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Soil amendments and watering influence the incidence of endophytic fungi in *Amaranthus hybridus* in South Africa

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Abstract

A study was conducted to determine the influence of soil amendments and irrigation on the incidence of endophytic fungi in *Amaranthus hybridus*. Five- and 6-month-old, asymptomatic tissues from *A. hybridus* were sampled from cultivated plots at Potchefstroom, South Africa in 1997 and 1998, respectively. Soil treatments consisted of the addition of commercial fertilizer or manure to irrigated soils, and wood ash to nonirrigated soils; control plots were neither amended nor irrigated. Ten leaves, 10 petioles, and 10 roots from each of five plants per soil treatment were surface disinfested and small sections from each were placed on corn-meal agar (8000 isolation attempts). After 5 days, the resulting fungal colonies were counted. Significant differences in recovery of fungi occurred among the soil treatments ($P < 0.01$) and among plant parts ($P < 0.01$). The highest recovery occurred from the commercial fertilizer and watered treatment (least stressed) for leaves and petioles in both years. Higher fungal recovery also occurred in the wettest year from leaves and petioles for all soil treatments. In contrast, roots yielded higher fungal recovery in the driest year for all soil treatments. These results show that soil attributes can influence frequency of endophytic fungi in both above- and below-ground tissues.

Keywords: *Alternaria tenuissima*; Amaranth; Fertilizer; Fungal ecology; Latent-infecting pathogen; Soil treatments

1. Introduction

The genus *Amaranthus* includes species cultivated as leafy vegetables and/or for their grain (i.e., pseudocereal) in several developed and developing countries (Harlan, 1992). *Amaranthus hybridus* (common name: smooth amaranthus or amaranth) is a highly nutritious, fast growing, annual, leafy-vegetable crop (Rawate, 1983). It grows well in semiarid regions such as southern Africa and its commercial production is increasing throughout the world as an important alternative food source (Kauffman and Haas, 1983; Kauffman and Weber, 1990; Rawate, 1983). An understanding of the abiotic and biotic factors that affect *A. hybridus* in South Africa will help in sustainable pest and disease management. Plant–fungal relationships are especially relevant, since they are known to play important roles in the biology and ecology of most cultivated plants.

Endophytic fungi, for example, can play important roles in plant health. These fungi live within asymptomatic plant tissues, and may or may not elicit disease symptoms during their life-cycle (Petrini, 1986, 1996; Siegel et al., 1987; Verhoeff, 1974; Wilson, 1995). When induced by environmental or nutritional conditions, or

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host maturity, some endophytic fungi act as latent-infecting pathogens eliciting disease symptoms (Petrini, 1986; Verhoeff, 1974; Wilson, 1995). Although mutualistic effects of endophytes in annual plants are unknown, mutualistic effects of endophytes in perennial grass can occur (Redlin and Carris, 1996). For example, the clavicipitaceous endophytes of grasses can inhibit phytophagous insects and some pathogenic fungi, but other endophytes cause disease (Johnson et al., 1985; Clay, 1989; Latch et al., 1985; Siegel et al., 1987). Neutral fungal symbionts also occur in plants (Dix and Webster, 1995).

The endophytic fungi of *A. hybridus* in South Africa were identified in field surveys conducted in 1996 and 1997 (Blodgett et al., 2000). The primary fungal species group in asymptomatic *A. hybridus* leaves and petioles are the *Alternaria tenuissima*-like grouping of species. This species group has been shown to extensively colonize asymptomatic leaves of *A. hybridus* in a manner consistent with other endophytic fungi (Blodgett and Swart, 2002). The *A. tenuissima*-like group, *Phoma* spp., and species of *Fusarium*, including *Fusarium oxysporum*, are the most common species in asymptomatic roots (Blodgett et al., 2000). These endophytic fungi are believed to be a natural component of *A. hybridus.*

Although the potential importance of plant–fungal relationships in ecosystem health is recognized, little is known about the environmental conditions conducive to asymptomatic plant–fungal associations (Rodriguez and Redman, 1997). The goal of this field experiment was to quantify the effects of different fertilizer and irrigation treatments on the incidence of asymptomatic fungal colonization under conditions experienced by small-scale farmers in rural African areas. The specific objectives of this study were to: (a) quantitatively test if soil amendments and irrigation influence the incidence of endophytic fungi in *A. hybridus* leaves, petioles, and roots, (b) correlate soil properties with fungal frequencies on the different plant parts, and (c) determine the distribution of these fungi within plants.

2. Materials and methods

Plots representing four treatments were established at Potchefstroom, North-West Province, South Africa in 1996. The experiment was repeated in 1997 using the same plots the following year. Plot treatments were selected to simulate conditions employed by small-scale farmers in rural areas of South Africa. Therefore, wood ash and manure were included as organic treatments. Soil treatments included the addition of: (a) commercial fertilizer to irrigated soils, (b) manure to irrigated soils, (c) wood ash to nonirrigated soil, and (d) control plots were neither amended nor irrigated. Given the soil treatments used, our study cannot separate the effects due to irrigation from the effects of the soil organic amendments. Therefore, the results of this experiment compare specific soil amendment/watering treatments used in South Africa.

All plots were mechanically cultivated using identical cultivation practices for the entire trial area. A 5-tine ripper was used for primary cultivation to break up deeper soil layers at an operating depth of 45 cm. Before each planting, a chisel plough was used at an operating depth of 35 cm, followed by a field tiller (for planting preparation) at a shallow depth. Commercial fertilizer (4:1:0, N:P:K; Kynoch, South Africa) was periodically applied to meet a nitrogen requirement of 160 kg ha^{-1}. Manure was applied at a rate of 8350 kg ha^{-1} to produce a nitrogen level equal to 180 kg ha^{-1}. Manure was incorporated into topsoil with hand spades 4 weeks before planting to ensure breaking down of organic matter. Wood ash was applied at a rate of 237 ml per plant into planting holes and mixed with the soil. Drip-line irrigation was used for 2 h every other day with approximately 3.9 cm^3 water per irrigation (approximately 58.5 cm^3 per month). Drip-line irrigation is routinely used in rural farming in semiarid regions of the world.

No herbicides or insecticides were used for two reasons: (a) nothing is registered for use on cultivated amaranth in South Africa, and (b) amaranth leaves are eaten in Africa, which make it unlikely that insecticides would be used in production. Weed control was done manually for the first month after transplanting. Weeds do not present problems later in the year, since the plants grow fast, form a canopy, and thus eliminating weed competition.

Legumes were planted on the trial site prior to amaranth planting. Legumes were planted on small plots and included: dry beans, cow peas, velvet beans, green gram, and black gram. However, nothing was planted on the trial site 1 year prior to planting of the amaranth trial.

Amaranth seeds were sown in seed trays in sterilized peat and grown for 30 days in a greenhouse. Plants were watered to field capacity daily. The average greenhouse temperature was 25 °C during the day and 17 °C during the night.

The seedlings were transplanted to the field in mid November in both 1996 and 1997. After placing the plants, planting holes were filled with water and then with soil. The intra row spacing was 0.3 m. Plots were 7 m long and three rows wide, with a 1.5-m spacing between rows resulting in 70 plants per plot. A completely