Available online at www.sciencedirect.com
ScienceDirect
Applied Soil Ecology 35 (2007) 311–318
Applied Soil Ecology
www.elsevier.com/locate/apsoil

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

J.T. Blodgett [a,*], W.J. Swart [a], S.vdM. Louw [b], W.J. Weeks [c]

[a] *Department of Plant Pathology, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa*
[b] *Department of Zoology and Entomology, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa*
[c] *North West Technical Support Services, Private Bag X804, Potchefstroom 2520, South Africa*

Received 16 November 2001; accepted 28 July 2006

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.
© 2006 Elsevier B.V. All rights reserved.

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

* Corresponding author. Current address: USDA-Forest Service, Forest Health Management, 1730 Samco Road, Rapid City, SD 57702, United States.
E-mail address: jblodgett@fs.fed.us (J.T. Blodgett).

0929-1393/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2006.07.010

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

randomized design was used and the four plot treatments were replicated three times.

Five 5-month-old and 6-month-old *A. hybridus* plants were collected from each of the four plot treatments in April 1997 and May 1998, respectively. Five plants were randomly selected from center rows of each of the plot treatments, with at least one plant per randomly repeated plot treatment. Therefore, a completely randomized experimental design was used in the analyses, given the randomly dispersed collection locations.

Ten asymptomatic leaves, petioles, and roots from each plant that were of similar size among the soil treatments were surface disinfested. Surface disinfestation involved a series of 1 min in 96% ethanol, 5 min in a 3.5% NaOCl solution (w/v), and 30 s in 96% ethanol. This surface disinfestation method is effective at removing surface fungi on *A. hybridus* (Blodgett and Swart, 2002). Ten 0.5-cm diameter sections from each leaf were removed laterally (five sections) and along the mid-vein (five sections) of each leaf using a cork-borer. The accompanying petiole was cut into five 1-cm long segments. Roots were washed with tap water, surface disinfested, and five 1-cm long segments were cut from each. Root sections selected had similar diameters as the petioles selected. All sections were placed on corn-meal agar (Oxoid, Basingstoke, England) plates containing 0.3 ml Novo-Strep l^{-1} of streptomycin sulphate at 1 g per 3 ml active ingredients and incubated at 24 °C (8000 isolation attempts). After 5–9 days, fungi growing from the plant parts were counted. Plates with no growth after 9 days were also evaluated at 15 and 30 days. All fungal colonies growing from the tissue pieces were counted (i.e., some individual pieces had as many as three colonies). In 1997, all fungal colonies growing from the tissue pieces were transferred from colony margins to 1.5% water agar (WA; Oxoid). Isolates were identified using methods previously described (Blodgett et al., 2000).

Additional leaves were collected for comparisons of stomatal densities (number of stomata mm^{-2}). Stomata are the sites of leaf infection for the most common leaf endophyte (*A. tenuissima*) of *A. hybridus* (Blodgett and Swart, 2002). Additional leaves, petioles, and roots were measured in the field for comparisons of relative surface areas and volumes of the plant parts collected for isolations. Stomata counts were made from lower-leaf surfaces at the top, middle, and bottom thirds of two leaves at both leaf margins (edges) and leaf centers (close to main vein). The thicknesses of two leaves were measured with calipers at the ten locations used for isolations. Measurements were taken from leaves that were still attached to plants by making small cuts in the leaves. Petiole diameters were measured with calipers from the two leaves at the center of section locations used for isolations. Two measurements were made at each petiole location (dorsal–ventral and horizontal). Roots were washed with water and diameters were measured with calipers at approximately the center of locations used for isolations, with two measurements each at 90° angles.

The soil is classified as a Bainsvlei type (orthic A horizon, red apedal (structureless) B horizon, soft plintic B horizon; FAO classification, oxisol) with an effective depth 60 cm. The soil pH, before the soil treatments were applied, averaged 5.3 for soils collected from the upper 30 cm.

