Chemical and Hot Water Treatments to Control *Rhizoctonia* AG P Infesting Stem Cuttings of Azalea

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Abstract. In the southern and eastern United States, azalea stems cut during the spring for propagation may be infested with *Rhizoctonia* spp. Multiple methods were evaluated in a series of laboratory experiments for the purpose of eliminating *Rhizoctonia* from stem cuttings of *Rhododendron* L. ‘Gumpo White’ [‘Gumpo White’ (Satsuki) azalea] to prevent spread of azalea web blight during the propagation phase of nursery production. Leafless stem sections were inoculated with an isolate of binucleate *Rhizoctonia* anastomosis group P (AG P). Disinfectants (sodium hypochlorite, hydrogen dioxide, and quaternary ammonium chloride) or fungicides (chlorothalonil + thiophanate-methyl and flutolanil) applied at several rates (below, at, and above label rates) did not eliminate *Rhizoctonia* AG P from stem sections. Recovery of *Rhizoctonia* AG P was not reduced by submersing stem pieces in 45 °C water, but was eliminated at water temperatures of 50 °C or greater. Mortality of *Rhizoctonia* infesting azalea stem pieces was best explained by a cubic regression model. Mortality increased with increasing time (0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 15, 18, and 21 min) in water at 50 and 55 °C and with increasing temperatures (52, 55, 58, 61, 64, 67, and 70 °C) when stem pieces were submerged for 30 and 60 s. The duration of hot water treatment at which 99% of stem pieces were predicted to be free of *Rhizoctonia* was 20 min 16 s at 50 °C and 5 min 19 s at 55 °C. The average water temperature at which 99% of the stem pieces were predicted to be free of *Rhizoctonia* was 60.2 and 56.9 °C when stem pieces were submerged for 30 and 60 s, respectively. Only minor leaf damage occurred on terminal, leafy stem cuttings when submerged in 50 °C water after 40 min. Severe leaf damage did occur if cuttings were submerged long enough in water of 55 °C or greater. Leaf damage was predicted to exceed a proportional leaf damage value of 0.25 (indicating severe damage) when leafy stem cuttings were submerged in 55 °C water for longer than 13 min 54 s or for 30 and 60 s with water temperature greater than 57.4 and greater than 56.8 °C, respectively. Of the methods tested, submersion in hot water has the greatest potential for eliminating *Rhizoctonia* AG P from azalea stem cuttings. Submerging stem pieces in 50 °C water for 21 min eliminated *Rhizoctonia* and provided the least risk for development of severe leaf damage.

Azalea web blight is an annual problem on some evergreen azalea cultivars grown in containerized nursery production in the

southern and eastern United States. *Rhizoctonia* AG P (equivalent to *Rhizoctonia* AG U) is the primary anastomosis group (AG) of the binucleate *Rhizoctonia* species that causes web blight on azalea (Rinehart et al., 2007). From field observations made during a study by Rinehart et al. (2007), it was noticed that azalea plants symptomatic of web blight were randomly distributed within blocks of the same cultivar yet regularly distributed across most azalea cultivars of differing susceptibility, plants of differing age (from newly potted cuttings to 4-year-old plants), and blocks arbitrarily located across nurseries that may be greater than 100 acres at a single site (Frisina and Benson, 1989; Rinehart et al., 2007). The mechanisms by which *Rhizoctonia* spp. are spread in agricultural field and row crops are generally known (Tsrar and Peretz-Alon, 2005; Yuan et al., 2006). In field and row crops, soil character-

istics and ecology affect conduciveness and pathogen distribution within a field, factors that have been used to develop recommendations such as deep plowing of crop residues (Lewis et al., 1983). Part of the disparity in pathogen dispersion concepts between field- and nursery-grown crops occurs because *Rhizoctonia* spp. are soilborne organisms. In nursery crops in the southern and eastern regions of the United States, pine bark is the most common growing substrate component and new bark-based substrate is used for each year's crop. Container-grown, woody ornamental crops also differ from field crops in that their production time extends over more than a single year, plants are physically handled multiple times during production, and plants are freely relocated to accommodate changing space and plant inventory needs at a nursery. Part of the distribution pattern of *Rhizoctonia* AG P may be explained by the discovery that as many as 20% of stems with new stem growth can be colonized by *Rhizoctonia* AG P during the spring when those stems are harvested for vegetative propagation (W.E. Copes, unpublished data). *Rhizoctonia* spp. are spread on cuttings of a variety of ornamental plants (Benson, 1992; Chase, 1991; Smith and Neely, 1981).

Best management practices for propagation of pathogen-free plants include the use of clean rooting substrate, the collection of cuttings from disease-free plants, and the use of sanitary propagation facilities. If the first two conditions cannot be guaranteed, generally accepted practices are to pasteurize substrate, treat stock plants with fungicides, and/or dip cuttings in a disinfectant or fungicide solution (Daughtrey and Benson, 2005; Williams-Woodward and Jones, 2001). A number of methods, including treatment with disinfectants or fungicides, submersion in hot water, and exposure to ultraviolet radiation, have been used for disinfecting a variety of plant material such as fruits, ornamental plants (bulbs, corms, stem cuttings), seeds, and vegetables (du Toit and Hernandez-Perez, 2005; Johnson et al., 2006; Keck et al., 1995; Lurie, 2006; Mannini, 2007; Mari et al., 2007; Porat et al., 2002; Sharma and Tripathi, 2008; Tsang et al., 2003; Ukuku, 2006). Various disinfectants and fungicides have been used to sanitize carrots, poinsettia cuttings, seed potatoes, and sugar beets of *Rhizoctonia* spp. (Amadioha, 1998; Benson, 1991; European and Mediterranean Plant Protection Organization, 1994; Fleury, 1992; Ricker and Punja, 1991; Weinhold et al., 1982). *R. solani* has been reported to be thermally killed in soils with sustained temperatures of 53 °C (Baker and Roistacher, 1972).

