Evaluating a Novel Method to Introduce a Mycorrhizal-like Fungus, *Piriformospora indica*, via an Inoculated Rooting Substrate to Improve Adventitious Root Formation

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Additional index words. **arbuscular mycorrhizal fungi, auxin, *Crossandra infundibuliformis*, *Dahlia* ×*hybrida*, endophyte, *Euphorbia pulcherrima*, *Lantana camara*, *Osteospermum* ×*hybrida*, perlite, propagation, rooting hormone, *Scaevola aemula*, unrooted cuttings**

Summary. **The mycorrhizal-like fungus *Piriformospora indica* has demonstrated potential to enhance adventitious root formation (ARF) and increase root weight when applied to the propagation substrate of unrooted cuttings (URCs). Experiments were conducted to determine the effect of *P. indica* on ARF of six floriculture species: cape daisy (*Osteospermum* ×*hybrida* 'Side Show White'), crossandra (*Crossandra infundibuliformis* 'Orange Marmalade'), dahlia (*Dahlia* ×*hybrida* 'Dahlietta Margaret'), lantana (*Lantana camara* 'Lucky Yellow'), poinsettia (*Euphorbia pulcherrima* 'Champion Fire', 'Premium White', and 'Supreme Bright Red'), and scaevola (*Scaevola aemula* 'Fan Dancer'). The treatments consisted of a peat-based growing medium that contained 5%, 10%, 20%, or 30% perlite colonized with *P. indica* (volume of colonized perlite/volume of growing medium). Inoculation with 10% to 20% colonized perlite significantly increased the root fresh weight for one cultivar, Supreme Bright Red poinsettia, whereas the 20% colonized perlite treatment resulted in a decrease in root fresh weight of scaevola and cape daisy. Rooting percentage of 'Champion Fire' poinsettia and dahlia showed a benefit at specific *P. indica* treatments, whereas cape daisy displayed a decrease in rooting percentage. Conventional rooting hormone treatment showed beneficial responses for dahlia, and 'Champion Fire' poinsettia rooting percentage and a negative response on lantana root fresh weight. This project demonstrates a novel method for delivering a root endophyte to URCs for the purpose of increasing ARF, and the results suggest the potential for *P. indica* usage for ARF enhancement. However, the results were not consistently beneficial across the eight cultivars tested, so growers would need to conduct in-house trials to identify the best treatments across a range of crop species and cultivars.**

Shoot tip cuttings are a common means of asexual propagation of ornamental plants. After a cutting is removed from the stock plant, it must form adventitious roots to become a new plant. Adventitious root formation is a complex process regulated by environmental and endogenous factors. Higher endogenous auxin concentrations or exogenously applied auxin can speed up the process of ARF (Went et al., 1938). Although most herbaceous annual species root easily from URCs, some species benefit from the exogenous application of auxin (i.e., rooting hormone). Rooting hormone application is a common propagation technique that is typically accomplished by dipping the base of the cutting stem into an auxin powder or solution before inserting the cutting stem into the propagation medium (Preece, 2003).

Fungal symbionts such as arbuscular mycorrhizae (AM) and the mycorrhizal-like fungus *P. indica* have been examined for their ability to enhance ARF and increase root weight when applied to the propagation substrate of URCs (Druege et al., 2007). *Piriformospora indica*, a root endophyte, was originally isolated from the Thar Desert in India (Verma et al., 1998). Root endophytes reside only in the roots, whereas endophytic fungi are able to spread throughout the plant. *Piriformospora indica* is commonly referred to as an AM-like plant symbiont although it actually is an endophyte. *Piriformospora indica* has been shown to increase nutrient uptake (Gosal et al., 2010), disease suppression (Deshmukh and Kogel, 2007), and biomass production (Baldi et al., 2010). Unlike AM fungi, *P. indica* can grow without a plant host. This characteristic is advantageous for mass production and potential commercialization because production does not require the additional complexity of growing plants to rear the fungus. *Piriformospora indica* also colonizes plants that AM fungi cannot, such as members of the Brassicaceae family (Deshmukh, 2006). *Piriformospora indica* is a Basidiomycota yet expresses similar characteristics to the Glomeromycota, which contains all AM fungi, yet endophytic fungi may occupy all phyla of the fungus kingdom.