In June 1997 and 1998, soils were collected from the upper 30 cm from each of the four soil treatments. Three soil cores were randomly collected from center rows, at least 1 m from the row ends, of each of the three plots per soil treatment. The three soil cores from within a plot were combined to produce one representative sample per plot (12 total combined samples per year).

Soil pH and P, K, Ca, Mg, Na, and NO_3 concentrations were determined using soil analysis methods described by Black et al. (1965) and performed according to procedures in the Handbook of Standard Soil Testing Methods (The Non-Affiliated Soil Analysis Work Committee, 1990). Soils were analyzed by the National Department of Agriculture, stationed at North West Technical Support Services in Potchefstroom, South Africa. Soil pH was determined by an electrometric procedure with a KCl soil–water mixture; extractable *P* was determined by extraction with Bray P_1 and measured with a photoelectric colorimeter; extractable cations (K, Ca, Mg, Na) were determined by atomic absorption spectroscopy after ammonium acetate extraction; and NO_3 concentrations were determined by colorimetric methods using a reduction column.

Climatic data were obtained from a weather station approximately 1 km from the study site. Data included precipitation and air temperatures for November 1996 to April 1997 and November 1997 to May 1998. Thirty-year-average precipitations (1969 to 1998) for November to April and November to May were supplied by the Institute for Soil Climate & Water.

2.1. Statistical analyses

Recovery frequencies per plant part (i.e., leaves, petioles, and roots) were converted to percentage recovery per plant part to allow for an analysis of variance, and to standardize sample sizes among the plant parts. This gives an even sample size of 50 individual

leaves, petioles, and roots per four-soil treatment per 2 year (1200 total plant parts). Percentage recovery from individual leaves, petioles, and roots were analyzed by two-factor analysis of variance with interactions. Factors used as main effects were plant part and soil treatment. Percentage recovery data were analyzed after an arcsine($\sqrt{}$(x/100)) transformation was applied (Snedecor and Cochran, 1989). The transformation was used to normalize the distribution of the percentage data. Means were separated using Fisher's least significant differences (LSD) at $P = 0.05$. χ^2 analyses were used to examine the incidence of fungal recovery from individual plant parts in relation to: time (year) and location in plant parts. Simple linear regression analyses were used to examine relationships between incidence of fungal recovery from plant parts in relation to soil pH/minerals. Analyses of variance (using the general linear model procedure), χ^2 (using the cross tabulation procedure), and linear regression analyses (using the regression procedure) were performed using Minitab for Windows, release 10.2 (Minitab Inc., State College, PA).

3. Results

Precipitation was highest (9.8 cm per month) between November 1996 and April 1997, and lowest (6.0 cm per month) between November 1997 and May 1998. The 30-year average precipitation for November to April is 8.7 cm per month, and for November to May is 7.8 cm per month. Average temperatures during the two growing seasons were similar (20 °C for both growing seasons), with only a slight difference between average minimum and average maximum temperatures in 1997 (13 and 27 °C) compared to 1998 (12 and 28 °C).

Fungi were recovered from asymptomatic, surface disinfested leaves, petioles, and roots of plants grown in all soil treatments in both years. However, significant differences in recovery between years occurred among the plant parts (Table 1). In 1997, higher percentages of recovery occurred from leaves and petioles for all soil treatments compared to 1998 ($P < 0.01$ for all soil treatments). In 1998, higher percentages of recovery occurred from roots for all soil treatments compared to 1997 ($P < 0.01$ for all soil treatments).

In the first year of this study, the *Alternaria tenuissima*-like group dominated in the leaves (86%) and petioles (70%) for all soil treatments, and the *A. tenuissima*-like group (28%), *Phoma* (25%) and *Fusarium* species (18%) dominated in roots for all soil treatments. The small number of dominant fungal species observed in this study is consistent with the high specificity for plant parts previously reported (Blodgett et al., 2000). Since there were no differences in compositions of the dominant fungal species among the soil treatments in 1997, and since species identification is time consuming (8000 isolation attempts in 1997), identification of fungi was not attempted in 1998.