The objective of this work was to evaluate the efficacy of disinfecting methods to eliminate *Rhizoctonia* from azalea stems without damaging leaf tissue of azalea stem cuttings. An initial series of experiments was performed to evaluate efficacy with a small number of levels (rates, temperatures, submersion times) of different types of disinfecting methods. When certain methods were shown

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to cause significant reduction in the recovery of *Rhizoctonia* AG P from azalea stem pieces, further experiments were constructed with a broader range of factor levels. The results from this research will be tested in the field in subsequent studies.

Materials and Methods

Stem preparation and inoculation. In preparation for inoculation of stem pieces, pathogen-infested rice was prepared by placing 25 g dry white rice grains and 18 mL deionized water in a 250-mL Erlenmeyer flask and closing the flask with a foam plug. The flask and contents were autoclaved at 125 °C for 60 min. After cooling to room temperature, the rice was stirred using aseptic methods in a biohazard IIA hood. Three 0.49-cm² square blocks of potato dextrose agar (PDA) with the leading edge of mycelium of 3- to 5-d-old cultures of *Rhizoctonia* AG P (isolate RHF076WAZ4, collected from azalea) were aseptically dropped onto the moistened rice of a single flask. Cultures were grown for 7 to 10 d at 23 °C in the dark. One to two flasks of inoculum were started in advance of each experiment.

Stem cuttings for all experiments were collected from a single group of 'Gumpo White' azalea plants grown in 3.8-L containers and maintained outside from May to September and in a greenhouse from October to November at the USDA-ARS Thad Cochran Southern Horticultural Laboratory in Poplarville, MS. Stem sections of variable length and 3 mm or less diameter were obtained from plants on the same day of inoculation. Leaves were removed before stem pieces were inoculated with *Rhizoctonia* AG P. Studies were performed from May 2008 to Nov. 2008.

Two inoculation procedures were used: a buried stem inoculation (BSinoc) method and an upright stem inoculation (USinoc) method. The BSinoc method was used for most experiments, because it was easy to set up and maintain. For the BSinoc method, a 1.2-cm-deep layer of moistened peat-based substrate (Fafard 2B; Conrad Fafard, Agawam, MA) was placed in the bottom of a 25.4 cm × 25.4-cm plastic tray. Infested rice grains (20 g) were evenly distributed on the substrate layer and then covered with a 0.6-cm-deep layer of the peat-based substrate. Stem pieces were laid horizontally and evenly distributed on the second layer of substrate and then covered with a 1.2-cm-deep top layer of the peat-based substrate. Deionized water was added as needed using a trigger spray bottle. The tray was covered with a clear plastic lid to reduce water loss from evaporation and maintained at 21 °C on a laboratory shelf. After stem pieces were exposed to colonization by *Rhizoctonia* AG P for 3, 5, or 7 d, stem pieces were removed with forceps and gently brushed to remove most of the substrate and cut to 3-cm lengths.

The purpose of the USinoc method was an attempt to produce a mycelium growth habit

similar to what would occur on stems in the canopy of an azalea plant. The USinoc method involved a bottom 1.2-cm-deep layer of peat-based substrate, a layer of infested rice (20 g), and a 1.2-cm-thick top layer of the peat-based substrate in a 25.4 cm × 25.4-cm plastic tray. Vertically orientated stem pieces were inserted into the peat until the cut ends butted against the bottom of the tray. Stem pieces were arranged in rows that alternated between strips of fiberglass air filter material (Ace 10004.011425 Furnace Filter, 35.6 × 63.5 × 2.5 cm; Flanders-PrecisionAire, Inc., Washington, NC). A fiberglass filter strip ≈1.5 cm wide was set on the surface of the substrate against a side of the plastic tray. A straight row of 10 vertical stem pieces was inserted into the substrate so that they pressed against the fiberglass filter. Another fiberglass filter strip was set on the surface of the substrate and pressed against that row of stem pieces. The process was repeated until ending with a fiberglass filter strip at the opposite side of the tray. The tray was maintained on a greenhouse bench at 25 °C and watered with an overhead, intermittent misting system. Irrigation mist was applied for 10-s durations at 30-min intervals from 700 HR to 2100 HR. The purpose of the fiberglass filter was to provide large air spaces that restricted air flow around the stem pieces to create a higher relative humidity microenvironment than ambient air, similar to the environment found within the canopy of an azalea plant. After 5 to 9 d, stem pieces were removed with forceps and the lower 2.5 cm that was buried in substrate and uppermost section that extended above the filter layer were cut off with pruning shears, resulting in 3-cm stem pieces. After each inoculation procedure for both inoculation methods, three stem pieces were arbitrarily selected and microscopically examined. Stem pieces were used in studies when mycelium growth was regularly distributed around the entire circumference and length of the stem pieces. Mycelium distribution was similar for both inoculation methods.

Stem cutting preparation. Terminal stem cuttings, 3 cm in length, were used to evaluate leaf damage in response to hot water treatments. These cuttings were collected from the same 'Gumpo White' azalea plants from which stem pieces were obtained for inoculation as previously described. Cuttings were collected during the morning, placed in a plastic bucket, and kept covered with a moist paper towel. Leaves were removed from the basal 0.5-cm portion of the stem in the laboratory. Cuttings with leaves were not inoculated with *Rhizoctonia* AG P or subjected to chemical treatments.

Chemical treatments. A series of experiments (Expts. 1 to 4) were conducted using selected chemicals (disinfectants and fungicides) and rates applied to stem pieces inoculated with *Rhizoctonia* AG P (Table 1). A total of 250 mL of each treatment solution was prepared and poured into a 600-mL beaker. Six stem pieces were randomly selected for each treatment, placed in

the solution, and swirled to provide thorough wetting. Stem pieces were submersed for selected durations (Table 1) by setting a second beaker into the solution, dispersing trapped air, and pushing downward until solution rose between the walls of the two beakers. Each experiment (except Expt. 2) was repeated with repetitions designated as Run 1 and Run 2.

