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Growth Promotion of Highbush Blueberry by Fungal and Bacterial Inoculants

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Abstract. **The highbush blueberry cultivar Bluecrop was inoculated with potential plant growth–promoting (PGPR) candidates, including bacterial inoculants *Pseudomonas fluorescens* (Migula) (strains Pf 5, PRA 25, 105, or 101), *Bacillus pumilus* (Mayer and Gottheil) (strain T4), *Pseudomonas corrugata* (Roberts and Scarlett) (strain 114), and fungal isolates *Gliocladium virens* (Miller et al., Von Arx) (strain Gl.21) and *Trichoderma harzianum* (Rifai) (strain T 22). Addition of *G. virens* to pasteurized soil increased leaf area and the number of leaves produced in a 4-month growth period, as well as shoot content of P, Zn and Cu in 1997. Treatment with *P. fluorescens* Pf 5 increased leaf area and stem diameter. In nonpasteurized soil, plants inoculated with *G. virens* had greater leaf area, stem diameter, shoot and root dry weight, and more leaves per plant. These results demonstrate the potential of *G. virens* for increasing growth when used to inoculate blueberry plants in the nursery or at transplanting.**

The blueberry industry in Arkansas has recently developed into a million dollar per year industry (Clark et al., 1989). Each year the cultivated area is increasing, especially in northwest Arkansas. Although yields per hectare are comparable with those of other blueberry producing states, production problems do exist. Blueberries in Arkansas are produced mainly on mineral soils (Moore, 1976) and in soils with high clay content (Sterne, 1982). However, blueberries are best adapted to peaty soils containing high organic matter (Eck, 1988); these do not exist in Arkansas. Phytophthora root rot has been associated with these clay soils, as well as with some mineral soils with poor drainage, and can reduce yields (Sterne, 1982).

Rhizosphere-inhabiting microorganisms interact with the root system and with each other to influence plant growth and productivity (Gaskins et al., 1985; Parke, 1990; Whipps and Lynch, 1986). Stimulatory effects of these organisms could be the result of biocontrol of soil-borne diseases (Keel et al., 1992; Handelsman and Stabb, 1996), production of phytohormones, or facilitation of the uptake of certain nutrients from the environment (Burr and Caesar, 1984; Glick, 1995).

Glick (1995) reported that pathogens could reduce yields by 25% to 75%. Blueberry plants are grown in peat before transplanting to the field, and Moore (1979) recommended that blueberries be planted in holes filled with peat. Plant growth–promoting rhizobacteria (PGPR) increase yields of beet (*Beta vulgaris* L.) (Suslow and Schroth, 1982) and potato (*Solanum tuberosum* L.) (Frommel et al., 1993). *Trichoderma* sp. stimulate growth of bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), and pepper (*Capsicum annum* L.) (Inbar et al., 1994). Significantly higher dry weights of apple (*Malus ×domestica* Borkh.) seedlings have been achieved by treating with *G. virens* (Smith et al., 1990). The objective of this study was to examine the ability of selected nonmycorrhizal or nonendophytic microorganisms to promote growth of blueberry.

Materials and Methods

Source of microorganisms. Indigenous blueberry plants were collected from Washington County, Ark. The roots were shaken to remove soil particles, weighed, submerged in 200 mL of sterile water, and shaken on an orbital shaker. An aliquot was serially diluted, and suspensions from the 10^{-6} dilutions were plated using a pour plate method in 1/3 tryptic soy agar (TSA) and incubated at room temperature. Single bacterial colonies were arbitrarily selected, stored in dimethyl sulfoxide (DMSO), and frozen at –80 °C. The bacterial isolates *Pseudomonas corrugata* strain 114 and *P. fluorescens* strains 105 and 101 were selected from all isolates that showed high antagonism in vitro to *Phytophthora cinnamomi* (Rands), a serious pathogen on blueberry in Arkansas (Sterne, 1982).

Rhizobacterial isolates *P. fluorescens* 105 and 101 were identified by the Biolog colorimetric method (similarity index 0.61 and 0.57, respectively), using microplates (Biolog, Hayward, Calif.) and *P. corrugata* 114 by fatty acid profiling (similarity index 0.36) on a MIDI DOS system (Midi Labs, Newark, Del.) at the Disease Diagnostic Laboratory, Texas A&M Univ.. Bacterial strain *P. fluorescens* Pf 5 was obtained from M.D. Henkels (U.S. Dept. of Agriculture, Agricultural Research Service, Horticultural Crops Research Laboratory, Corvallis, Ore.), *Bacillus pumilus* T4 from J.W. Kloepper (Auburn Univ., Auburn, Ala.), and *P. fluorescens* PRA 25 from J.L. Parke (Univ. of Wisconsin, Madison). The fungal candidates consisted of *G. virens* Gl.21, (W.R. Grace and Co., Columbia, Md.) and *Trichoderma harzianum* T 22 obtained from C.S. Rothrock (Univ. of Arkansas, Fayetteville).