Soil treatment had a significant effect ($P < 0.01$) on the recovery of fungi from plant parts in both years (Table 1). However, in 1998, petioles could not be separated by soil treatment based on LSD at $P = 0.05$. Significant differences ($P < 0.01$) in recovery also occurred among leaves, petioles, and roots in both years (Table 1). Leaf sections yielded fungi 95 and 44% of the time, petioles 80 and 20%, and roots 58 and 80% in 1997 and 1998, respectively. Leaf sections yielded fungi more often than petioles in both years. However, leaf sections yielded fungi more often than roots in 1997 (wetter year) and less often than roots in 1998 (drier year). The interaction between soil treatment and plant part was significant ($P < 0.01$ in both years), indicating that the different plant parts responded differently depending on the soil treatment.

Table 1
Percentages of field samples from which fungi were isolated from asymptomatic, surface disinfested *Amamnthus hybridus* leaves, petioles, and roots of plants associated with four-soil treatments[a]

Year[b]/plant part	Soil treatment[a]			
	Commercial fertilizer irrigation	Manure irrigation	Wood ash no irrigation	Control no irrigation
1997[b]				
Leaves	99 g[c]	95 fg	91 f	93 f
Petioles	96 fg	69 cd	71 cd	84 e
Roots	74 d	34 a	56 b	67 c
1998[b]				
Leaves	49 c	43 bc	38 b	44 bc
Petioles	23 a	15 a	21 a	20 a
Roots	91 f	58 d	96 f	74 e

[a] Treatments include the addition of: commercial fertilizer to irrigated soils, manure to irrigated soils, or wood ash to nonirrigated soils; control plots were neither amended nor irrigated.

[b] For all individual comparisons between plant parts from the same soil treatment the differences between the 2 years were significant ($P < 0.01$), based on χ^2 analyses of incidence of fungal recovery (4000 isolation attempts per year).

[c] Values are mean percentages from 50 leaves, 50 petioles, and 50 roots of each soil treatment in each year (600 total plant parts per year). Within a year, percentages followed by different letters, both among soil treatments and among plant parts, are significantly different based on Fisher's least significant difference at $P = 0.05$. In both years, the probability that there was no difference among soil treatments was $P < 0.01$, and the probability that there was no difference among plant parts was $P < 0.01$ based on two-way analyses of variance with soil treatment and plant part as factors.

Table 2
Correlations and probabilities for the relationships between potassium concentration[a] in soils and fungal recovery from asymptomatic, surface disinfested *Amaranthus hybridus* leaves, petioles, and roots of plants associated with four-soil treatments[b]

Year/plant part	Correlation coefficient	P^c
1997		
Leaves	−0.939	0.06
Petioles	−0.996	<0.01
Roots	−0.942	0.06
1998		
Leaves	−0.773	0.21
Petioles	−0.988	0.01
Roots	−0.830	0.17

[a] Potassium soil concentrations (mg kg^{-1}) were determined by atomic absorption spectroscopy after ammonium acetate extraction.
[b] Treatments include the addition of: commercial fertilizer to irrigated soils, manure to irrigated soils, or wood ash to nonirrigated soils; control plots were not amended nor irrigated.
[c] Probability that there is no difference in the linear relationship, based on simple linear regression. The sample size is 20 for each plant part in each year.

Although not always significant at $P < 0.05$, the recovery rates from leaves, petioles, and roots were always negatively correlated with soil potassium concentration (Table 2). Recovery rate from leaves was positively correlated with soil phosphorus concentrations in 1997 (correlation coefficient = 0.987; $P = 0.01$) and with NO_3 in 1998 (correlation coefficient = 0.998; $P < 0.01$). Other soil properties (pH, Ca, Mg, and Na) did not produce significant correlations with fungal recovery from plant parts.