Stem pieces treated with a disinfectant (sodium hypochlorite, hydrogen peroxide, or quaternary ammonium chloride) were set on water agar (WA) medium within minutes after treatment. Stem pieces treated with a fungicide (chlorothalonil + thiophanate-methyl or flutolanil) were allowed to dry for at least 2 h before setting on WA medium. Plates were examined daily for 7 d for the presence of *Rhizoctonia*. *Rhizoctonia* was verified based on observing macroscopic mycelium branching traits in WA by viewing the reverse side of plates held toward a fluorescent light and microscopic examination of mycelium. A small number of samples were additionally plated on half-strength PDA to verify identification.

Hot water treatments. For hot water treatments, 19-L and 15-L circulating water baths (Models 260 and 280, respectively; Precision-NAPCO, A Division of Joshua, Inc., Winchester, VA) were used to bring deionized water to the desired treatment temperature (Table 1). Approximately 800 mL of water was scooped from the hot water bath into each of two 1000-mL glass beakers and then the beakers were set down into the hot water bath. Leafless stem pieces inoculated by the BSinoc method were dropped into water in a separate beaker than noninoculated, terminal stem cuttings with leaves. Stem pieces and cuttings were stirred for 2 to 3 s until submerged. A metal lid was used to cover the opening of the hot water bath and lifted each time stem pieces were removed. Water temperature was recorded using a partial immersion thermometer in the hot water bath and in the beakers at the start and finish of a replication. Before the next replication, water within the beakers was discarded, deionized water was added back to the hot water bath and allowed to reach the required temperature, and then the process was repeated.

Stem pieces and cuttings were removed from the water with forceps. Leafless stem pieces were immediately set on WA medium, whereas cuttings with leaves were placed in humid chambers (31-L and 16-L plastic boxes with snap-closure lids and with wet paper towels lining the bottom on the inside of the box). WA plates were checked daily for 7 d for the presence of *Rhizoctonia* as previously described. Leaf damage on the cuttings was rated after 24 h by counting the number of leaves per cutting that appeared healthy (H), that exhibited damage (leaf discoloration) on less than 50% leaf area (LDlt50), and that exhibited damage on greater than 50% leaf area (LDgt50). A proportional leaf damage (Lfd) value was calculated per cutting using the following

Table 1. Experiment number, treatment description, and type of tissue treated in a series of experiments examining efficacy of chemicals (disinfectants and fungicides) and hot water exposures (temperature and duration) for eliminating *Rhizoctonia* AG P from stem cuttings of 'Gumpo White' azalea.

Expt. ²	Chemical and hot water treatments	Tissue
1	Sodium hypochlorite ³ at 0 ⁴ , 3.05, 6.10 ^w , 9.15, or 12.20 mL·L ⁻¹ a.i. for 10 min; hydrogen dioxide ^u at 0, 1.35, 2.70 ⁴ , 13.50, or 27.00 mL·L ⁻¹ a.i. for 10 min; quarternary ammonium chloride ⁵ at 0, 0.5, 1.0 ⁴ , 5.0, or 10.0 mL·L ⁻¹ a.i. for 10 min	Stem pieces inoculated ^v for 7 d
2	Chlorothalonil + thiophanate-methyl ⁶ at 0, 0.431, 0.863 ⁴ , or 1.726 ⁴ g·L ⁻¹ a.i. + 0.108, 0.216, or 0.431 g·L ⁻¹ a.i., respectively, for 3 to 4 s; flutolanil ^q at 0, 157.5 ⁴ , 315, or 630 ⁴ mg·L ⁻¹ a.i. for 3 to 4 s	Stem pieces inoculated ^v for 7 d
3	Deionized water for 10 min; sodium hypochlorite at 12.20 mL·L ⁻¹ a.i. for 10 min; flutolanil at 0.315 g·L ⁻¹ a.i. for 3 to 4 s	Stem pieces inoculated ^p for 3, 5, and 7 d
4	Sodium hypochlorite at 0 or 12.20 mL·L ⁻¹ a.i. for 10 min; sodium hypochlorite at 12.20 mL·L ⁻¹ a.i. + Surf-Ac ^o 820 at 1.92 ⁴ mL·L ⁻¹ a.i. for 10 min; hot water at 45 or 55 °C for 5, 25, or 45 min	Stem pieces inoculated ^v for 7 d; terminal stem cuttings ⁿ
5	Hot water at 50 °C for 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 15, 18, or 21 min; hot water at 55 °C for 0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5.5, 6.5, or 7.5 min; hot water at 50 and 55 °C for 0, 1, 3, 5, 7, 9, 15, 20, 25, 30, 35, or 40 min	Stem pieces inoculated ^v for 7 d; terminal stem cuttings
6	Hot water at 52, 55, 58, 61, 64, 67, or 70 °C for 0, 30, and 60 s	Stem pieces inoculated ^v for 7 d; terminal stem cuttings

²All experiments, except Expt. 2, were repeated in two runs.

³Clorox bleach.

⁴"0" value is deionized water only.

^wCommon rate used by the industry, 9:1 (v:v) water:product.

^vStem pieces were infested with *Rhizoctonia* AG P by covering with moist peat-based substrate containing colonized rice grains as inoculum. Substrate containing the stem pieces was hand-misted periodically, enclosed in a covered plastic container, and maintained at 21 °C on a laboratory shelf.

^uZerotol (Biosafe Systems, Glastonbury, CT).

⁶Registered rate listed on Environmental Protection Agency-approved label. Consult labels for specific use recommendations.

^oGreen Shield (Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO).

^qSpectro 90 (Cleary Chemical, Dayton, NJ).

