Interaction of *Calibrachoa* and Selected Root and Foliar Pathogens in Greenhouse Settings

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Abstract

***Calibrachoa* (*Calibrachoa* × *hybrida*) is a popular annual ornamental that was introduced in the late 1990s by the greenhouse ornamental industry. Little is published about its interaction with pathogens commonly associated with greenhouse production. We report here for the first time the response of *Calibrachoa* to infection by pathogens that may be introduced in greenhouse production cycles through the use of infested soil, contaminated tools, infected cuttings, and contaminated irrigation water. Rooted cuttings of 'Colorburst Violet' were artificially inoculated with isolates from *Phytophthora*, *Pythium*, *Verticillium* and *Botrytis*. Symptoms expressed in response to infection included interveinal chlorosis of young leaves, wilting and necrotic root tips with fewer or no secondary or tertiary roots. Non-challenged plants had healthy root systems with an abundance of primary, secondary, and tertiary roots. We observed a 12 to >80% decrease in root fresh weight in symptomatic plants compared to plants that showed no disease symptoms. All isolates from infected plants were recovered and identities confirmed. Greenhouse managers and clinicians should be aware that *Calibrachoa* is susceptible to several important plant pathogens and should scout regularly for them in order to exclude them as much as possible from their production systems.**

INTRODUCTION

Calibrachoa (*Calibrachoa* × *hybrida*) is a solanaceous plant with some similarities to petunia (*Petunia* spp.) (Becktell et al., 2006). Both *Calibrachoa* and petunia do well in USA hardiness zones 10-11, and so are considered to be annuals in temperate regions. However, the two are different in that *Calibrachoa* is predominantly woody while petunias are mostly herbaceous (Reis et al., 2002.). Although *Calibrachoa* has become a popular ornamental since its introduction in the late 1990s by the greenhouse industry, little has been reported about its response to infection by plant pathogens that can be introduced through the use of infested substrates, contaminated tools, or contaminated water supply.

Many species of the water mold pathogens *Phytophthora* and *Pythium* that are pathogenic or can be pathogenic to nursery and greenhouse crops (Ali-Shtayeh et al., 1991; Hong and Moorman, 2005), were often detected in irrigation water from nursery and greenhouse effluents, and from dust and soil-mix particle samples from walkways, floors and beds within the greenhouse and also from seedling flats (Stephens et al., 1983).

Species of *Phytophthora* commonly cause root and crown rot in plants and at times foliar dieback when inoculum from the substrate or contaminated irrigation water is splashed onto susceptible stems and leaf tissue (Benson and von Broembsen, 2002; Bush, et al., 2006). *Pythium* species, on the other hand, infect plants in seedbeds, and seedlings and cuttings in the greenhouse, resulting in damping-off, decay and rotting of the seed before germination, or rotting of seedlings below the soil line (Moorman, 2002). Moreover, *Pythium* propagules in partially rotted plants and cuttings may be carried to

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other plants, infest the substrate mix, or contaminate the tools and equipment.

Botrytis cinerea (Pers.:Fr.), which causes Botrytis gray mold symptoms on flowers, leaves, and stems of many greenhouse ornamentals and can quickly render the plants unsalable, is favored by conditions common in many greenhouse settings, such as high relative humidity, standing moisture on the leaves, and the presence of susceptible plants adjacent to other plants (Sirjusingh and Sutton, 1996). Although disease symptoms may not be detected in the greenhouse during the production period, symptoms may become visible in the post-harvest phase as the conditions become more conducive for *B. cinerea* (Moorman and Chastagner, 2002; Dik and Wubben, 2004).

Verticillium dahliae (Kleb.) causes wilt disease on a number of solanaceous plants, like tomato and potato. Although *V. dahliae* has not been reported on *Calibrachoa*, the pathogen could be of interest due to the relatedness of the latter to potato and tomato.

These pathogens are present in many greenhouses under conditions that favor their survival and spread. However, their interaction with *Calibrachoa* is not well documented.

Our objective was to evaluate the interaction between *Calibrachoa* cuttings and a variety of greenhouse pathogens with different modes of infection and symptom expression. The pathogens tested included root rot pathogens, a foliar pathogen and a vascular wilt pathogen, with special emphasis on water mold pathogens. While it is not possible to evaluate all greenhouse pathogens, the ones selected for this study represent and highlight pathogen classes that are routinely recovered in greenhouses. This will provide a better understanding of the response of this popular plant to some of the pathogens that are commonly present during the production process.