Differences in fungal recovery occurred within leaves ($P < 0.01$ and $P = 0.02$; 1997 and 1998, respectively) and within petioles ($P < 0.01$; both years). Leaf margins had the lowest recovery (average 84 and 35%; 1997 and 1998, respectively) while the middle portions had the highest recovery (average 100 and 63%; 1997 and 1998, respectively). The petiole segments nearest the leaf blade had the highest recovery rates (average 90 and 47%; 1997 and 1998, respectively), and petiole segments nearest the stems had the lowest recovery rates (52 and 8%; 1997 and 1998, respectively). Differences in recovery within roots were not significant ($P = 0.53$ and $P = 0.11$; 1997 and 1998, respectively).

Leaf stomatal frequencies were higher at leaf centers (212 ± 21 stomata mm^{-2}) compared to leaf margins (167 ± 16 stomata mm^{-2}). The surface area of leaf, petiole, and root sections averaged 0.48 ± 0.001 cm^2 (standard error), 0.63 ± 0.01, and 0.62 ± 0.04 cm^2, respectively. The surface areas of leaf sections did not differ between leaf centers and leaf margins. However, the surface areas of petiole and root sections were greater closer to the stems (0.67 cm^2 versus 0.60 cm^2 for petiole sections; 0.79 cm^2 versus 0.51 cm^2 for root sections). The volume of leaf, petiole, and root sections averaged 0.0090 ± 0.0001, 0.026 ± 0.001, and 0.025 ± 0.003 cm^3, respectively. The volumes of leaf sections did not differ between leaf centers and leaf margins. However, the volumes of petiole and root sections were greater closer to the stems (0.030 cm^3 versus 0.024 cm^3 for petiole sections; 0.041 cm^3 versus 0.018 cm^3 for root sections).

4. Discussion

This study indicates that environmental factors are important in infection and/or colonization of host tissues by endophytic fungi in *A. hybridus*. Environmental conditions such as water and temperature extremes, soil nutrients, and light have been shown to affect plant disease incidence and severity in other hosts (Schoeneweiss, 1975, 1981). Although disease incidence and severity can be affected by both water extremes and soil nutrients, little research has been conducted on how these factors might affect asymptomatic fungal infection and colonization in host plants. The relationships among factors affecting disease incidence and severity, and factors affecting endophytic infection and colonization are unknown. However, since we could find no previous published experimental results on the effects of water extremes or soil nutrients on endophytic fungal frequencies, comparisons with diseases are used in this discussion. The most common endophytic fungal species of *A. hybridus* leaves and roots (Blodgett et al., 2000) also have been shown to cause significant disease in the field (Blodgett et al., 1998, 1999) when induced by wounding, indicating that these endophytic fungi can act as latent-infecting pathogens.

Experimental studies and field surveys have found correlations of environmental factors with frequencies of plant endophytes. For example, air pollutants can reduce (Helander et al., 1993, 1994, 1996) or increase (Magan et al., 1995) the frequencies of endophytic fungi in plants. Also, a field survey conducted at more than 200 sites found that fewer endophytic fungi are recovered from trees on dry sites and at high elevations (Carroll and Carroll, 1978). However, other site factors such as precipitation, humidity, light, available soil nutrients, and soil/air temperature cannot be excluded when different locations are used. These other factors can directly or indirectly influence fungal inoculum production, spore dispersal and germination, infection, and colonization of host tissues.

In the present experimental study, plots were within the same field and collections were made from randomly dispersed locations within a field reducing the effect of site. The drip-line irrigation used in our study prevents direct wetting of leaf and petiole surfaces that can influence germination and infection of plants by fungi. However, we examined fertilizers and irrigation regimes under conditions used by small-scale farmers in rural African areas. Therefore, our study cannot separate the effects due to irrigation from the effects of soil amendments. The randomized experimental design of this field experiment compares specific soil amendment/watering treatments, eliminating some environmental variation.