^pContrast (Scotts-Sierra Crop Protection Co., Marysville, OH).

ⁿLeafless stem pieces were infested with *Rhizoctonia* AG P by vertical insertion in moist peat-based substrate containing colonized rice grains as inoculum. A higher-than-ambient relative humidity around the aerial portion of the stem was favored by contiguous covering of the peat surface with a 2.54-cm-thick strip of fiberglass furnace filter. Stem pieces were incubated in a greenhouse under an intermittent mist system.

^oSurf-Ac 820 (Drexel Chemical Co., Memphis, TN).

ⁿTerminal stem cuttings were used to assess leaf damage in response to hot water treatment and not used with solutions containing sodium hypochlorite.

formula (where TLN is the total leaf number per cutting): $LfD = [(LD_{50}/TLN) \times 0.25] + [(LD_{gt50}/TLN) \times 0.75]$. The midpoint value of each category was selected as multipliers to assess leaf damage. LfD is a "leaf damage" value, whereby the likelihood of a stem cutting to not successfully develop roots would be correlated to the proportional number of leaves per stem that were healthy, had less than 50% necrotic area, and had greater than 50% necrotic area. Damage to stem cuttings was categorized as none (all leaves green and healthy in appearance), moderate (LfD 0.25 or less; should not affect success of rooting), and severe (LfD greater than 0.25; likely to affect rooting success).

Statistical analysis. In all six experiments, treatment efficacy was measured as a binary response of recovery or no recovery of *Rhizoctonia* (with no recovery also referred to as elimination of *Rhizoctonia*). In Expts. 4 to 6, leaf damage data were collected as a continuous response variable, the calculation of which was described in the previous section.

A completely randomized design was used in Expts. 1 to 3 with random assignment of stem pieces or stem cuttings to experimental treatments. No statistical analysis was conducted in Expts. 1 to 3 because there was no elimination of *Rhizoctonia* from stem pieces with any treatment.

A completely randomized design was used in Expt. 4. Associations were analyzed with Mantel-Haenszel χ^2 tests using the FREQ procedure of SAS (Version 9.1.3; SAS Institute, Inc., Cary, NC), because stratified variables were ordinarily scaled and the

response was dichotomous. Data were treated as nonparametric standardized midrank scores, using modified Ridit scores, to avoid potential distribution problems. For the binary response of recovery of *Rhizoctonia*, the null hypothesis was one of general association. For the ordinal leaf damage response, the null hypothesis was one of shifts in row mean scores. When more than one-third of the treatment responses had a frequency of five or less, exact *P* values were calculated using Fisher's exact test.

A split plot design was used in Expts. 5 and 6 with a main plot factor, subplot factor, and replication as a block effect by time. In Expt. 5, the main plots (two temperatures) were replicated four times, and the subplots (12 durations of exposure) were replicated three times within each main plot. In Expt. 6, the main plots (two durations of exposure) and subplots (seven temperatures) were replicated 12 times in Run 1 and seven times in Run 2.

For Expt. 5, the binary response of elimination of *Rhizoctonia* was analyzed using probit regression with the PROBIT procedure of SAS to calculate lethal dose curves and to compare intercept and slope parameters between factors and runs. In Expt. 6, the binary response of elimination of *Rhizoctonia* was analyzed with generalized linear mixed models (with a binomial distribution and probit link) with the GLIMMIX procedure of SAS.

In Expt. 5, leaf damage data were analyzed using the MIXED procedure of SAS with independent variables being temperature and linear, quadratic, and cubic effects of duration of exposure. Duration of exposure

was treated as a continuous variable in the model statement. Random effects were experiment, experiment with replication and subplot replication, and the previous effects with temperature and duration of exposure. In Expt. 6, leaf damage data were analyzed using the MIXED procedure of SAS with independent variables being duration of exposure and linear, quadratic, and cubic effects of temperature. Temperature was treated as a continuous variable in the model statement. Heterogeneous variances were fit using the Toeplitz covariance structure. Replication was defined as an independent random effect using the SUBJECT option in a REPEATED statement.

Results

In Expts. 1 and 2, none of the disinfectant or fungicide treatments (Table 1) resulted in elimination of *Rhizoctonia* AG P; rather, *Rhizoctonia* was recovered from all stem pieces (data not shown). These results were unexpected, because it might be assumed that one or more of these treatments would have reduced recovery of the pathogen.

In Expt. 3, the duration of stem exposure to colonization by *Rhizoctonia* AG P (3, 5, and 7 d) resulted mostly in no differences among treatments (deionized water, sodium hypochlorite, and flutolanil) (data not shown). *Rhizoctonia* AG P was recovered from 100% of the leafless stem pieces in Run 1 and from 100% of the leafless stem pieces treated with deionized water and sodium hypochlorite in Run 2. A reduction in the recovery of *Rhizoctonia* from leafless stem cuttings

treated with flutolanil was negatively correlated ($P < 0.0001$) with increasing number of days that stem pieces were colonized by the fungus.

In Expt. 4, response frequencies were nearly identical between the two runs for each treatment; therefore, data were combined across levels. Treatments (deionized water, sodium hypochlorite, 45 °C water, and 55 °C water) were different ($P < 0.0001$) (Table 2). *Rhizoctonia* was not recovered from stem pieces submersed in 55 °C water whether for 5, 25, or 45 min, but was recovered at a high frequency from all other treatments.

In Expt. 4, the leaf damage responses to hot water treatments were similar between runs; therefore, data were combined across runs for analysis. LfD values tended to remain below the 0.25 level, then increase to above the 0.25 level, and then increase readily to the 0.75 level; therefore, the 0.25 value was selected as the demarcation between moderate and severe leaf damage (Figs. 1 and 2). Treatments [deionized water (21 °C), 45 °C water, and 55 °C water] across ranked LfD values were different ($P < 0.0001$) (Table 2). No leaf damage resulted from submersion in 21 °C water, very minor damage occurred from submersion in 45 °C water, and pronounced leaf damage resulted from submersion in 55 °C water. An additional analysis was done for leaf damage in association with the duration of submersion at 55 °C. Treatments (submersion for 0, 5, 25, and 45 min) were different ($P < 0.0001$) (Table 3). The frequency of severe leaf damage per stem increased with increasing duration of submersion in 55 °C water.