Mutualistic fungi, such as AM fungi and *P. indica*, added to propagation media have been shown to increase root weight and root initiation in a range of plant species, including dwarf umbrella tree (*Schefflera arboricola*), anglojap yew (*Taxus* ×*media*), rose (*Rosa* 'Scarlet Cupido'), bearberry (*Arctostaphylos uva-ursi*), geranium (*Pelargonium* sp.), malabar nut (*Adhatoda vasica*), and poinsettia (Druege et al., 2007; Fatemeh and Zaynab, 2014; Rai and Varma, 2005; Scagel, 2004a, 2004b; Scagel et al., 2003). These studies suggest that *P. indica* may be an alternative

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This research was supported by the Clemson University Experiment Station Project #1700539 (Tech. Contribution #6626), the USDA-ARS Floriculture and Nursery Research Initiative, and the Fred C. Gloeckner Foundation.

We thank Vijay Rapaka for valuable comments and Kelly Lewis for technical assistance.

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https://doi.org/10.21273/HORTTECH03914-17

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
10	%	$g \cdot L^{-1}$	0.1
29.5735	fl oz	mL	0.0338
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
28.3495	oz	g	0.0353
1	ppm	$mg \cdot L^{-1}$	1
10.7639	W/ft^2	$W \cdot m^{-2}$	0.0929
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

to chemical auxin application while also imparting additional benefits such as disease suppression or nutrient uptake.

The proposed mechanism for *P. indica* effects on ARF is that *P. indica* not only produces and excretes auxin but also upregulates auxin biosynthesis genes inside the plant cell (Dutra et al., 1996; Felten et al., 2010; Hilbert et al., 2012; Lee et al., 2011; Ludwig-Müller and Güther, 2007; Schafer et al., 2009; Vadassery et al., 2008). Levels of auxin production induced by *P. indica* vary with plant host (Sirrenberg et al., 2007; Sukumar et al., 2013; Vadassery et al., 2008). For example, it was found that auxin levels in *P. indica*–colonized chinese cabbage (*Brassica campestris* ssp. *chinensis*) roots were 2-fold compared with that in the control (Lee et al., 2011).

The objectives of this project were to test a novel method of introducing *P. indica* to URCs in propagation via a colonized rooting substrate and to quantify the subsequent effect of the *P. indica*–colonized rooting substrate on the ARF of the URCs of eight species and cultivars that are commonly propagated in the floriculture industry.

Materials and methods

Inoculation methodology. *Piriformospora indica* was obtained from P. Franken through the U.S. Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS application no. P526-120104-009) as a single culture on agar medium. *Piriformospora indica* was maintained in culture on sterile potato dextrose agar (39 g·L^{-1}; Becton, Dickson and Co., Sparks, MD) in 9-cm-diameter petri dishes. A 1-cm-diameter plug from the youngest growth region of *P. indica* was placed in the center of each petri dish and grown for 14 d at 21 °C in the dark. For liquid culture, potato dextrose broth (24 g·L^{-1}; Becton, Dickson and Co.) was sterilized in 200-mL flasks. Once cooled, five 1-cm-diameter plugs from the youngest growth region of *P. indica* were aseptically transferred into each flask. The flasks with inoculum were placed on an orbital shaker and maintained at 150 rpm for 21 d in the dark at 21 °C. Twenty mushroom spawn incubation bags, measuring 21 × 8.25 × 4.75 inches (Fungi Perfecti, Olympia, WA) with a microporous filter patch, were each filled with 5 L of perlite (S&B Industrial Minerals, Vero Beach, FL), moistened with 3 L of potato dextrose broth (24 g·L^{-1}), and sterilized for 1 h at 121 °C. *Piriformospora indica* grown in potato dextrose broth was blended aseptically with a magnetic stir bar and stirrer for 30 s to break up the fungal pellets. Each bag was inoculated with 25 mL of blended *P. indica*, sealed with an impulse sealer, and grown at 25 °C in the dark. After 21 d, when the perlite was fully colonized, it was incorporated into a commercial soilless growing medium (Fafard Germination Mix; Sun Gro Horticulture, Belton, SC) to function as both a source of inoculum and a media component. The commercial growing medium consisted of 64% fine-grade peat, 22% fine perlite, and 14% vermiculite. At the start of the experiment, the growing medium had a pH of 6.1, an electrical conductivity of 0.86 mS·cm^{-1}, and a starter charge of nutrients that included nitrogen, phosphorus, potassium, magnesium, and calcium at 29, 8, 48, 55, and 47 mg·L^{-1}, respectively.