Water stress is associated with the enhancement of disease and fungal colonization of many plant species by several pathogens (Appel and Stipes, 1984; Bagga and Smalley, 1974; Bertrand et al., 1976; Blodgett et al., 1997; Pusey, 1989; Schoeneweiss, 1981, 1983). However, water stress can have either a neutral or negative influence on plant disease development (Biggs et al., 1983; Jacobi and Riffle, 1989; Swart et al., 1992; Tippett et al., 1987). *Alternaria* diseases, for example, can be enhanced by high or low soil moisture extremes (Rotem, 1994). Our results suggest that the abundance of endophytic fungi in *A. hybridus* was influenced in both years by changes in the soil environment, and potentially by soil irrigation (moisture). The differences in fungal recovery between years in our study might be explained by the differences in precipitation between years. The differences between years stress the importance of the effect of yearly variation in the environment on fungal infection and colonization. Although there were differences in colonization between the 2 years, the relative recoveries among the four soil treatments were similar between the years. Therefore, water availability might explain the differences in frequencies of asymptomatic fungal colonization both among soil treatments and between the years in our study. This conclusion is consistent with the previous field survey by Carroll and Carroll (1978) of tree endophytes that found lower frequencies of endophytic fungi from tree leaves on dry sites than on sites of moderate moisture. Lower host water potentials might reduce fungal colonization of host tissues or, through stomatal closure due to host water conservation, reduce host infection. Asymptomatic infections of field collected *A. hybridus* leaves have only been observed through stomata (Blodgett and Swart, 2002).

Increased disease incidence and severity has been associated with high soil nutrition including nitrogen and phosphorus (Huber, 1980; Van Dijk et al., 1992). This is consistent with the higher incidence of asymptomatic fungal colonization observed in the commercial fertilizer and irrigation soil treatment in our study. However, of the soil minerals tested in our study, potassium concentration had the most consistent correlation with fungal recovery and was inversely correlated with recovery from leaves, petioles, and roots. High potassium concentration might inhibit infection and/or colonization of hosts. This is consistent with previous studies of pathogenic fungi that found inverse correlations of potassium concentration with disease incidence and severity (Huber, 1980). Potassium and nitrogen deficiency predisposed beans to infection by the pathogen *A. alternata* (Saad and Hagedorn, 1996) and potash applications reduced disease by *A. macrospora* in cotton (Hillocks and Chinodya, 1989).

Although it might be expected that a high degree of fungal colonization would result in depletion of host resources and host stunting, or that more vigorous plants (i.e., commercial fertilized and watered) might overcome the invading fungi resulting in less colonization of hosts by virtue of balanced antagonism (Schulz et al., 1999), this was not observed. In fact, the commercial fertilized and watered plants had greater fungal colonization and greater biomass production. The addition of fertilizer and/or water might provide additional resources that both plants and associated fungi can use. This could explain the higher recovery of fungi from asymptomatic plant parts in above-ground tissues when plant vigor is high (i.e., the commercial fertilizer and irrigation treatment). Although plants with limited resources (i.e., less water and soil nutrients) might support fewer endophytic fungi in above-ground tissues, this might not be the case in below-ground tissues as indicated by the higher recovery of fungi from asymptomatic roots in the drier year compared to the moist year in our study. In this context, the quantitative incidence of asymptomatic fungal colonization might be a bio-indicator of host vigor.

The distribution of stomata may determine the distributions of endophytic fungi in above-ground plant parts. In the present study, higher fungal recovery occurred from leaves compared with petioles, which have fewer stomata. Asymptomatic, *A. hybridus* stems contain few fungi (Blodgett et al., 2000). This is consistent with the frequencies of stomata and the higher rates of recovery from petiole sections nearest the leaf blades, compared with sections nearer the stems. Colonization from leaves into petioles might also account for the higher recovery from petiole sections near leaves. The higher frequency of leaf stomata at leaf centers compared to leaf margins can also explain the

higher fungal recovery from leaf centers. These observations are consistent with our observation that endophytic fungi enter asymptomatic *A. hybridus* leaves only through stomata (Blodgett and Swart, 2002).