In Expt. 5, *Rhizoctonia* was eliminated from azalea stem pieces with increasing duration of exposure to hot water at 50 and 55 °C (Fig. 1). The response was different between temperatures within Runs 1 and 2 as a result of differences in slopes ($P < 0.0001$ and $P = 0.0002$, respectively). The response was different between runs at each temperature but varied as to whether they differed as a result of the intercept or slope. At 50 °C, runs were different as a result of the intercept ($P = 0.0377$) but not the slope ($P = 0.8601$); the intercept (SE) and slope (SE) were -2.130 (0.329) and 0.286 (0.042) for Run 1 and

-3.360 (0.433) and 0.276 (0.038) for Run 2. At 55 °C, runs were different as a result of the slope ($P = 0.0085$) but not the intercept ($P = 0.9091$); the intercept (SE) and slope (SE) were -1.406 (0.415) and 1.481 (0.282) for Run 1 and -1.348 (0.279) and 0.691 (0.103) for Run 2. The LD₉₉ was 15 min 36 s and 20 min 16 s at 50 °C in Runs 1 and 2, respectively, and 2 min 31 s and 5 min 19 s at 55 °C in Runs 1 and 2, respectively (Fig. 1).

For leaf damage in response to treatments in Expt. 5, results from the two runs were not significant ($P = 0.5141$) so data were combined. A significant interaction occurred between temperature and both quadratic and cubic terms for duration of exposure ($P < 0.0001$ for the cubic response). Contrast statements showed the response was different between 50 and 55 °C ($P < 0.0001$). Minor leaf damage occurred with submersion of stem pieces in 50 °C water, but that damage did not significantly increase over 40 min ($P = 0.3429$). At 55 °C, leaf damage increased over time ($P < 0.0001$). Based on regression equations, plant damage exceeded the 0.25 level (selected as the maximum acceptable level) at 13 min 54 s submersion in 55 °C water (Fig. 1).

In Expt. 6, *Rhizoctonia* was eliminated from stem pieces with increasing water temperature when stem pieces were submerged for 30 s and 60 s (Fig. 2). Significant factors were duration of exposure ($P = 0.048$ in Run 1; $P = 0.038$ in Run 2) and water temperature ($P = 0.012$ in Run 1; $P = 0.0015$ in Run 2). The water temperatures at which a 30-s submersion was predicted to cause 50% and 99% mortality were, respectively, 57.0 and 59.7 °C in Run 1 and 53.9 and 60.7 °C in Run 2 (Fig. 2A). The water temperatures at which a 60-s submersion was predicted to cause 50% and 99% mortality were, respectively, 53.0 and 55.6 °C in Run 1 and less than 52.0 and 58.3 °C in Run 2 (Fig. 2B).

In Expt. 6, leaf damage increased with increasing water temperature when stem pieces were submersed for 30 s and 60 s ($P = 0.0003$ and $P = 0.0033$ for Expt. 1 and Expt. 2, respectively) with no interaction between temperature and duration of exposure (Fig. 2). Linear, quadratic, and cubic model terms for leaf damage in response to water temperature were significant ($P < 0.0001$ for the

cubic response in both experiments). Based on cubic regression equations, plant damage exceeded the 0.25 severity rating with submersion in 60.9 and 57.4 °C water for 30 s in Runs 1 and 2, respectively, and in 60.6 and 56.8 °C water for 60 s in Runs 1 and 2, respectively (Fig. 2).

Discussion

Based on results from these laboratory experiments, submersion in hot water was the only effective treatment for eliminating binucleate *Rhizoctonia* AG P, the cause of azalea web blight, from azalea stem cuttings used for propagation. However, hot water treatment carries the risk of damaging plant tissue. Based on these experiments, the safety margin in time between the point when *Rhizoctonia* is eliminated and the point when severe plant damage develops is greater at 50 °C than at 55 °C. At 50 °C, *Rhizoctonia* was eliminated after 20 min, whereas severe plant damage did not occur even at the maximum submersion time of 40 min. At 55 °C, *Rhizoctonia* was eliminated after only 5 min, but severe plant damage occurred after 13 min. With only a slight increase in water temperature to 58 and 60 °C, *Rhizoctonia* was eliminated in 60 s and 30 s, respectively. However, stem submersions for 60 s and 30 s are predicted to cause severe plant damage at 57 and 58 °C, respectively, which is slightly below the temperature predicted to eliminate *Rhizoctonia*. Obviously, greater safety margins are achieved with the cooler water temperatures. *Rhizoctonia* was not eliminated by submerging inoculated azalea stem pieces in 45 °C water for a maximum of 45 min. It is not known whether longer durations could have been lethal to the pathogen. *Botrytis allii* infections were reduced by submerging onions in 45 °C water for 8 to 12 h (Grondeau and Samson, 1994). Our estimation of how long to submerge stems to eliminate *Rhizoctonia* is likely conservative, because stem pieces were artificially inoculated. It is likely that new growth that is naturally infested in the nursery will have lower densities of mycelium present.

