Efficacy of Plantpro 45 as a Seed and Soil Treatment for Managing Fusarium Wilt of Basil

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ADDITIONAL INDEX WORDS. *Ocimum basilicum*, *Fusarium oxysporum* f.sp. *basilici*, iodine

SUMMARY. Plantpro 45, an iodine-based compound, was evaluated as a seed treatment for management of fusarium wilt caused by *Fusarium oxysporum* f.sp. *basilici* on basil (*Ocimum basilicum*) in greenhouse assays and for effects on growth of the pathogen in vitro. Plantpro 45 at a concentration of 300 mg·L⁻¹ (ppm) prevented fusarium hyphal growth in vitro. Seed treatments of 800 to 1000 mg·L⁻¹ eliminated fungal contamination of seed and increased germination by 27% compared to the nontreated control. Basil transplants grown from seed treated with 400, 800, and 1000 mg·L⁻¹ were significantly taller, weighed more, exhibited larger leaf area, and had reduced wilt severity in the greenhouse compared to the nontreated control. Transplants grown in soil treated with increasing concentrations of Plantpro 45 had correspondingly decreased wilt severity, regardless of whether or not the seeds had been previously treated

with Plantpro 45. Further research and optimization of soil and foliar applications in combination with seed treatments are needed to provide a complete program for management of fusarium wilt of basil.

Sweet basil, a native of central Asia and northwestern India, is one of the principal fresh-market herbs in the world (Davis, 1998). Basil is an economically important herb crop in several Mediterranean countries with Italy being the largest producer (Garibaldi et al., 1997). Hawaii is currently the largest U.S. producer of basil with over 22.3 ha (55 acres) valued at over \$3.6 million (National Agricultural Statistic Service, 2001). Basil production in Florida is increasing because of the high cash value, small acreage requirement, and ideal growing conditions (Hochmuth and Davis, 1999). Domestic basil yields as much as three times the price of imported basil and is highly acclaimed for its rich color, sweet flavor, and uniformity of milling (Food Product Design, 2000).

Fusarium wilt and crown rot of basil is an emerging disease that has become a serious problem in commercial production worldwide. Fusarium wilt of basil has been reported in Russia (Kvartskhara, 1957), Italy (Grasso, 1975), France (Mercier and Pionnat, 1982), Canada, Australia, and Israel (Trueman and Wick, 1995). In the U.S., the disease was first discovered in Massachusetts (Wick and Haviland, 1992) and later in eleven other states (Trueman and Wick, 1995).

On young seedlings and mature basil plants, *F. oxysporum* f. sp. *basilici* is capable of infecting the crown, root, and above-ground plant parts (Gamliel et al., 1996). Infected plants show epinasty, asymmetric growth, curling, chlorosis, vascular discoloration, and wilt, starting from the apical leaves. Necrosis follows and extends basipetally from the vegetative apex to the entire plant, while the roots and the base of the stem remain symptomless until the final disease stage. Young plants will die 4 to 7 d after the first symptoms develop while older plants may survive longer (Garibaldi et al., 1997). Host resistance has been successfully bred into basil (Reuveni et al., 1997), but due to horticultural characteristics, *F. oxysporum* f. sp. *basilici* resistant cultivars have not

yet attained the popularity of susceptible cultivars.

Basil seed lot contamination worldwide averages between 1% and 27% (Trueman and Wick, 1995). Initial introduction of the pathogen into fields is typically by *F. oxysporum* f. sp. *basilici* infested seed (Kvartskhara, 1957; Trueman and Wick, 1995). Basil seed treatment with sodium hypochlorite or hot water has been attempted. However, sodium hypochlorite is only effective at low levels of infestation (Martini and Gullino, 1991) and control with hot water treatment is highly variable due to the heat sensitivity of basil seed (Wick 1997). To date, there are no consistently effective chemical seed treatments registered for the control of fusarium wilt on basil (Garibaldi et al., 1997; Minuto, et al., 1997; Wick, 1997).