Preparation of inoculum. The rhizobacteria were grown on V-8 agar, containing 163 mL of V-8 juice (Campbell Soup Co., Camden, N.J.), 2.4 g calcium carbonate, and 12 g of agar (Difco Laboratories, Detroit) per liter for 2 d, and six loops were added to 200 mL of tryptic soy broth and grown at 25 °C for 2 d. The cultures then were centrifuged at $6800 \times g_n$ for 20 min and resuspended in sterile water. The procedure was repeated. The bacterial suspensions were serially diluted and plated. Percentage of transmittance at 590 nm of the dilutions were read with a spectrophotometer (Bausch & Lomb, Rochester, N.Y.), and a standard curve was obtained by plotting number of colony-forming units (cfu) against percentage of transmittance for each dilution. The bacterial candidates then were standardized using the standard curve before applying to the soil.

Inoculum of *T. harzianum* was prepared by suspending spores from 4-day-old cultures on corn meal agar (CMA) using 10 mL of sterile water. The spore concentration was adjusted to 10^8 per mL by adding sterile water. Alginate pellets containing *T. harzianum* were made as follows. Sodium alginate (Sigma Chemical Co., St. Louis) was dissolved in distilled water (10 g·L^{-1}), and corn meal (Associated Wholesale Grocers, Kansas City, Kans.) (10 g), and kaolinite (Fluka Chemika, Buchs, Switzerland) (75 g) were added and blended for 1 min. Spores of *T. harzianum* were added to provide a spore concentration of 10^6 mL, and the mixture was comminuted for 20 s in the blender. The prilles were formed using 0.25 M $CaCl_2$ (Fisher Scientific, Springfield, N.J.).

Pasteurized and nonpasteurized soil study. Field soil (Typic Hapludults) (1 kg) from the Agricultural Experiment Station, Fayetteville, Ark., was pasteurized by microwaving at 800 W for 4 min (Ferriss, 1984), and the fungal inoculum of *T.*

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harzianum or *G. virens* Gl.21 (10^6 spores/g) was mixed with the soil at the rate of 1% dry weight. Bacterial treatments consisted of *B. pumilus* strain T4, *P. fluorescens* PRA 25, *P. fluorescens* Pf 5, and the selected rhizobacteria isolated from blueberry, *P. corrugata* 114 and *P. fluorescens* strains 105 and 101. The bacterial strains were added at the rate of 10^6 to 10^7 cfu/g of soil after transplanting, and the soil was flushed with water. The control treatment consisted of plants without any inoculations. Peters (20N–8.8P–16.6K) (United Industries Corp., St. Louis) fertilizer at the rate of 200 $mg \cdot L^{-1}$ of nitrogen was applied each week. Nine-month-old, bare-rooted, uniform-sized plants of the cultivar Bluecrop (Highlander Nursery, Pettigrew. Ark.) were planted in 15-cm-diameter pots and the experiments were initiated on 4 Apr. 1996 and 8 Apr. 1997. Both experiments were conducted in a greenhouse and treatments were replicated three times in a randomized complete-block design. An additional study was done using nonpasteurized soil on 20 Apr. 1998 with *G. virens* and *P. fluorescens* Pf 5 in the greenhouse. These two treatments were chosen based upon the first-year results. The control treatment was not inoculated. The treatments were replicated five times in a randomized complete-block design.

Measurements. Stem diameter and number of leaves were recorded after planting and again before harvest. Plants were harvested on 6 Aug. 1996, 10 Aug. 1997, and 22 July 1998 for the three experiments, respectively. The leaf area was measured with a leaf area meter (Lambda Institute Corp., Lincoln, Neb.). After harvest, plants were oven-dried at 80 °C for 3 d and shoot and root dry weights recorded. Shoots and leaves were ground using a Wiley mill (Arthur Thomas, Philadelphia) to pass a 16-mesh (1.02-mm pores) screen. Nutrient analysis was then determined by inductively coupled plasma atomic emission spectrometry after HNO_3 digestion at the Univ. of Arkansas Altheimer Laboratory, Fayetteville.

Data analysis. The data for 1996 and 1997 were combined for analysis, as there were no interactions or treatment differences between years. Nutrient analysis was done only on the 1997 experiment. Data analysis was performed by SAS GLM procedure (SAS Institute, Cary, N.C.) and mean separation was by $LSD_{0.05}$.

Results

Effects of inoculants on growth of blueberry in pasteurized soil. Treatment of blueberry plants with *G. virens* and *P. fluorescens* Pf 5 increased leaf area ≈60% (Table 1), whereas treatment with *T. harzianum* reduced it. Plants treated with *G. virens* produced 82 leaves vs. only 22 for control plants during the 4-month growing period, whereas *T. harzianum*–treated plants produced only three leaves. Increase in stem diameter of *P. fluorescens* Pf 5–treated plants was more than twice that of the control.

Effects of inoculants on dry weight. Treatment with *G. virens* or with *P. fluorescens* Pf 5 increased both shoot and root dry weight.

Nutrient content. Treatment with *G. virens* significantly increased shoot content of P, Zn, and Cu in 1997 (Table 2). Uptake of Cu and P also was increased by treatment with *P. fluorescens* PRA 25; however, N, K, Ca, and S content was not affected (data not shown).