Submerging stem cuttings of 'Gumpo White' azalea in 50 °C water for 21 min caused only minor leaf damage with a LfD less than 0.1 that is not expected to affect rooting ability. The LfD value, which ranges from 0 to 0.75, was used to quantify leaf damage resulting from hot water treatments. Research (Reuveni and Raviv, 1981; van Overbeek et al., 1946) shows that a reduction in leaf area can negatively impact rooting, and this general knowledge has been noted previously (Baker, 1962; Riehl, 1956). Because published information relating leaf area damage to rooting efficacy was not found, the 0.25 LfD value (also the midpoint value of the lower leaf damage category) was selected as a conservative threshold based on distribution of LfD values in Figures 1 and 2. The effect of leaf damage on root development still needs to be critically evaluated. Further study is also needed to establish

Table 2. Percent recovery of *Rhizoctonia* AG P from inoculated, leafless stem pieces of 'Gumpo White' azalea treated with sodium hypochlorite or submerged in hot water for a duration of time, and frequency of damage to terminal stem cuttings resulting from submersion in hot water.

Treatment	n ²	Recovery (%)	Frequency of cuttings with leaf damage ³		
			None	Moderate	Severe
Deionized water	12	83	12	0	0
Sodium hypochlorite	24	75	Not tested	Not tested	Not tested
45 °C water ⁴	36	72	35	1	0
55 °C water ⁴	36	0	7	9	20

¹Includes combined data from two runs (repeated experiment).

²Damage to stem cuttings was categorized as none (all leaves green and healthy in appearance), moderate (LfD 0.25 or less; should not affect success of rooting), and severe (LfD greater than 0.25; likely to affect rooting success). LfD is a "leaf damage" value whereby the likelihood of a stem cutting to not successfully develop roots would be correlated to the proportional number of leaves per stem cutting that were healthy, had less than 50% necrotic area, and had greater than 50% necrotic area.

³Frequencies include combined data for 5-, 25-, and 45-min durations.

Fig. 1. Elimination of *Rhizoctonia* AG P from stem pieces (long-dashed lines; diamonds = Run 1 and squares = Run 2) and leaf damage (rating of 0 to 0.75) on terminal stem cuttings (dotted lines; circles = runs combined) of 'Gumpo White' azalea in response to submersion for 0 to 40 min in hot water at (A) 50 °C and (B) 55 °C. Open symbols are means and filled symbols are predicted values. Leaf damage above 0.25, represented by the horizontal dashed and dotted line, is considered the point at which damage to the stem cutting may decrease stem survivability.

sensitivity of azalea cultivars, other than 'Gumpo White', to thermotherapy.

Accuracy of water temperature and duration of submersion is critical for achieving pathogen mortality without damaging plant tissue (Grondeau and Samson, 1994; Lurie, 2006). Water temperature and duration of exposure found to eliminate *Rhizoctonia* from azalea stem pieces generally fall within the range of temperatures and durations that have been successful with other pathogens and plant material (Yarwood, 1975). Various plant species are tolerant of a water temperature that reduces pathogen viability such as onions in 45 °C water for 8 to 12 h to reduce infection by *Botrytis allii* (Grondeau and Samson, 1994), bare-rooted palms in 50 °C water for 15 min to control burrowing nem-

atode (Tsang et al., 2003), grapevine cuttings in 50 °C water for 30 min to control *Phomopsis viticola* (Clarke et al., 2004), seed potatoes in 55 °C water for 5 min to control *R. solani* and three other pathogens (Dashwood et al., 1991), gladiolus corms in 55 °C water for 25 min to control *Fusarium oxysporum* (Sharma and Tripathi, 2008), and Irish potatoes in 60 °C water for 5 min to control *Fusarium oxysporum* and four other pathogens (Salami and Popoola, 2007). In some cases, plant sensitivity to hot water requires use of temperatures that result in reduction, but not elimination, of pathogen populations. Johnson et al. (2006) reduced incidence of *Colletotrichum acutatum* by nearly 80% by submerging strawberry runner cuttings in a prewarming treatment of 35 °C water for 7

min followed by 50 °C water for 3 min, but vigor and survival of some cultivars were reduced at those temperatures and durations. Vegetative growth of strawberry nursery stock was minimally damaged when bagged nursery plants were exposed to dry or wet heat of 44 °C for 4 h or 48 °C for 2 h (Turechek and Peres, 2009). These temperatures and durations did not eliminate the pathogen, but caused a reduction of 10^5 or 10^6 colony-forming units/mL of *Xanthomonas fragariae*, the cause of angular leaf spot.

It is unfortunate that *Rhizoctonia* AG P was not inhibited by treatment of stem pieces with disinfectants (sodium hypochlorite, hydrogen dioxide, and quaternary ammonium chloride) and fungicides (chlorothalonil + thiophanate methyl, and flutolanil), because these would have provided additional treatment options. Reasons why posttreatments were ineffective was not determined. One possible explanation is the pathogen avoided contact with the chemicals by the mycelium growing within the irregular bark crevices of the azalea stem or penetrating into intracellular or intercellular plant spaces. If this occurred, the untreated mycelium could retain viability even if some mycelium died from chemical contact. A surfactant was mixed with sodium hypochlorite for one treatment in Expt. 4, but this did not reduce recovery of *Rhizoctonia* AG P. Other research approaches such as application of a disinfectant as a gas and histopathologic evaluation of plant tissue were not attempted and would have provided additional experimental evidence.

Although disinfectants can effectively kill pathogens (Copes, 2004), other studies have reported variable levels of control responses. Chlorine and hydrogen peroxide reduced *Salmonella* on muskmelon surfaces, yet a greater reduction resulted from submersion in 96 °C water for 2 min (Ukuku, 2006). Chlorine treatments reduced the incidence of three fungal pathogens on spinach seed but were not as effective as submersion in 40 to 60 °C water for 10 to 30 min depending on the pathogen (du Toit and Hernandez-Perez, 2005). Submersion of onion seed in 50 °C water for 20 min reduced incidence of two pathogens more consistently than sodium hypochlorite or fungicides (Aveling et al., 1993). Postharvest treatment of seed potatoes with a combination of disinfectants and fungicides reduced rot severity by *R. solani* (Burth et al., 1978; Errampalli et al., 2006). Errampalli et al. (2006) deduced that chlorine dioxide may have killed the majority of sclerotia, whereas thiophanate-methyl in combination suppressed growth of *R. solani*. Some studies have reported improved disease control by combining chemical and hot water treatments (Porat et al., 2002; Sharma and Tripathi, 2008). Hot water (41 °C) submersion of acorns for 2.5 h prolonged seed storage by controlling *Sclerotinia pseudotuberosa* (Finch-Savage et al., 2003). The addition of fungicides (benomyl and/or thiram) to the hot water further improved the storage life of acorns.