A primary problem with chemical soil treatments for control of pathogens on young basil plants is phytotoxicity (Keinath, 1994). Soil disinfection with methyl bromide has provided some control of fusarium wilt of basil in the field without phytotoxicity (Garibaldi, et al., 1997). However, even with methyl bromide fumigation, once fields become infested, growers often experience complete crop loss due to the rapid spread of inoculum. Because methyl bromide was found to be an ozone depleting substance, it will not be available for use in the U.S. after 2005. The rapid aerial spread of inoculum and the loss of methyl bromide amplify the importance of using pathogen-free seed.

Plantpro 45 (PP45) (Ajay North America, Powder Springs, Ga.), a water soluble iodine-based compound, is currently being tested as a reduced-risk methyl bromide alternative for the control of soilborne pathogens. PP45 at ≥5 mg·L⁻¹ a.i. suppressed conidial germination of *Fusarium moniliforme* in vitro, and at 250 mg·L⁻¹ reduced hyphal growth from infested maize (*Zea mays*) seed by 50% without reducing seedling survival or development (Yates et al., 2000).

The objectives of this research were to 1) determine if PP45 adversely affects *F. oxysporum* f. sp. *basilici* hyphal growth, 2) evaluate PP45 as a seed treatment for management of *F. oxysporum* f. sp. *basilici* on thai basil (bai grapao, *Ocimum basilicum* var. *sanctum*) seed, and 3) evaluate PP45 as a soil treatment for effects on seedling development and fusarium wilt incidence and severity.

This research was performed under a Cooperative Research and Development Agreement (CRADA No. 58-3K95-9-739) with the USDA, ARS and Ajay North America, LLC. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture. The authors would like to acknowledge Amanda Rinehart for assistance with data analysis and manuscript preparation, Sissy Ivey for technical assistance, and George Vogel for providing expertise in cultivation and disorders of basil.

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Materials and methods

EFFECTS ON HYPHAL GROWTH. A virulent *F. oxysporum* f. sp. *basilici* isolate was obtained from symptomatic stem tissue of thai basil grown in western Florida. Pathogenicity was confirmed and virulent fungal isolates were submerged in 50:50 (v/v) water and glycerol solution and stored at -80°C (-112°F). One milliliter (0.034 fl oz) of sterile deionized water or PP45 solution was spread over the potato dextrose agar (PDA) plates and allowed to dry in the dark overnight before inoculation with *F. oxysporum* f. sp. *basilici*. Plugs [4-mm (0.16-inch)] from 7-d-old cultures were inverted onto 80% PDA plates amended with 0, 30, 60, 120, 240, and 300 $\text{mg}\cdot\text{L}^{-1}$ of active ingredient PP45. Treatments were replicated three times, arranged in a completely randomized design, and incubated at 25°C (77°F) under 8 h light/16 h dark. After 14 d, colony radius was measured for each plug. Fungal plugs were excised from treated plates and placed onto nontreated PDA plates to observe regrowth of the fungus.

SEED GERMINATION AND CONTAMINATION. Two milliliters of sterile deionized water or PP45 solution were applied to 1 g (0.035 oz) of basil seed at concentrations of 0, 50, 100, 200, 400, 800, and 1000 $\text{mg}\cdot\text{L}^{-1}$ and mixed thoroughly. Seeds were allowed to absorb all liquid and were spread out to dry overnight, after which 20 to 25 seeds from each treatment were placed onto petri plates containing either water agar or Komada's medium (Komada, 1975). Treatments were replicated five times, arranged in a completely randomized design, and incubated at 25°C with 12 h of light/12 h dark. After 5 d, the percentage of seed exhibiting fungal growth was recorded for both media. After 14 d, the percentage of germinated seed was determined.

