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 Gotthel) (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 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 the root system and with each other to influence plant growth and productivity (Gaskins et al., 1985; Parke, 1990; Whipp and Lynch, 1986). Stimulatory effects of these organisms could be the result of biocontrol of soil-borne diseases (Keel et al., 1992; Handelmsman 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 saxodentica Borkh.) seedlings were 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 10⁻⁴ 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 ºC.

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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. Kloeper (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 ºC for 2 d. The cultures then were centrifuged at 6800 × g, 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 10⁶ mL−1 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 10⁶ mL⁻¹, and the mixture was comminuted for 20 s in the blender. The prills were formed using 0.25 m CaCl₂ (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 microwave at 800 W for 4 min (Ferris, 1984), and the fungal inoculum of T.
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 for microbial growth.
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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