Fig. 2. Elimination of *Rhizoctonia* AG P from stem pieces (long-dashed lines; diamonds = Run 1 and squares = Run 2) and leaf damage (rating of 0 to 0.75) on terminal stem cuttings (dotted lines; triangles = Run 1 and circles = Run 2) of 'Gumpo White' azalea in response to submersion in hot water at 52 to 70 °C for (A) 30 s and (B) 60 s. Open symbols are means and filled symbols are predicted values. Leaf damage above 0.25, represented by the horizontal dashed and dotted line, is considered the point at which damage to the stem cutting may decrease stem survivability.

Table 3. Frequency of damage to terminal stem cuttings of 'Gumpo White' azalea resulting from submersion in 55 °C water for various durations.

Duration (min)	n ^z	Frequency of cuttings with leaf damage ^y		
		None	Moderate	Severe
0	12	12	0	0
5	12	7	5	0
25	12	0	4	8
45	12	0	0	12

^zFrequencies are combined data from two runs (repeated experiment).

^yDamage to stem cuttings was categorized as none (all leaves green and healthy in appearance), moderate (Lfd 0.25 or less; should not affect success of rooting), and severe (Lfd greater than 0.25; likely to affect rooting success). Lfd is a "leaf damage" value whereby the likelihood of a stem cutting to not successfully develop roots would be correlated to the proportional number of leaves per stem that were healthy, had less than 50% necrotic area, and had greater than 50% necrotic area.

We have determined from other research that the *Rhizoctonia* population persists from year to year within a container-grown azalea plant (W.E. Copes, unpublished data). Use of

Rhizoctonia-infested azalea stem cuttings for propagation allows spread of the pathogen into the propagation facility. The moisture and temperature conditions in propagation

greenhouses are conducive to spread of *Rhizoctonia* spp. from cutting to cutting, possibly similar to the colonization of foam rooting cubes (Benson, 1991, 1992). It is likely that additional mechanisms of spread, from routine plant handling activities and wind-disseminated plant debris, may contribute to the wide dispersal of *Rhizoctonia* on azaleas in nurseries. The long-term goal of achieving *Rhizoctonia*-free plants involves first obtaining pathogen-free propagation material.

Although posttreatment of azalea stem pieces with disinfectants and fungicides was not effective, additional research is planned to investigate field application of fungicides and posttreatments with alternative application methods and chemical mixtures. We used hot water submersion as the form of thermotherapy; however, further study could be valuable in developing more comprehensive guidelines, including use of alternative heat application methods such as hot air and vapor-heat treatments.

Literature Cited

- Amadihoa, A.C. 1998. Control of post harvest tuber rot of potato incited by *Rhizoctonia bataticola*. Arch. Phytopathol. Plant Prot. 31: 225-231.
- Aveling, T.A.S., H.G. Snyman, and S.P. Naude. 1993. Evaluation of seed treatments for reducing *Alternaria porri* and *Stemphylium vesicarium* on onion seed. Plant Dis. 77:1009-1011.
- Baker, K.F. 1962. Thermotherapy of plant material. Phytopathology 52:1244-1255.
- Baker, K.F. and C.N. Roistacher. 1972. Heat treatment of soil, p. 123-137. In: Baker, K.F. (ed.). The U.C. system for producing healthy container-grown plants. Calif. Agr. Exp. Sta. Manual 23. University of California Division of Agricultural Sciences, Berkeley, CA.
- Benson, D.M. 1991. Control of *Rhizoctonia* stem rot of poinsettia during propagation with fungicides that prevent colonization of rooting cubes by *Rhizoctonia solani*. Plant Dis. 75: 394-398.
- Benson, D.M. 1992. Detection by enzyme-linked immunosorbent assay of *Rhizoctonia* species on poinsettia stem cuttings. Plant Dis. 76:578-581.
- Burth, U., J. Pfaumbaum, and G. Brazda. 1978. Preservation of healthy planting material for disinfection. Nachrich. Pflanz. DDR 32:105-110.
- Chase, A.R. 1991. Characterization of *Rhizoctonia* species isolated from ornamentals in Florida. Plant Dis. 75:234-238.
- Clarke, K., V. Sergeeva, R.W. Emmett, and N.G. Nair. 2004. Survival of *Phomopsis viticola* in grapevine cuttings after hot water treatment. Australas. Plant Pathol. 33:317-319.
- Copes, W.E. 2004. Dose curves of disinfectants applied to plant production surfaces to control *Botrytis cinerea*. Plant Dis. 88:509-515.
- Dashwood, E.P., E.M. Burnett, and M.C.M. Perombelon. 1991. Effect of a continuous hot water treatment of potato tubers on seed-borne fungal pathogens. Potato Res. 34:71-78.
- Daughtrey, M.L. and D.M. Benson. 2005. Principles of plant health management for ornamental plants. Annu. Rev. Phytopathol. 43: 141-169.
- du Toit, L.J. and P. Hernandez-Perez. 2005. Efficacy of hot water and chlorine for eradication