SEEDLING, TRANSPLANT, AND DISEASE DEVELOPMENT. Nontreated seed and seed treated with 400, 800, and 1000 $\text{mg}\cdot\text{L}^{-1}$ PP45 as previously described were planted into 128-cell styrofoam trays (Speedling, Sun City, Fla.) containing a peat-based transplant medium (Speedling Peat Lite Mix, Bushnell, Fla.). At 21 d after planting, shoot height, total plant fresh weight, and leaf area measurements were recorded for 20 arbitrarily selected seedlings per treatment. After 28 d, five plants from each seed treatment were

individually transplanted into 10-cm (4-inch) pots containing soil from a commercial basil production field in western Florida with a history of severe fusarium wilt. Soil was treated with PP45 at 0, 80, 120, and 300 $\text{mg}\cdot\text{L}^{-1}$ 14 d before planting and watered daily. Plants were fertilized once per week with Peter's 20-20-20 solution (20N-8.7P-16.6K; United Industries, St. Louis, Mo.). Wilt ratings were recorded 19 d after transplanting using a scale where 1 = 0% to 9% wilt, 2 = 10% to 24% wilt, 3 = 25% to 49% wilt, 4 = 50% to 74% wilt, and 5 = 75% to 100% of plant material wilted. Treatments were replicated five times and arranged in a completely randomized design in the greenhouse.

All experiments were repeated at least once. Data were analyzed using the general linear model procedure of SAS (SAS, Institute Inc., Cary, N.C.) and the least significant difference test for separation of treatment means at $P \leq 0.05$.

Results

In vitro mycelial growth of *F. oxysporum* f. sp. *basilici* on PDA decreased linearly with increasing concentrations of PP45 ($R^2 = 0.80$) (Fig. 1). PP45 applied to agar at 300 $\text{mg}\cdot\text{L}^{-1}$ completely inhibited growth (Fig. 1). However, when transferred to nontreated PDA plates, all colonies grew normally except fungi whose growth had previously been completely inhibited (data not shown).

On both fusarium selective and nonselective media, fungal contamination of seed decreased with increasing concentrations of PP45 ($R^2 = 0.89$ for

Komada's medium, $R^2 = 0.93$ for water agar) (Fig. 2A and 2B). The two highest concentrations of PP45 applied to seed (800 and 1000 $\text{mg}\cdot\text{L}^{-1}$) were 100% fungicidal when seed were treated and then placed on Komada's medium and on water agar (Fig. 2A and B). Fungal contamination of nontreated basil seed averaged 92% on the nonselective water agar (Fig. 2B). Correspondingly, basil seed germination on water agar increased with increasing concentrations of PP45, from about 53% in nontreated seed to about 74% in seed treated with 1000 $\text{mg}\cdot\text{L}^{-1}$ PP45 ($R^2 = 0.63$) (Fig. 3).

Seedlings produced from seed treated with 800 and 1000 $\text{mg}\cdot\text{L}^{-1}$ PP45 were significantly taller and weighed more than plants grown from nontreated seed (Table 1). Seedlings produced from seed treated with 400 $\text{mg}\cdot\text{L}^{-1}$ PP45 had plant heights comparable to the nontreated control, but had significantly greater fresh weight and leaf area. Plants from seed treated with 1000 $\text{mg}\cdot\text{L}^{-1}$ PP45 had significantly larger leaf area measurements than plants from all other treatments including the nontreated control.

At 19 d after transplanting, fusarium wilt severity in transplants in the nontreated control soil averaged a maximum wilt rating of 5 regardless of whether seed were nontreated or treated with 400, 800, or 1000 $\text{mg}\cdot\text{L}^{-1}$ PP45 (Table 2). For all seed treatments, wilt severity was significantly

Fig. 1. Effect of increasing concentrations of Plantpro 45 on hyphal growth of *Fusarium oxysporum* f. sp. *basilici* in vitro after 14 d; 1.0 cm = 0.39 inch.

Fig. 2. Effect of increasing concentrations of Plantpro 45 on (A) growth of *Fusarium* spp. from seed plated on Komada's medium, and (B) fungal incidence from seed placed on water agar.

reduced with PP45 preplant soil treatments of 120 and 300 mg·L⁻¹ (Table 2). The greatest reduction in wilt severity was observed in transplants in 300 mg·L⁻¹ treated soil (Table 2). No seed treatments evidenced significantly different wilt ratings within a given soil treatment (data not shown).