Results of this study provide quantitative data on the frequencies of endophytic fungi associated with *A. hybridus*, and shows that the soil environment can influence both above- and below-ground infection and/or colonization of *A. hybridus* by these fungi. However, since endophytic and latent-infecting fungi, fungal pathogens, and phytophagous insects of this host have not been extensively studied, additional questions need to be addressed. One implication of these findings is, if these fungi protect *A. hybridus* from insect herbivory and/or disease, then healthy plants (i.e., fast growing, well watered and fertilized plants) that contain more endophytic fungi in leaves and petioles should be more resistant to these harmful organisms. Saprophytic strains of *A. alternata* reduced disease of pathogenic strains of *A. alternata* on tobacco (Spurr, 1977). A saprophytic *A. tenuissima* can inhibit germination of the pathogen *A. zinniate*, in bean (van den Heuvel, 1970). Given the extensive colonization of the *A. tenuissima* group in *A. hybridus*, the potential that it might inhibit other fungal pathogens should be examined. Preliminary trials suggest that the most common endophyte of leaves (isolates of the *A. tenuissima* group) might provide protection from pig weevil (*Hypolixus haerens*) herbivory (Blodgett and Swart, unpublished). However, since tissue wounding is conducive to leaf-spot symptoms caused by isolates of the *A. tenuissima* group (Blodgett et al., 1999), healthy plants might be more likely to exhibit disease. Even if no visible symptoms are observed, the incidence of these fungi inside plant tissues may reduce crop yields due to their interactions and nutrient utilization. On the other hand, some endophytic fungi can reduce the negative effects of abiotic stresses of their hosts. Therefore, data on asymptomatic fungal colonization of amaranth, as influenced by soil conditions, might serve as a useful indicator of plant health.

Acknowledgements

We thank P. Bonello and S. Nameth for presubmission reviews, and [editor] and anonymous reviewers for their many helpful suggestions. We also thank P. Mohasi, Z. van der Linde, M. Islam, and M. Molemela for technical assistance. Financial support was provided by the National Research Foundation (former Foundation for Research Development) of South Africa.

References

Appel, D.N., Stipes, R.J., 1984. Canker expansion on water-stressed pin oaks colonized by *Endothia gyrosa*. Plant Dis. 68, 851–853.

Bagga, D.K., Smalley, E.B., 1974. The development of hypoxylon canker of *Populus tremuloides*: role of interacting environmental factors. Phytopathology 64, 658–662.

Bertrand, P.F., English, H., Uriu, K., Schick, F.J., 1976. Late season water deficits and development of *Cytospora* canker in French prune. Phytopathology 66, 1318–1320.

Biggs, A.R., Davis, D.D., Merrill, W., 1983. Cutting development and restriction of wound-associated infection in *Populus*. Can. J. Plant Pathol. 5, 269–272.

Black, C.A., Evans, D.D., Ensminger, L.E., White, J.L., Clark, F.E., Dinauer, R.C., 1965. Methods of Soil Analysis, Part 2. American Society of Agronomy, Madison, WI, 1572 pp.

Blodgett, J.T., Kruger, E.L., Stanosz, G.R., 1997. Effects of moderate water stress on disease development by *Sphaeropsis sapinea* on red pine. Phytopathology 87, 422–428.

Blodgett, J.T., Swart, W.J., 2002. Infection, colonization, and disease of *Amaranthus hybridus* leaves by the *Alternaria tenuissima* group. Plant Dis. 86, 1199–1205.

Blodgett, J.T., Swart, W.J., Louw, S.vdM., 1998. First report of *Fusarium sambucinum*, *F. oxysporum*, and *F. subglutinans* associated with stem decay of *Amaranthus hybridus* in South Africa. Plant Dis. 82, 1062.

Blodgett, J.T., Swart, W.J., Louw, S.vdM., Weeks, W.J., 2000. Species composition of endophytic fungi in *Amaranthus hybridus* leaves, petioles, stems, and roots. Mycologia 92, 853–859.