Experimental treatments. Treatments consisted of noncolonized media, noncolonized media plus rooting hormone (Hormodin #1; OHP, Mainland, PA) applied to the URCs, and four rates of *P. indica*–colonized media (Table 1). The rooting hormone was a powder formulation that contained 0.1% indole-3-butyric acid and was applied as a dip to the bottom 1 cm of the stem before inserting the URC into the propagation medium. To keep the growing medium matrix consistent throughout the experiments, all treatments consisted of growing media containing 30% sterilized perlite and 70% commercial growing media measured by volume. This amount of perlite was used because commercial growing media contain no more than 30% perlite on a volume basis. Each *P. indica* treatment contained different percentages of colonized perlite, whereas the total percentage of perlite in the growing media was constant at 30%. For example, the 10% colonized perlite treatment contained 10% colonized perlite, 20% sterilized noncolonized perlite, and 70% growing media, whereas the 20% colonized perlite treatment contained 20% colonized perlite, 10% sterilized noncolonized perlite, and 70% growing media. The 30% colonized perlite treatment contained 30% colonized perlite and 70% growing media.

The specific treatments varied with each plant species. For example, poinsettia treatments consisted of noncolonized media, noncolonized media plus rooting hormone applied to the URCs, and media inoculations with each of three rates of *P. indica* (10%, 20%, or 30% colonized perlite). Crossandra and cape daisy URC treatments consisted of noncolonized media and media inoculations with each of three rates of *P. indica* (10%, 20%, or 30% colonized perlite). Scaevola URC treatments consisted of noncolonized media and media inoculations with each of three rates of *P. indica* (5%, 10%, or 20% colonized perlite). Dahlia and lantana treatments consisted of noncolonized media, noncolonized media plus rooting hormone applied to the URCs, and media inoculations with each of three rates of *P. indica* (5%, 10%, or 20% colonized perlite). For poinsettia, 28 URCs were used per treatment, whereas 50 URCs per treatment were used for all other species. Each experiment was repeated twice for each species.

Plant materials. Unrooted cuttings of 'Side Show White' cape daisy; 'Orange Marmalade' crossandra; 'Dahlietta Margaret' dahlia; 'Lucky Yellow' lantana; 'Champion Fire', 'Premium White', and 'Supreme Bright Red' poinsettia; and 'Fan Dancer' scaevola were delivered via airfreight by 2-d delivery. All were URCs of commercial quality. The propagation environment for all URC species consisted of a mist system, a retractable shade curtain (55% light reduction) that was engaged when solar radiation measured outdoors exceeded 450 W·m^{-2}, heating/ventilation set points at 22 °C/26 °C, and bottom heat that provided a growing media minimum temperature of 24.4 ± 1.2 °C. The URCs received no additional fertilizer beyond the initial nutrients provided in the growing media starter charge.

Data collection. Cuttings were evaluated for ARF on different dates for each cultivar and experiment depending on the timing of root initiation. Cape daisy cuttings were measured at 19 and 21 d after sticking, crossandra at 20 and 21 d, dahlia

Table 1. The propagation medium components used in treatments to examine the effect of a mycorrhizal-like fungus, *Piriformospora indica*, on adventitious root formation of unrooted cuttings. All treatments contained a 70% (volume) commercial growing medium (Fafard Germination Mix; Sun Gro Horticulture) mixed with an additional 30% sterilized perlite. The commercial growing medium consisted of 64% fine-grade peat, 22% fine perlite, and 14% vermiculite. The perlite component contained different percentages (by volume) of perlite colonized and noncolonized with *P. indica*. The rooting hormone control was a powder formulation containing 0.1% indole-3-butyric acid applied to the base of the cutting stem.

Treatment	Growing medium (% vol)	Perlite (% vol)	
		Colonized	Noncolonized
Noncolonized control	70	0	30
Noncolonized control + rooting hormone	70	0	30
5% colonized perlite	70	5	25
10% colonized perlite	70	10	20
20% colonized perlite	70	20	10
30% colonized perlite	70	30	0

at 30 and 33 d, lantana at 14 and 16 d, poinsettia at 15 and 21 d, and scaevola at 16 and 18 d. At harvest, root fresh weight and the percentage of rooted cuttings were quantified. Root fresh weight was obtained after gently rinsing the roots with water and then excess water was absorbed with a paper towel. Roots were sampled to determine root colonization with *P. indica*. To accomplish this, roots were submerged in 10% potassium hydroxide (w/v) for 3 h in a water bath at 60 °C to remove any pigmentation. Roots were then rinsed with deionized water twice and stained with 0.05% trypan blue for 24 h. Roots were mounted in 50% glycerol (Phillips and Hayman, 1970) and observed with a compound light microscope (Motic model BA210; Speed Fair Co., Hong Kong, China). Colonization was confirmed to be successful by the presence of chlamydospores.