Discussion

Our research indicates that PP45 completely inhibits growth of *F. oxysporum* f. sp. *basilici* in vitro and that PP45 is fungicidal at concentrations of 300 mg·L⁻¹. These data support results obtained by Yates et al. (2000) with *F. moniliforme*-infected

maize seed. Additionally, the regrowth of previously treated hyphae indicates that PP45 is a contact disinfectant that must totally inhibit fungal growth to be completely effective.

Most previously examined treatments have been inadequate at controlling *Fusarium* spp. on basil seed (Reuveni et al., 1997; Wick, 1997). Seed treatment with PP45 at rates of 800 to 1000 mg·L⁻¹ controlled *Fusarium* spp. and other fungi on basil seed, and increased seed germination as well as transplant growth. It should be noted that pathogenicity of all *Fusarium* spp. isolated from seed was not determined.

When transplanting occurred at 14 d after PP45 soil treatment in greenhouse experiments a significant reduction in fusarium wilt severity occurred in PP45 treated soil compared to the nontreated control. Although the seed and soil treatments did not have a synergistic effect, both treatments can significantly contribute to the management of this important pathogen of basil. PP45 as a seed treatment for control of *F. oxysporum* f. sp. *basilici* on basil can significantly reduce initial contamination or reintroduction of the pathogen into fields via seed. As a pre-plant soil disinfectant in greenhouse trials, PP45 has shown potential to reduce fusarium wilt severity, but requires further investigation to determine its potential for use in the field for management of this pathogen. Further study is also needed to determine optimum soil application rates and planting intervals.

Because *F. oxysporum* f. sp. *basilici* attacks the foliage of basil, spread of the pathogen is difficult to control once a field becomes infested. Experimentation with PP45 foliar applications is necessary to determine efficacious rates for management of aerial spread of *F. oxysporum* f. sp. *basilici*. An integrated program of PP45 seed treatment, soil application, and foliar sprays has potential to eventually provide effective management of fusarium wilt of basil.

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Fig. 3. Effect of increasing concentrations of Plantpro 45 on basil seed germination.

Table 1. Effects of increasing concentrations of Plantpro 45 applied to basil seed on seedling growth in a peat-based transplant medium at 21 d after planting.

Plantpro 45 [mg L ⁻¹ (ppm)]	Ht (cm) ^z	Fresh wt (g) ^y	Leaf area (cm ²) ^x
0	8.19 b ^w	1.49 c	8.69 c
400	8.96 ab	1.78 b	10.71 b
800	9.35 a	1.89 ab	9.39 c
1000	9.40 a	2.05 a	12.09 a
LSD (0.05)	0.87	0.25	0.87

^z1.00 cm = 0.394 inch.

^y1.00 g = 0.035 oz.

^x1.00 cm² = 0.155 inch².

^wMeans are for 20 arbitrarily selected seedlings per treatment; treatments in columns followed by the same letters are not significantly different at $P = 0.05$.

Table 2. Effects of increasing concentrations of Plantpro 45 seed and soil treatments on incidence of fusarium wilt of basil at 19 d after transplanting into infested field soil.

Soil treatment [mg L ⁻¹ (ppm)]	Seed treatment [mg L ⁻¹ (ppm)]			
	0	400	800	1000
0	5.00 ^z a ^y	5.00 a	5.00 a	5.00 a
80	3.20 b	3.18 b	2.72 b	4.20 a
120	1.40 c	2.00 bc	2.40 bc	2.20 b
300	1.00 c	1.00 c	1.00 c	1.00 b
LSD (0.05)	1.20	1.46	1.49	1.64

^zWilt ratings were recorded using a scale where 1 = 0% to 9% wilt, 2 = 10% to 24% wilt, 3 = 25% to 49% wilt, 4 = 50% to 74% wilt, and 5 = 75% to 100% of plant material wilted.

^yTreatments in columns followed by the same letters are not significantly different at $P = 0.05$.

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