Blodgett, J.T., Swart, W.J., Chen, Weiqun, 1999. First report of *Alternaria tenuissima* as a leaf pathogen of *Amaranthus hybridus*. Plant Dis. 83, 878.

Carroll, G.C., Carroll, F.E., 1978. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. Can. J. Bot. 56, 3034–3043.

Clay, K., 1989. Clavicipitaceous endophytes of grasses: their potential as biocontrol agents. Mycol. Res. 92, 1–12.

Dix, N.J., Webster, J., 1995. Fungal Ecology. Chapman & Hall, London, 549 pp.

Harlan, J.R., 1992. Crops and Man, 2nd ed. American Society of Agronomy, Madison, WI, 284 pp.

Helander, M.L., Neuvonen, S., Ranta, H., 1996. Ecology of endophytic fungi: effects of anthropogenic environmental changes. In: Redlin, S.C., Carris, L.M. (Eds.), Endophytic Fungi in Grasses and Woody Plants: Systematics, Ecology, and Evolution. APS Press, St. Paul, MN, pp. 197–208.

Helander, M.L., Ranta, H., Neuvonen, S., 1993. Responses of phyllosphere microfungi to simulated sulphuric and nitric acid deposition. Mycol. Res. 97, 533–537.

Helander, M.L., Sieber, T.N., Petrini, O., Neuvonen, S., 1994. Endophytic fungi in Scots pine needles: spatial variation and consequences of simulated acid rain. Can. J. Bot. 72, 1108–1113.

Hillocks, R.J., Chinodya, R., 1989. The relationship between Alternaria leaf spot and potassium deficiency causing premature defoliation of cotton. Plant Pathol. 38, 502–508.

Huber, D.M., 1980. The role of mineral nutrition in defense. In: Horsfall, J.G., Cowling, E.B. (Eds.), Plant Disease: An Advanced Treatise, vol. 5. Academic Press, New York, pp. 381–406.

Jacobi, W.R., Riffle, J.W., 1989. Effects of water stress on Thyronectria canker of honeylocusts. Phytopathology 79, 1333–1337.

Johnson, M.C., Dahlman, D.L., Siegel, M.R., Bresh, L.P., Latch, G.C.M., Potter, D.A., Varney, D.R., 1985. Insect feeding deterrents

in endophyte-infected tall fescue. Appl. Environ. Microbiol. 49, 568–571.

Kauffman, C.S., Haas, P.W., 1983. Grain amaranth: a crop with low water requirements and high nutritional value. In: Lockeretz, W. (Ed.), Proceedings of the 4th International Federation of Organic Agriculture Movements Conference on Environmentally Sound Agriculture, Selected Papers, New York, August 1982. Praeger, Cambridge, MA, pp. 299–313.

Kauffman, C.S., Weber, L.E., 1990. Grain amaranth. In: Janick, J., Simon, J.E. (Eds.), Proceedings of the 1st National Symposium on New Crops, Research, Development, Economics. Advances in New Crops, Indianapolis, IN, October 1988. Timber Press, Portland, OR, pp. 127–139.

Latch, G.C.M., Hunt, W.F., Musgrave, R.R., 1985. Endophyte fungi affect growth of perennial ryegrass. NZ J. Agric. Res. 28, 165–168.

Magan, N., Kirkwood, I.A., McLeod, A.R., Smith, M.K., 1995. Effect of open-air fumigation with sulphur dioxide and ozone on phyllosphere and endophytic fungi of conifer needles. Plant Cell Environ. 18, 291–302.

Petrini, O., 1986. Taxonomy of endophytic fungi of aerial plant tissues. In: Fokkema, N.J., Heuvel, J., van den, (Eds.), Microbiology of the Phyllosphere. Cambridge University Press, Cambridge, pp. 175–187.

Petrini, O., 1996. Ecological and physiological aspects of host specificity in endophytic fungi. In: Redlin, S.C., Carris, L.M. (Eds.), Endophytic Fungi in Grasses and Woody Plants: Systematics, Ecology, and Evolution. APS Press, St. Paul, MN, pp. 87–100.