Effects of inoculants on plants in nonpasteurized soil. In nonpasteurized soil, inoculation with *G. virens* increased leaf area, stem diameter, and root and shoot dry weight (Table 3). Inoculated plants produced 78 leaves in the 4-month growing period vs. 41 for the control plants.

Discussion

Many fungi identified as potential antagonists against plant pathogens have also been reported to enhance plant growth (Meera et al., 1994). In these studies with both pasteurized and nonpasteurized soil, *G. virens* stimulated the growth of blueberry plants. These results agree with those from previous work with wheat (*Triticum aestivum* L.) (Shivanna et al., 1994). Higher uptake of some nutrients by blueberry plants also occurred with application of *G. virens* and *P. fluorescens* Pf 5, which also has been observed in wheat (de Freitas and Germida, 1992). Although *P. fluorescens* Pf 5 increased plant dry weights in pasteurized soil, the effects in nonpasteurized soil were nonsignificant. This could have been due to competition from natural flora, unfavorable environmental conditions, inadequate distribution of the organism (Schippers et al., 1987) or low root colonizing ability of the microorganism (Loper et al., 1984). The stimulation of growth achieved with *G. virens* treatments was greater than that obtained by addition of fertilizer. These results agree with a study done with *G. roseum* on tomato (*Lycopersicon esculentum* Mill.) by Sivapalan et al. (1994). Although the mechanism of growth promotion was not studied, it could have resulted from suppression of plant pathogens (Lumsden et al., 1992) or production of growth-regulating substances (Brown and Surgeoner, 1991). The ability of *G. virens* to grow and sporulate in natural soil is well-documented (Lewis et al., 1996).

Blueberry plants in Arkansas are grown in peat, which is a good growth medium for a microbial inoculant, as it provides organic matter, moisture, and a suitable environment

Table 1. Effects of inoculation with microorganisms on growth of blueberry.[z]

Treatment	Leaf area (cm^2)	No. of leaves	Increase in stem diam (mm)	Dry wt (g) Shoot	Dry wt (g) Root
G. virens	774 a[y]	82 a	0.28 ab	16.1 a	9.5 ab
P. fluorescens Pf 5	748 a	37 b	0.40 a	13.0 ab	12.7 a
P. corrugata 114	589 ab	18 bc	0.23 ab	10.0 bc	7.4 bc
P. fluorescens PRA25	488 bc	30 b	0.23 ab	12.1 abc	5.7 bc
P. fluorescens 101	380 bc	24 bc	0.22 ab	9.5 bc	4.0 c
P. fluorescens 105	364 cd	24 bc	0.29 ab	9.1 bc	4.8 c
B. pumilus T4	286 cd	25 bc	0.12 b	10.2 bc	4.8 c
T. harzianum	153 d	3 c	0.10 b	9.3 bc	4.1 c
Control	470 bc	22 bc	0.09 b	8.3 c	4.4 c

[z]Mean of two experiments, each with three replications, one in 1996 and one in 1997. Data recorded 6 Aug. 1996 and 10 Aug. 1997.
[y]Mean separation within columns by LSD, $P \leq 0.05$.

Table 2. Effects of inoculation with microorganisms on total nutrient content (µg) of blueberry shoots in 1997.[z]

Treatment	Zn	Cu	P
G. virens	320 a[y]	130 a	16600 a
P. fluorescens PRA 25	280 ab	100 ab	14200 ab
P. fluorescens 101	220 abc	60 bc	9700 abc
P. fluorescens Pf 5	200 bc	50 c	7600 bc
B. pumilus T4	180 bc	60 bc	10500 abc
P fluorescens 105	180 bc	50 c	7900 abc
T. harzianum	170 bc	60 bc	10300 abc
P. corrugata 114	130 c	40 c	4800 c
Control	170 bc	30 c	4100 c

[z]Shoot samples collected from all plants after harvest on 10 Aug. 1997.
[y]Mean separation within columns by LSD, $P \leq 0.05$. n = 3.

Table 3. Effect of inoculation with microorganisms on blueberry in nonpasteurized soil.

Treatment	Leaf area (cm^2)[z]	No. of leaves[y]	Stem diam (mm)[y]	Dry wt (g)[z] Shoot	Dry wt (g)[z] Root
G. virens	759 a[x]	78 a	2.40 a	20.5 a	18.7 a
P. fluorescens Pf 5	478 b	27 b	1.20 b	13.6 b	7.3 b
Control	517 b	41 b	0.77 b	14.0 b	8.0 b

[z]Determined on 22 July 1998 (after harvest).
[y]Increase in a 4-month growing period, as determined on 20 July 1998.
[x]Mean separation within columns by LSD, $P \leq 0.05$. n = 5.

for establishment. We also found that treatment of blueberry plants with *G. virens* increased shoot content of nutrients essential for growth. In Arkansas, blueberry plants are transplanted into a planting hole filled with peat. The fungus could be applied in the nursery or could be added into the planting hole before transplanting. Although fungi reportedly can survive in diverse environments and in many ecological niches (Brown and Surgeoner, 1991), field studies should be undertaken to cast more light on their growth–promoting ability.

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