MATERIALS AND METHODS

Preparation of Rooting Substrate and Plant Maintenance

Cuttings of *Calibrachoa* 'Colorburst Violet' were rooted in Oasis Growing Cubes (Smithers Oasis, Kent, OH), placed in a tray in a mist chamber, and allowed to root for 3 weeks before being transplanted to 10-cm pots containing peat-based substrate. This substrate consisted of sphagnum peat and perlite in a 70:30 (v:v) ratio and was amended with 75 g dolomite lime and 11.25 g micronutrient mix (Micromax, Scotts Co., Marysville, OH) per bushel. Soluble fertilizer (20:20:20 Peters Fertilizer, Scotts Co.) was applied weekly at a rate of 125 mg N-P-K L^{-1} in irrigation water to maintain fertility levels within the recommended range (Hamrick, 2003). Control of aphids, whiteflies and other greenhouse insects was done as needed. All plants were treated once with iron chelate (Fe-EDTA) 10% Fe due to yellowing of the leaves, which was attributed to iron deficiency (Fisher et al., 2005). At the end of each trial, shoots and roots from each treatment-replication were removed, washed and weighed in order to determine the effect of treatments on shoot and root fresh weights. The trial was repeated three times.

Pathogens Tested

The pathogens tested represent a diverse group with different modes of infection and symptom expression. They included *Pythium aphanidermatum*, *Pythium ultimum*, *Phytophthora cactorum*, *Phytophthora cinnamomi*, *Phytophthora citrophthora*, *B. cinerea* and *V. dahliae*. A *Phytophthora* species, isolated from infected petunia plants and identified as *Phytophthora nicotianae* was tested in the 2nd and 3rd trials. Cultures of species of *Phytophthora* and *Pythium* were grown for 7-10 days at 25°C on potato dextrose agar (PDA) (DIFCO, Sparks, MD). At the time of transplanting, four to five 1-cm diameter disks from the edge of the colonies were placed at the stem-root interface of each plant in a 10-cm pot and covered lightly with the rooting substrate to reduce desiccation. Control plants were treated with four to five 1-cm diameter disks of sterile PDA as described above. The pots were then placed in plastic trays containing about 2 cm of water depth for 48 h to allow for the release and spread of zoospores and sporangia. The pots were then removed from the trays, returned to the greenhouse bench, and watered as needed for the duration of the study.

Calibrachoa plants challenged with *V. dahliae* were grown in the substrate mix described above, and amended with microsclerotia of *V. dahliae* at a concentration of 10^5 g m^{-1} mix, prior to transplanting the rooting cuttings. No microsclerotia were added to the substrate mix for the control plants.

B. cinerea was isolated from infected begonia leaves and cultured for 7-10 days at 25°C on PDA. Plates were then flooded with sterilized distilled water and conidia were dislodged with a glass rod. The conidial suspension was adjusted to 2.5×10^5 ml^{-1} by use of a hemacytometer, and sprayed to run-off with a hand-held spray bottle. Control plants were sprayed with sterile distilled water as described above. The plants were then placed in a mist chamber for 48 h to create a high level of humidity in order to enhance infection and symptom development (Moorman and Chastagner, 2002). All challenged plants were arranged on greenhouse benches in a randomized complete block design with four blocks (1^{st} trial), and three blocks (2^{nd} and 3^{rd} trials).

Pathogens tested were re-isolated from symptomatic tissues of treated plants, plated on PDA, and their identity was confirmed with light microscopy based on the structure of sporangia for water molds, the color and structure of conidia and conidiophores for *B. cinerea* (Trolinger and Strider, 1985), and the verticillate arrangement of the phialides on the conidiophore and the shape and color of microsclerotia for *V. dahliae*.