Pusey, P.L., 1989. Influence of water stress on susceptibility of nonwounded peach bark to *Botryosphaeria dothidea*. Plant Dis. 73, 1000–1003.

Rawate, P.D., 1983. Amaranth (pigweed): a crop to help solve the world protein shortage. In: Lockeretz, W. (Ed.), Proceedings of the 4th International Federation of Organic Agriculture Movements Conference on Environmentally Sound Agriculture, Selected Papers, Cambridge, MA, August 1982. Praeger, New York, pp. 287–298.

Redlin, S.C., Carris, L.M., 1996. Endophytic Fungi in Grasses and Woody Plants: Systematics, Ecology and Evolution. APS Press, St. Paul, MN, 223 pp.

Rodriguez, R.J., Redman, R.S., 1997. Fungal life-styles and ecosystem dynamics: biological aspects of plant pathogens, plant endophytes and saprophytes. Adv. Bot. Res. 24, 169–193.

Rotem, J., 1994. The Genus *Alternaria*: Biology, Epidemiology and Pathogenicity. APS Press, St. Paul, MN, 326 pp.

Saad, S., Hagedorn, D.J., 1996. Symptomatology and epidemiology of Alternaria leaf spot of bean *Phaseolus vulgaris*. Phytopathology 59, 1530–1533.

Schoeneweiss, D.F., 1975. Predisposition, stress, and plant disease. Annu. Rev. Phytopathol. 13, 193–211.

Schoeneweiss, D.F., 1981. The role of environmental stress in diseases of woody plants. Plant Dis. 65, 308–314.

Schoeneweiss, D.F., 1983. Drought predisposition to Cytospora canker in blue spruce. Plant Dis. 67, 383–385.

Schulz, B., Römmert, A.K., Dammann, U., Aust, H.J., 1999. The endophyte-host interaction: a balanced antagonism? Mycol Res. 103, 1275–1283.

Siegel, M.R., Latch, G.C.M., Johnson, M.C., 1987. Fungal endophytes of grasses. Annu. Rev. Phytopathol. 25, 293–315.

Snedecor, G.W., Cochran, W.G., 1989. Statistical Methods, 8th ed. Iowa State University, 503 pp.

Spurr Jr., H.W., 1977. Protective application of conidia of nonpathogenic *Alternaria* sp. isolates for the control of tobacco brown spot disease. Phytopathology 67, 128–132.

Swart, W.J., Conradie, E., Wingfield, M.J., Venter, W.B., 1992. Effect of water stress on the development of cambial lesions caused by *Cryphonectria cubensis* on *Eucalyptus grandis*. Plant Dis. 76, 744–746.

The Non-Affiliated Soil Analysis Work Committee, 1990. Handbook of Standard Soil Testing Methods. Soil Science Society of South Africa, Pretoria, South Africa, 146 pp.

Tippett, J.T., Crombie, D.S., Hill, T.C., 1987. Effect of phloem water potential on the growth of *Phytophthora cinnamomi* in *Eucalyptus marginata*. Phytopathology 77, 246–250.

van den Heuvel, J., 1970. Antagonistic effects of epiphytic microorganisms on infection of dwarf bean leaves by *Alternaria zinniae*. Phytopathol. Lab. Willie Commelin Scholten, Mededeling No. 84, 84 pp.

Van Dijk, H.F.G., Van Der Gaag, M., Perik, P.J.M., Roelofs, J.G.M., 1992. Nutrient availability in Corsican pine stands in the Netherlands and the occurrence of *Sphaeropsis sapinea*: a field study. Can. J. Bot. 70, 870–875.

Verhoeff, K., 1974. Latent infection by fungi. Annu. Rev. Phytopathol. 12, 99–110.

Wilson, D., 1995. Endophyte—the evolution of a term, and clarification of its use and definition. Oikos 73, 274–276.