- of *Cladosporium variabile*, *Stemphylium botryosum*, and *Verticillium dahliae* from spinach seed. Plant Dis. 89:1305–1312.
- Errampalli, D., R.D. Peters, K. MacIssac, D. Darrach, and P. Boswall. 2006. Effect of a combination of chlorine dioxide and thiophanate-methyl pre-planting seed tuber treatment on the control of black scurf of potatoes. Crop Prot. 25:1231–1237.
- European and Mediterranean Plant Protection Organization. 1994. Guidelines on good plant protection practice: Potato. Bull. OEPP 24: 825–845.
- Finch-Savage, W.E., H.A. Clay, S.P. Budge, K.C. Dent, J.P. Clarkson, and J.M. Whipps. 2003. Biological control of *Sclerotinia pseudotuberosa* and other fungi during moist storage of *Quercus robur* seeds. Eur. J. Plant Pathol. 109: 615–624.
- Fleury, P. 1992. Flutolanil: A fungicide for treatment of potato plants. Phytoma 82:65–66.
- Frisina, T.A. and D.M. Benson. 1989. Occurrence of binucleate *Rhizoctonia* spp. on azalea and spatial analysis of web blight in container-grown nursery stock. Plant Dis. 73:249–254.
- Grondeau, C. and R. Samson. 1994. A review of thermotherapy to free plant materials from pathogens, especially seeds from bacteria. Crit. Rev. Plant Sci. 13:57–75.
- Johnson, A.W., D.W. Simpson, and A. Berrie. 2006. Hot water treatment to eliminate *Colletotrichum acutatum* from strawberry runner cuttings. Acta Hort. 708:255–257.
- Keck, M., R. Chartier, W. Zislavsky, P. Lecomte, and J.P. Paulin. 1995. Heat treatment of plant propagation material for the control of fire blight. Plant Pathol. 44:124–129.
- Lewis, J.A., R.D. Lumsden, G.C. Papavizas, and J.G. Kantzes. 1983. Integrated control of snap bean diseases caused by *Pythium* spp. and *Rhizoctonia solani*. Plant Dis. 67:1241–1244.
- Lurie, S. 2006. The effect of high temperature treatment on quality of fruits and vegetables. Acta Hort. 712:785–792.
- Mannini, F. 2007. Hot water treatment and field coverage of mother plant vineyards to prevent propagation material from phytoplasma infections. Bull. Insectology 60:311–312.
- Mari, M., F. Neri, and P. Bertolini. 2007. Novel approaches to prevent and control postharvest diseases of fruits. Stewart Postharvest Rev. 3: 11–13.
- Porat, R., A. Daus, B. Weiss, L. Cohen, and S. Droby. 2002. Effects of combining hot water, sodium bicarbonate and biocontrol in postharvest decay of citrus fruit. J. Hort. Sci. Biotechnol. 77:441–445.
- Reuveni, O. and M. Raviv. 1981. Importance of leaf retention to rooting of avocado cuttings. J. Amer. Soc. Hort. Sci. 106:127–130.
- Ricker, M.D. and Z.K. Punja. 1991. Influence of fungicide and chemical salt dip treatments on crater rot caused by *Rhizoctonia carotae* in long-term storage. Plant Dis. 75:470–474.
- Riehl, G. 1956. The effect of various environmental conditions on the propagation of ornamental plants by cuttings with the aid of sub-irrigation. Arch. Gartenb. 4:433–522.
- Rinehart, T.A., W.E. Copes, T. Toda, and M.A. Cubeta. 2007. Genetic characterization of binucleate *Rhizoctonia* species causing web blight on azalea in Mississippi and Alabama. Plant Dis. 91:616–623.
- Salami, A.O. and O.O. Popoola. 2007. Thermal control of some post-harvest rot pathogens of Irish potato (*Solanum tuberosum* L.). J. Agr. Sci. 52:17–31.
- Sharma, N. and A. Tripathi. 2008. Integrated management of postharvest Fusarium rot of gladiolus corms using hot water, UV-C and *Hyptis suaveolens* (L.) Poit. essential oil. Postharvest Biol. Technol. 47:246–254.
- Smith, M.A.L. and D. Neely. 1981. Screening woody ornamental cuttings for propagation diseases. Plant Dis. 65:893–895.
- Tsang, M.M.C., A.H. Hara, and B. Sipes. 2003. Hot-water treatments of potted palms to control the burrowing nematode, *Radopholus similis*. Crop Prot. 22:589–593.
- Tsror, L. and I. Peretz-Alon. 2005. The influence of the inoculum source of *Rhizoctonia solani* on development of black scurf on potato. J. Phytopathol. 153:240–244.
- Turechek, W.W. and N.A. Peres. 2009. Heat treatment effects on strawberry plant survival and angular leaf spot, caused by *Xanthomonas fragariae*, in nursery production. Plant Dis. 93: 299–308.
- Ukuku, D.O. 2006. Effect of sanitizing treatments on removal of bacteria from cantaloupe surface, and re-contamination with *Salmonella*. Food Microbiol. 23:289–293.
- van Overbeek, J., S.A. Gordon, and L.E. Gregory. 1946. An analysis of the function of the leaf in the process of root formation in cuttings. Amer. J. Bot. 33:100–107.
- Weinhold, A.R., T. Bowman, and D.H. Hall. 1982. *Rhizoctonia* disease of potato: Effect on yield and control by seed tuber treatment. Plant Dis. 66:815–818.
- Williams-Woodward, J. and R.K. Jones. 2001. Sanitation: Plant health from start to finish, p. 384–368. In: Jones, R.K. and D.M. Benson (eds.). Diseases of woody ornamentals and trees in nurseries. APS Press, St. Paul, MN.
- Yarwood, C.E. 1975. Temperature coefficients in plant pathology. Phytopathology 65:1198–1201.
- Yuan, G.Q., A. Kamio, B.S. Sharma, Y. Sagara, M. Arakawa, and K. Inagaki. 2006. Survival and subsequent dispersal of rice sclerotial disease fungi, *Rhizoctonia oryzae* and *Rhizoctonia oryzae-sativae*, in paddy fields. Plant Dis. 90: 615–622.