Evaluation of Disease Severity

The susceptibility of *Calibrachoa* plants to the tested pathogens was determined by visual inspection of leaves for the presence of chlorotic and/or wilting symptoms for plants challenged with water mold pathogens, wilting for plants challenged with *V. dahliae* and the presence of necrotic lesions or gray mold for plants treated with *B. cinerea*. In the first trial, symptoms were rated on a scale of 1 to 6 with 1=healthy, symptomless plants; and 6=dead or dying plants. To allow for a wider range of symptom expression, the rating scale for disease severity for the 2^{nd} and 3^{rd} trials was adjusted to reflect percent of area infected of each plant with 0 indicating healthy, symptomless plant; and 100%=dead or dying plant. The effect of treatments on disease severity and shoot and root fresh weights was subjected to analysis of variance (ANOVA) using the Statistix 8 Analytical Software (Tallahassee, FL). Values for the area under the disease progress curve (AUDPC) were calculated for each plant using the mid-point analysis (Campbell and Madden, 1990). Separation of means was determined by LSD-test (P=0.05). Due to the use of two rating systems to evaluate disease severity, as exhibited by the AUDPC values, data for the first trial was analyzed separately (Fig. 1A), and averaged for the 2^{nd} and 3^{rd} trials (Fig. 1B).

RESULTS AND DISCUSSION

Disease Severity

In the 1^{st} trial, the response of *Calibrachoa* plants to tested pathogens fell into three distinct categories (Fig. 1A):

1) Severe disease symptoms on plants treated with *P. citrophthora* resulted in significantly (P<0.05) higher AUDPC values. These plants showed severe necrosis of the roots, with little to no secondary and no tertiary root development.
2) Moderate disease severity was observed on plants treated with *B. cinerea*, *P. cinnamomi* and *V. dahliae*. Plants in this category were characterized by roots with secondary development but no or little tertiary development.
3) Very little or no symptom development, resulting in AUDPC values that were not significantly different (P=0.05) from that of the control, was observed in plants challenged with *P. cactorum*, *P. ultimum* and *P. aphanidermatum*. Most of the plants in this category had well developed secondary and tertiary root systems.

In the 2^{nd} and 3^{rd} trials, *P. nicotianae* was the most aggressive among the pathogens tested (Fig. 1B), showing AUDPC values that were significantly higher

(P<0.05) than that of *P. citrophthora*, the most aggressive in the 1st trial. Aside from the high disease severity rating for plants challenged with *P. nicotianae,* we observed similar, although not identical, trends in disease severity for the pooled data of the 2nd and 3rd trials to that of the 1st trial. Plants challenged with *P. citrophthora* had the highest AUDPC values, followed by plants challenged with *P. cinnamomi* and *V. dahliae*. Plants treated with these pathogens showed extensive chlorosis and wilting and most of the plants failed to bloom. Disease severity in plants treated with *P. aphanidermatum* was not significantly different from that of the non-treated control plants (Fig. 1B).

Symptoms of Botrytis blight developed on the foliage of *Calibrachoa* plants within 3 days after inoculation with *B. cinerea*. Three weeks post-inoculation, plants treated with *B. cinerea* were not salable due to gray mold on the foliage. *V. dahliae* seemed to be aggressive on *Calibrachoa,* causing leaves to wilt 4-5 weeks after inoculation.

Data presented here show that *Calibrachoa* is prone to infection by a diverse group of pathogens. Three of the *Phytophthora* species tested (*P. nicotianae*, *P. citrophthora* and *P. cinnamomi*) killed the plants within 2-5 weeks. Similar findings were reported with *Phytophthora drechsleri.* In a fungicide trial (Dik and Wubben, 2004), *Phytophthora drechsleri* killed 50% of *Calibrachoa* plants in 3 weeks. In another study (Slinski et al., 2003), *Calibrachoa* plants challenged with *P. drechsleri* began to die about one week after inoculation and by week four, 88% of the plants were dead. In comparison, the two *Pythium* species tested in our study (*P. aphanidermatum* and *P. ultimum)* had little effect on *Calibrachoa*. We attribute the inability of the two *Pythium* species tested to infect *Calibrachoa* to the woody nature of *Calibrachoa* roots. *Phytophthora* species tested were known to infect woody plants (Elena and Paplomatas, 1999). In addition to wilting, chlorosis of lower leaves and stunting growth, we also observed a delay in the onset of blooming for plants treated with *P. nicotianae*, *P. citrophthora* and *P. cinnamomi.* Such characteristics will no doubt reduce the plants aesthetic value and render them unsalable.

