary, NC).

During the test period Cone-tainers™ were waterlogged for 3 days per week by submerging bottoms in plastic cups filled with water. When not subjected to waterlogged conditions, plants were irrigated when the growing medium began to dry.

Disease assessment and pathogen isolation. Plants that were completely wilted and collapsed, were considered dead and were removed from the experiment for root assay to ascertain the causal agent. Roots were washed with tap water to remove soilless media, blotted dry on paper towels, and plated on P$_5$ARP+H+V8 (Appendix 1, A1.2), which was modified with 10 ppm benomyl (E. I. DuPont DeNemours and Co.; Wilmington, DE). Plates were examined daily for colony growth and identification. Pathogenicity experiments were terminated 27 to 39 days after inoculation, when disease progress had ceased and remaining plants' shoots were asymptomatic. [Appendix 4, Table A4.1]. At the termination of an experiment fresh weights of roots and shoots were recorded for each remaining plant if the mortality rate of the treatment was not significantly ($P \leq 0.05$) higher when compared to the control. Roots were washed with tap water to remove soilless media and blotted dry on paper towels before weighing. Roots of all remaining plants were plated on P$_5$ARP+H+V8, modified with 10 ppm benomyl. A portion of the recovered colonies was purified and subcultured for identification and comparison with the inoculated species. Comparison of percent mortality of plants within a treatment and percent recovery of *Phytophthora* spp. from roots of all plants in the respective treatment was made.

Comparison of pathogenicity of different isolates of *P. citrophthora* and *P. drechsleri.* Different isolates of *P. citrophthora* (isolate #s: 103, 118, 119, 283, 290, 372) and *P. drechsleri* (isolate #s: 106, 138, 173, 174, 247, 303) were also tested for pathogenicity to sage along with a non-treated control and a positive control of *P. cactorum* (isolate #: 65). These tests were conducted as outlined above except that plants were inoculated 21 to 22 days after seeding and the duration of the experiments ranged from 24 to 25 days (Appendix 4, Table A4.2). Additionally, plants were not waterlogged, but irrigated only when medium in Cone-tainers™ began to dry, since I had found in informal, preliminary work that waterlogged conditions, although commonly used in *Phytophthora*-pathogenicity tests, were not necessary for disease. Roots of asymptomatic plants remaining at the termination of the experiment were not assayed for *Phytophthora*, nor were fresh weights of roots and shoots recorded.

Statistical analysis. Statistical analyses of pathogenicity tests were performed using JMP® software, 2nd edition (SAS Institute, Inc.; Cary, NC). Fisher's exact test was used to determine differences in mortality between treatments ($P \leq 0.05$). The Pearson test was used to determine differences between mortality and isolation rates. Analysis of variance was used to separate means and determine 95% confidence intervals for fresh root and shoot weights among treatments ($P \leq 0.05$).

RESULTS AND DISCUSSION

Greenhouse conditions. Ambient temperatures over the duration of all tests with *P. cactorum, P. capsici, P. citrophthora* (isolate #s: 67 and 288), *P. cryptogea, P. drechsleri* (isolate #: 179), and *P. nicotianae* ranged from 14 to 36°C with an average weekly minimum and maximum of 18 and 32°C, respectively (Appendix 4, Table A4.1). The percentage relative

humidity over the duration of all tests ranged from 20 to 90% with an average weekly minimum and maximum of 27 and 73%, respectively. Ambient temperatures over the duration of the two tests with other isolates of *P. citrophthora* and *P. drechsleri* ranged from 13 to 36°C with an average weekly minimum and maximum of 16 and 29°C, respectively (Appendix 4, Table A4.1). The percentage relative humidity over the duration of the two tests ranged from 16 to 64% with an average weekly minimum and maximum of 15 and 42%, respectively. Temperature and relative humidity fluctuated on a daily basis for all tests, with the high temperature and low humidity generally corresponding to afternoon.

Mortality of sage plants. Isolates of *P. cactorum*, *P. capsici*, *P. citrophthora,* and *P. nicotianae* tested were pathogenic to sage (Fig. 15). However, the disease severity did not always increase with increasing concentration of inoculum. *P. drechsleri* (isolate #:179) caused only limited disease on sage, but other isolates of this species and *P. cryptogea* (isolate #: 139) caused no mortality under the conditions of this study (Fig. 15).

P. cactorum (isolate #: 65) produced abundant asexual and sexual structures rapidly, which would allow both rapid inoculum increase and longevity of this aggressive pathogen (Fig. 15) in the various environs of a nursery. Isolates of *P. cactorum* are reported to colonize multiple host species (Erwin and Ribeiro, 1996). This ability makes this water-borne pathogen a potential threat to larger numbers of hosts in a nursery. Lower mortality rates in the repeated test on sage could be due to high ambient temperature, which rose to 33°C in the greenhouse [Appendix 4, Table A4.1]--a temperature higher than the reported maximum growth temperature of 31°C for this species (Erwin and Ribeiro, 1996).