Data were analyzed using JMP (version 10; SAS Institute, Cary, NC). Analysis of variance was performed with means separation by Tukey's honestly significant difference test ($P \leq 0.05$). Treatments were provided on the propagation bench in a completely randomized design.

Results and discussion

Root fresh weight of 'Supreme Bright Red' poinsettia cuttings increased significantly when colonized with *P. indica* at the 10% and 20% perlite inoculation levels in comparison with the noncolonized control (Table 2). 'Supreme Bright Red' poinsettia propagated with the *P. indica* treatments also had higher rooting percentages than the hormone control (Table 3). Root fresh weight of 'Champion Fire' poinsettia did not respond significantly to *P. indica* treatments or the rooting hormone treatment. Significantly higher rooting percentages were produced by the 'Champion Fire' poinsettia 20% perlite treatment and by the rooting hormone control when compared with the noncolonized control. 'Premium White' poinsettia did not respond to *P. indica* treatments or the rooting hormone treatment. For poinsettia, no negative responses were observed with the *P. indica* treatments, and positive responses were observed on some *P. indica* treatments compared with the hormone treatments and the noninoculated treatments. Therefore, *P. indica* can be a treatment to enhance ARF during poinsettia propagation. Similarly, Druege et al. (2007) found that *P. indica* enhanced root number and length of poinsettia cuttings.

Cape daisy and scaevola were the only species to show a negative response to *P. indica* treatments. Specifically, the 20% colonized perlite treatment had a lower root fresh weight than the control for cape daisy and scaevola (Table 2), whereas the 10% and 20% colonized perlite treatments had negative effects on the rooting percentage of cape daisy compared with the control (Table 3).

The *P. indica* treatments did not affect dahlia in terms of mean root weight (Table 2), although the plants with rooting hormone displayed a significant increase in the rooting percentage (Table 3). Rooting percentage also increased with the 5% and 20% colonized perlite treatments. Dahlia had the lowest rooting percentage of all the species in this study with the noncolonized control at only 25.5% success, whereas the hormone control yielded 57% rooted cuttings. In general, the rooting hormone treatment was the best option overall among the treatments for dahlia.

Crossandra and lantana did not significantly respond to *P. indica* treatments in terms of fresh root weight or rooting percentage. However, application of rooting hormone on lantana negatively affected root fresh weight compared with the noncolonized control and the 5% and 10% colonized perlite treatments.

Inoculation of URCs with *P. indica* has not yet been carried out commercially, but this research demonstrates a novel method of inoculating URCs before root formation in propagation in a manner that is commercially feasible. In a previously reported laboratory experimentation, solutions containing mycelium fragments of *P. indica* were blended into clay granules before propagating the URCs (Druege et al., 2007). Clay granules are not a commercially viable propagation medium, so our method sought to provide a practical technique for introducing the *P. indica* inoculum to the URCs in a standard commercial growing medium. Sterilized perlite was inoculated with *P. indica* that colonized the bags in 3 weeks. The inoculated perlite was then easily incorporated into a standard peat-based growing medium in which the URCs were inserted. Colonization of the URCs with *P. indica* was confirmed in all of the inoculated treatments, whereas the absence of *P. indica* colonization was also confirmed in all noninoculated treatments.

Table 2. The effect of *Piriformospora indica* inoculation rate in the propagation media on root fresh weight at harvest of six species: cape daisy, crossandra, dahlia, lantana, poinsettia, and scaevola. The components of the propagation media are described in Table 1. The two control groups were propagated in a medium that did not contain perlite colonized with *P. indica*. The rooting hormone control was a powder formulation containing 0.1% indole-3-butyric acid applied to the base of the cutting stem; *n* = 56 for poinsettia, *n* = 100 for all other species.