Plant Vigor

For the 1st trial, the shoot fresh weight for the plants challenged with the two species of *Pythium* (*P. aphanidermatum* and *P. ultimum*) was higher and significantly different (P<0.05) from that of the plants challenged with *Phytophthora* (*P. cinnamomi)* trials (Fig. 2A). The shoot fresh weight was averaged for the 2nd and 3rd in comparison to plants challenged with *Phytophthora* spp., with plants challenged with *P. nicotianae* showing the lowest shoot biomass (Fig. 2B).

In trial 1, the plants treated with some of the pathogens had significantly larger shoot biomass than the un-inoculated control plants. We can only speculate about the reasons for this. It is possible that the treated plants received a chronic, low-level root stress causing the partitioning of more photosynthate into shoot growth resulting in larger shoot mass. However, this trend was not observed in trials 2 and 3.

The effect of the water molds as root pathogens was more pronounced in the root system of the treated plants. All the pathogen treatments were significantly different from the control (P<0.05). All water mold pathogens tested caused a decline in root fresh weight in comparison to the non-treated control and the plants challenged with the leaf pathogen *B. cinerea* (Fig. 2C and D). When *P. nicotianae* was tested in the 2nd and 3rd trials, plants challenged with this pathogen had the lowest root weight. We observed 86-89% reduction in root fresh weight in plants treated with *P. nicotianae* in comparison to plants treated with the two *Pythium* species; and 80-85% reduction in plants treated with the other three *Phytophthora* species (Fig. 2D).

CONCLUSION

Response of *Calibrachoa* plants to the pathogens tested fell into distinctive groups that ranged from no noticeable disease symptoms to chlorosis, stunting, wilting, and death in a matter of weeks. The few plants that managed to survive were covered with gray

mold, showed a delay in blooming or failed to bloom all together. Such appearances reduce the plants' attractiveness and salability. This will no doubt add to the cost of production for the growers.

Greenhouse managers and clinicians should be aware that *Calibrachoa* is susceptible to several important plant pathogens and should scout regularly for them. Those tested here may be introduced through the use of infested soil, contaminated tools, infected cuttings, and contaminated irrigation water. Steps should be taken, particularly by plant propagators, to exclude these pathogens as much as possible from their production systems.

ACKNOWLEDGEMENTS AND DISCLAIMER

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Figures

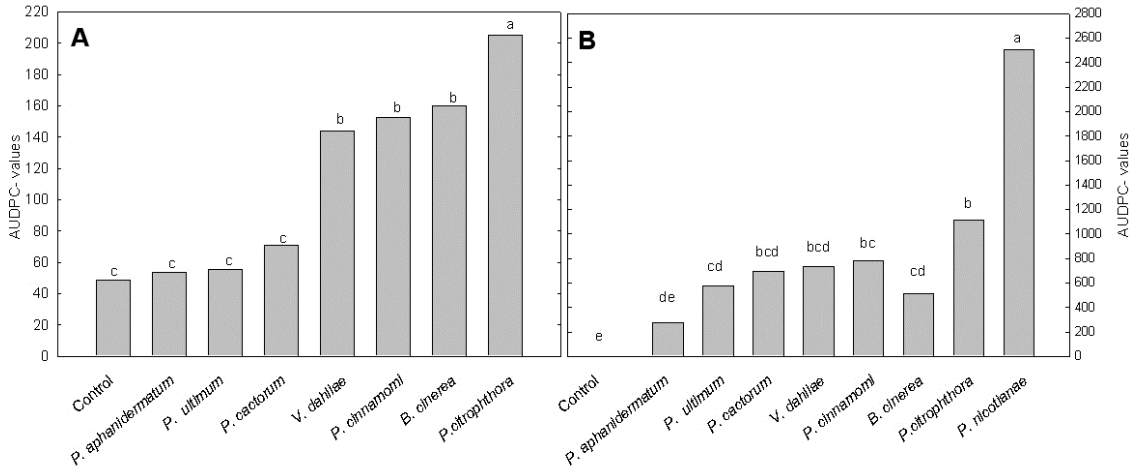

Fig. 1. Disease severity in response to challenge with pathogens. Symptoms were visually rated on a scale of 1 to 6 (A), and 0 to 100% (B). The area under disease progress curve (AUDPC) was calculated using the mid point analysis. Bars with the same letter are not significantly different ($P<0.05$). Treatments included un-inoculated control, *Pythium aphanidermatum*, *Pythium ultimum*, *Phytophthora cactorum*, *Phytophthora cinnamomi*, *Phytophthora citrophthora*, *Phytophthora nicotianae*, *Botrytis cinerea* and *Verticillium dahliae*.