P. capsici caused significantly higher levels of mortality of sage, compared to the control, at the highest inoculum level (Fig. 15). The non-persistent nature of the sporangia of this species

may allow transport of intact sporangia by splashing water, which could increase the probability of larger amounts of inoculum reaching a host synchronously. Although a relatively limited host range was reported for this species (Erwin and Ribeiro, 1996), the susceptibility of many ornamental plants to this species is yet to be tested.

P. citrophthora isolates (#: 288 and 67) caused significant mortality to sage and disease increased with increasing inoculum levels (Fig. 15). This species has a fairly broad range of plant hosts, including another member of the genus *Salvia, S. splendens* Sellow ex Roem. and Schult (Erwin and Ribeiro, 1996). Recovery of *P. citrophthora* in our irrigation water assays was relatively frequent and these tests indicate that irrigation water contamination by this species poses a significant threat to susceptible crop species.

P. nicotianae has an extremely broad host range, which includes many important ornamental crops, including sage (Erwin and Ribeiro, 1996). However, isolates have been found, in some cases, to be host-specific. This may limit the host range of strains in irrigation water (Erwin and Ribeiro, 1996). The isolate tested here showed limited pathogenicity on sage in the conditions of these trials (Fig. 15), but it is likely that other ornamental hosts would show various degrees of susceptibility to isolates of this species.

P. cryptogea is reported pathogenic to a broad range of plants, including many ornamental plants, while *P. drechsleri*'s host range is more limited. *G. jamesonii* is a reported host of both (Erwin and Ribeiro, 1996), but Farr (1995) reports only *P. cryptogea* as pathogenic on *G. jamesonii*. While the pathogenicity of *P. cryptogea* and *P. drechsleri* appeared lacking or limited, respectively, in these experiments, investigation with other ornamental crop hosts is warranted, since *P. cryptogea* and *P. drechsleri* were recovered frequently in water assays during 2000 and 2001. Certainly, more isolates of *P. cryptogea* should be tested. The experimental

protocols used in this work should also be modified with these species to include other timing and methods of inoculation, such as mycelial inoculation. However, work by Mills et al. (1991) and Hong et al. (1999) indicates that additional species may be currently lumped into these two species. Taxonomic work to delimit these groups further may be necessary before pathogenicity work can be meaningful.

Rate of *Phytophthora* sp. recovery from sage roots in comparison with mortality of sage plants. Isolation rates of *P. cactorum* (65), *P. citrophthora* (288), and *P. cryptogea* (139) from roots of sage were not significantly ($P \leq 0.05$) higher than mortality rates of sage in both experiments (Table 2). However, *P. capsici* (279) was isolated from assayed roots at significantly higher rates when compared to mortality rates in one greenhouse experiment at all three zoospore concentrations and in the repeated experiment at the 5,000 zoospores/plant concentration. Likewise, for *P. citrophthora* (67), isolation from sage roots was significantly higher than mortality rates in one experiment for all three zoospore inoculation concentrations and in the repeated experiment at the 5,000 zoospores/plant concentration. For both *P. drechsleri* (179) and *P. nicotianae* (100), isolation from sage roots was significantly higher than mortality rates in only two cases, both at the 10,000 zoospore/plant concentration.

Weight analyses of sage roots and shoots from plants exhibiting no aboveground symptoms. Root weight analyses demonstrated no significant ($P \leq 0.05$) effect on root weight of inoculated plants when compared to the control, except in a single case, when sage inoculated with 5,000 zoospores/plant had a mean significantly higher than the control (Appendix 5, Table A5.1). No trend is apparent in other means comparisons or in comparison of 95% confidence intervals of root weights.

Fresh shoot weight analyses reveal differing results depending on the host and *Phytophthora* sp. combination. *P. capsici* (279) demonstrated a stimulatory effect on sage shoot growth in both tests. Likewise, 95% confidence intervals of mean shoot weight of *P. citrophthora* (67 and 288) indicate higher shoot weights for *Phytophthora*-inoculated sage when compared to controls in both tests. However, in analyses of *P. cryptogea* (139), *P. drechsleri* (179), and *P. nicotianae* (100)--isolates less pathogenic in tests with sage compared to *P. capsici* (279) and *P. citrophthora* (67 and 288)--this trend is not apparent in all cases.

Comparison of pathogenicity of different isolates of *P. citrophthora* and *P. drechsleri* to sage. A range of aggressiveness was demonstrated in tests with six isolates of *P. citrophthora* recovered in irrigation water assays, but all isolates were pathogenic on sage (Fig. 16). Mortality rates of sage inoculated with *P. citrophthora*, ranged from 10 to 50% in the first experiment and 20 to 40% in the second experiment, whereas no isolate of *P. drechsleri* tested caused any plant death under the conditions of these tests (Fig. 16). During the first test only *P. citrophthora* isolates 119 and 372 caused mortality rates significantly ($P \leq 0.05$) higher than the control, whereas in the second test inoculation with *P. citrophthora* isolates 103 and 119 caused mortality rates significantly higher than the control. *P. citrophthora* isolates 118, 283, and 290 were less aggressive in both tests, with peak mortality levels of 20%.