		Noncolonized control	Rooting hormone control	*P. indica*–colonized perlite (% vol)			
				5	10	20	30
Species	Cultivar	Root fresh wt (g)[z]					
Cape daisy	Side Show White	0.157 a[y]	—[x]	—	0.156 a	0.046 b	0.196 a
Crossandra	Orange Marmalade	0.299 a	—	—	0.310 a	0.299 a	0.276 a
Dahlia	Dahlietta Margaret	0.055 b	0.187 a	0.056 b	0.053 b	0.060 b	—
Lantana	Lucky Yellow	0.159 ab	0.106 c	0.190 a	0.164 ab	0.140 bc	—
Poinsettia	Champion Fire	0.255 a	0.233 a	—	0.265 a	0.305 a	0.231 a
	Premium White	0.381 a	0.439 a	—	0.398 a	0.400 a	0.417 a
	Supreme Bright Red	0.286 b	0.392 ab	—	0.417 a	0.452 a	0.383 ab
Scaevola	Fan Dancer	0.164 a	—	0.126 ab	0.141 a	0.082 b	—

[z]1 g = 0.0353 oz.
[y]Numbers followed by the same letter within rows indicate no significant differences between treatments according to Tukey's honestly significant difference test ($P \leq 0.05$).
[x]Dashes indicate data were not recorded.

Table 3. The effect of *Piriformospora indica* inoculation rate in the propagation media on the percentage of rooted cuttings at harvest of six species: cape daisy, crossandra, dahlia, lantana, poinsettia, and scaevola. The components of the propagation media are described in Table 1. The two control groups were propagated in a medium that did not contain perlite colonized with *P. indica*. The rooting hormone control was a powder formulation containing 0.1% indole-3-butyric acid applied to the base of the cutting stem; *n* = 56 for poinsettia, *n* = 100 for all other species.

		Noncolonized control	Rooting hormone control	*P. indica*–colonized perlite (% vol)			
				5	10	20	30
Species	Cultivar	Rooted cutting (%)					
Cape daisy	Side Show White	86 a[z]	—[y]	—	72 b	46 c	74 ab
Crossandra	Orange Marmalade	100 a	—	—	100 a	100 a	100 a
Dahlia	Dahlietta Margaret	25 c	57 a	42 ab	35 bc	44 ab	—
Lantana	Lucky Yellow	98 a	95 a	97 a	95 a	98 a	—
Poinsettia	Champion Fire	79 b	96 a	—	95 ab	97 a	81 ab
	Premium White	89 a	92 a	—	91 a	89 a	96 a
	Supreme Bright Red	89 ab	82 b	—	98 a	95 a	97 a
Scaevola	Fan Dancer	82 a	—	86 a	82 a	76 a	—

[z]Numbers followed by the same letter within rows indicate no significant differences between treatments according to Tukey's honestly significant difference test ($P \leq 0.05$).
[y]Dashes indicate data were not recorded.

This technique would be readily scalable for commercial implementation.

Rooting hormone clearly can be beneficial to ARF as this research demonstrates; however, rooting hormone provided at the wrong concentrations can have detrimental responses as was also observed (Table 2). Similarly, this research demonstrates how *P. indica* can promote, inhibit, or confer no response in terms of ARF depending on the concentration, species, and cultivar. Druege et al. (2007) also reported that *P. indica* promoted ARF on two of three floriculture species tested. Geranium and poinsettia root number and length were enhanced, whereas petunia (*Petunia ×hybrida*) did not show positive responses from inoculation. Scagel et al. (2003) reported that arbuscular mycorrhizal fungal inoculum in the propagation media did not increase ARF of kinnikinnick (*A. uva-ursi*) cuttings, whereas adding arbutoid mycorrhizal fungus or inoculum consisting of colonized root fragments of kinnikinnick to the propagation media did increase ARF. Vosatka et al. (1999) reported no benefit observed on 'Peterstar Red' poinsettia cuttings propagated in a medium containing arbuscular mycorrhizal fungal inoculum containing spores, mycelium, and colonized root fragments, whereas verbena (*Verbena* sp.) exhibited increased shoot growth and flowering.

Commercial growers are often looking for rooting treatments that are effective across a range of species and cultivars so that each crop does not require a unique treatment. When applying a treatment to a wide range of species and cultivars, it is not usually possible to test every single one. So, the grower simply expects that the treatment has either a beneficial response or no response, but a negative response is certainly undesirable. As the data in this study demonstrate, both rooting hormone and *P. indica* can benefit ARF; however, negative results are possible with each approach.

In general, rooting substrate inoculated with *P. indica* demonstrated the most potential benefit in ARF with poinsettia. In dahlia, results from *P. indica* were mostly positive compared with those from the noncolonized control; however, the rooting hormone treatment proved to be the best overall treatment. Lantana also yielded some positive results, although the magnitude of the response was

rather small. Scaevola and crossandra showed no benefit to *P. indica* treatment, and the response of cape daisy was mostly negative.

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