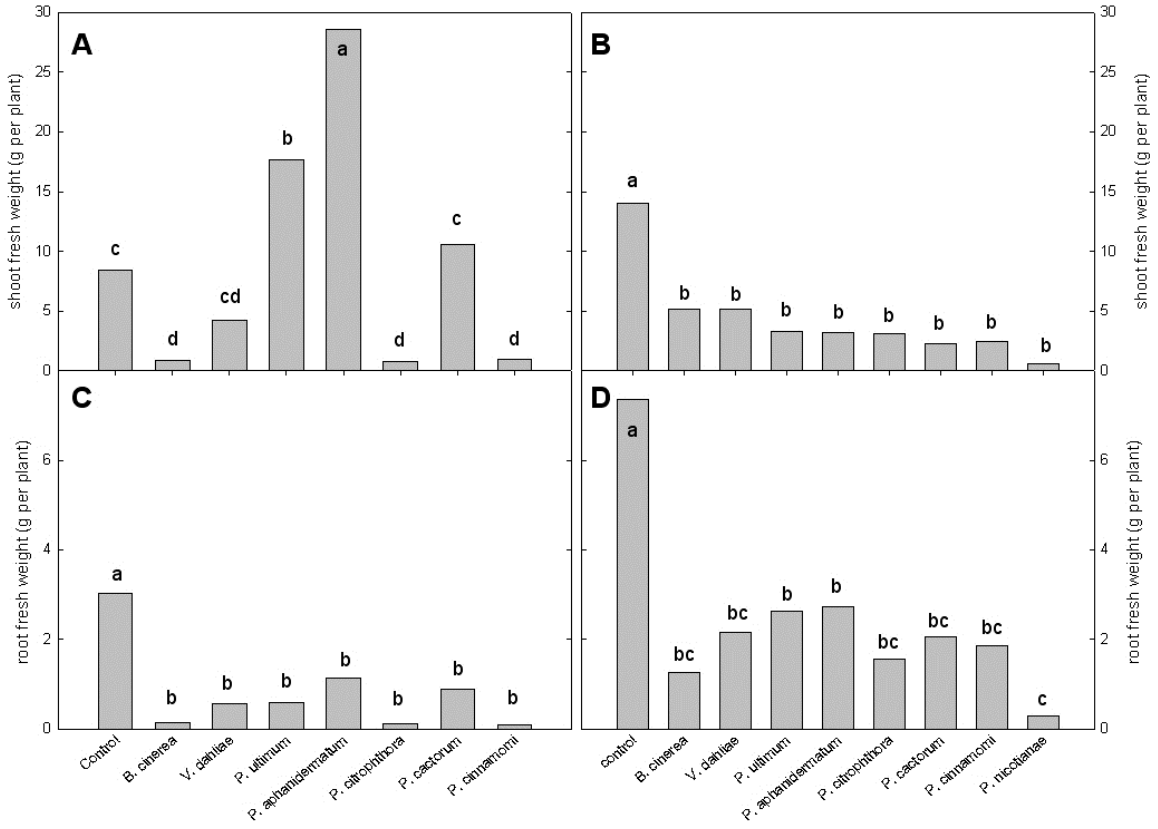

Fig. 2. Fresh weight (g) of *Calibrachoa* shoot system at harvest for the 1st trial (A) and the 2nd and 3rd trials (B) and root system for the 1st trial (C) and the 2nd and 3rd trials (D). Weight for each treatment was pooled and averaged for the 2nd and 3rd trials. Different letters indicate significant difference (P<0.05) based on LSD. Treatments included un-inoculated control, *Pythium aphanidermatum*, *Pythium ultimum*, *Phytophthora cactorum*, *Phytophthora cinnamomi*, *Phytophthora citrophthora*, *Phytophthora nicotianae*, *Botrytis cinerea* and *Verticillium dahliae*.