Mortality of gerbera plants. *P. cactorum* (65) at the two lower inoculum concentrations caused mortality rates of 8% in two separate tests; however, this result did not occur in the corresponding tests and these mortality rates were not significantly ($P \leq 0.05$) higher than mortality rates of the control. Gerbera is not reported as a host of *P. cactorum* (Farr et al., 1995; Erwin and Ribeiro, 1996); however, *P. cactorum* isolates are often able to colonize a variety of hosts (Erwin and Ribeiro, 1996). No mortality of inoculated gerbera plants was caused by any of

the other *Phytophthora* isolates tested in these experiments. Strains of *P. nicotianae* are sometimes host-specific (Erwin and Ribeiro, 1996), and this may explain gerbera's lack of susceptibility to this particular isolate despite the fact that *G. jamesonii* and *G.* sp. L. are reported hosts of *P. nicotianae* (Erwin and Ribeiro, 1996).

Recovery of inoculated *Phytophthora* isolates from gerbera roots of plants exhibiting no aboveground symptoms. *P. cactorum* , *P. citrophthora* , *P. cryptogea* , and *P. drechsleri* were not recovered from roots of asymptomatic gerbera plants (Table 2). Only three instances of isolation from roots of asymptomatic gerbera occurred--two with *P. citrophthora* and one with *P. capsici*.

Weight analyses of gerbera roots and shoots from plants exhibiting no aboveground symptoms. In gerbera tests mortality p-values for all treatments were greater than 0.05; therefore, weights of roots and shoots of all plants remaining at the termination of each experiment were recorded. No means of root weights of *Phytophthora*-inoculated plants were significantly higher or lower when compared to non-treated controls. Additionally, no trend could be discerned regarding 95% confidence intervals of root weights (Appendix 6, Tables A6.1, A7.2).

Results of fresh shoot weight analyses of *Phytophthora*-inoculated gerbera plants show a different trend from *Phytophthora*-inoculated sage plants. Shoot weights of gerbera plants inoculated with *P. cactorum, P. capsici, P. citrophthora, and P. nicotianae* generally were lower than those of non-treated controls. However, in only two cases, did treated plants have significantly ($P \leq 0.05$) lower shoot weight than the controls (Appendix 6, Table A6.2). The 95% confidence intervals for shoots of gerbera inoculated with isolates of these species are also generally lower when compared with the control plants, except in seven cases out of the thirty

analyzed. *P. cryptogea* and *P. drechsleri*-inoculated gerbera plants show 95% confidence intervals for shoot weights, in most cases, that are higher than non-treated control plants. A mean shoot weight significantly ($P \leq 0.05$) higher for gerbera plants inoculated with these two species occurs only in a single case when compared to the non-treated controls.

Conclusions. This is the first report on the pathogenicity of *P. cactorum*, *P. capsici*, and *P. citrophthora* to *S. officinalis*. Results from this work indicate the majority of tested isolates of *Phytophthora* spp. which were recovered from irrigation water during 2000, have the ability to cause substantial crop loss. Analyses of fresh root weights of controls and *Phytophthora*-inoculated asymptomatic sage and gerbera revealed no apparent effect on roots from *Phytophthora*-inoculation. However, under the duration and environmental conditions of these tests it appears that *Phytophthora* inoculation may either reduce or stimulate plant shoot growth, depending on the host and *Phytophthora* spp. combination, but generally little effect is apparent on roots. Therefore, interactions between *Phytophthora* spp. and host plants appear to be complex. The identification of the cause of stimulation of shoot elongation, which was exhibited by some isolate-host combinations, requires further investigation.

Recovery rates that were significantly higher ($P \leq 0.05$) than mortality rates suggest that asymptomatic plants could serve as hidden sources of inoculum in a nursery or greenhouse (Table 2). Although rates of isolation of *Phytophthora* from roots of both dead and asymptomatic (aboveground) sage plants was not always significantly higher than mortality rates, the general trend observed is that many sage plants, although not killed by *Phytophthora*, maintained inoculum long after *Phytophthora*-inoculation--between 26 to 39 days in these tests (Appendix 4, Table A4.1). Although infrequent instances of isolation of *Phytophthora* occurred with gerbera,

generally gerbera did not appear to maintain *Phytophthora* inoculum over the conditions and duration tested (Appendix 4, Table A4.1).

Asymptomatic plants harboring inoculum should be of concern to the ornamental horticultural industry. For growers and consumers, any source of inoculum could be significant if spread to highly susceptible crops occurs in a commercial situation or otherwise. Under poor drainage conditions, which are commonly encountered in the landscape after sale and transplant, inoculum production would be favored in wet conditions. This is an issue both for terms of on-site management practices and consumer perceptions of horticultural products. In a time of increasing ornamental plant trade, both within the United States and globally, this issue gains precedence. The potential for spread of new species and strains into new geographic areas not previously infested has increased. Therefore, in addition to monitoring and controlling *Phytophthora* spp. because of the economic bottom line, greater stringency would also be to avoid introduction of species or strains into new geographic regions.

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