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Species composition of endophytic fungi in *Amaranthus hybridus* leaves, petioles, stems, and roots

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Abstract: The objectives of this study were to identify and quantify fungi in asymptomatic, 5-mo-old *Amaranthus hybridus* plants grown in cultivated plots and in a greenhouse, and determine the species composition in different plant parts. Plants were sampled in South Africa from a plot at Potchefstroom in 1997, from two plots at Bloemfontein in 1997, and from one plot at Bloemfontein in 1996. Leaves, petioles, roots, stems, and seeds were collected, surface disinfested, and small sections were placed on corn-meal agar; seeds were not sectioned (9160 isolation attempts). All fungal colonies growing from the sections/seeds were transferred from colony margins onto separate agar plates and identified. Differences in species composition were found among plant parts ($P < 0.001$). The most common species isolated from leaves and petioles at both sites and both years were species of the *Alternaria tenuissima* group. Fungal genera other than *Alternaria* predominated in the roots with pronounced differences between the two sites. Isolation frequencies from stems and seeds were low. This paper identifies the endophytic fungi found in *A. hybridus* and sets the stage for further studies dealing with these fungi.

Key Words: *Alternaria tenuissima,* amaranth, fungal ecology, latent-infecting fungi

INTRODUCTION

Amaranthus hybridus (smooth amaranthus or amaranth) is a fast growing annual plant producing up to six generations per yr. It is eaten as a leafy-vegetable crop and is high in proteins, carbohydrates, and lipids (Rawate 1983). Amaranth grows well in South Africa and other semiarid regions of Africa, and its utilization is increasing throughout the world as an important alternative food source (Harlan 1992, Kauffman and Haas 1983, Kauffman and Weber 1990, Rawate 1983). However, the fungi associated with *A. hybridus* and their potential beneficial or detrimental effects on this host are unknown.

Endophytic fungi live within healthy plant tissues without eliciting disease symptoms (Bills 1996, Wilson 1995). They can play important roles in the health and vigor of many plants by affecting various ecological and physiological processes (Redlin and Carris 1996). In grasses, clavicipitaceous endophytes can benefit their hosts through inhibition of phytophagous insects and some pathogenic fungi, whereas others cause disease (Clay 1989, Latch et al 1985, Siegel et al 1987). It has been suggested that endophytic fungi infecting nongrass hosts may act as protective mutualists (Carroll 1986). Latent-infecting fungi also are endophytes that can elicit disease symptoms under certain environmental or nutritional conditions, or following host maturational changes (Petrini 1986, Verhoeff 1974, Wilson 1995). Other inconspicuous fungal colonists of plants are neutral symbionts (Dix and Webster 1995). Some investigators consider endophytic colonization clearly distinct from colonization by latent pathogens (Carroll 1986, Sinclair and Cerkauskas 1996). However, Bills (1996) points out that many fungi commonly found as symptomless colonists can cause disease.

Information currently available on mutualistic effects of endophytes is based on work with grass and perennial plant endophytes (Redlin and Carris 1996). The roles of endophytes in annual plants are unknown. The study objectives were to (i) identify and quantify the endophytic fungi associated with asymptomatic *A. hybridus* plants grown in cultivated plots, (ii) determine differences in species compositions among different plant parts (leaves, petioles, stems, roots, and seeds), (iii) test consistency in species occurrence between two field sites and 2 yr, and (iv) examine if fungal frequencies and/or species compositions differ between field and greenhouse grown plants. Studies were conducted in cultivated-

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monoculture fields at three sites in South Africa and in a greenhouse using potted plants grown from seed.

MATERIALS AND METHODS

Field plants.—Fields were established for sampling in a plot at Potchefstroom (site A) and in two plots at Bloemfontein (sites B and C), South Africa. Amaranth seeds were sown in seed trays in a sand-peat (50:50 v/v) mixture. Fields were mechanically cultivated using tine implements at all sites. Thirty-d-old seedlings were transplanted in mid-Nov at all three sites. After placing plants in the planting holes, the holes were filled with water and then with soil. The row spacing (distance between rows) was 1.5 m with an intra row spacing (distance between plants) of 0.3 m. Drip line irrigation was used for 2 h every other d with ca 40 mm water per irrigation at each site.

In Apr 1997, ten asymptomatic leaves, petioles, and roots from each of 20 and ten 5-mo-old *A. hybridus* plants were collected at sites A and B, respectively. The same sampling frequencies used at site B in 1997 also were used in a preliminary trial in Mar 1996. Asymptomatic stems of ten plants and four seeds from each of ten plants were collected at sites B and C in 1997. One hundred asymptomatic seeds were collected from each of ten plants at site B in 1996.

Plant parts were surface-disinfested using a series of 1 min in 96% ethanol, 5 min in a 3.5% NaOCl solution (m/v), and 30 s in 96% ethanol. Ten 5-mm-diam pieces from each leaf were removed laterally (5 sections) and along the midvein (5 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 before surface-disinfestation, and five 1-cm-long segments were then cut from each. Four small chips of ca $(5\ mm)^3$ were cut from each of the surface-disinfested stems. Surface-disinfested seeds were not sectioned.

Tissue pieces and seeds were then placed on corn-meal agar (CMAs; Oxoid, Basingstoke, England) containing streptomycin sulfate (0.3 mL Novo-Strep/L; at 1 g per 3 mL active ingredients) and incubated at 24 C (9160 total isolation attempts). After 5 d, all fungal colonies growing from the tissue pieces and seeds were transferred from colony margins to 1.5% water agar (WA; Oxoid). Percentage isolation can exceed 100% due to multiple colonies from individual pieces and seeds.

Greenhouse plants.—Plants were established in a soil mix (v/v) of 50% sand-loam (10–15% clay) and 50% peat in 1 L pots. Soils were steam sterilized twice, for 1 h at 80 C, before planting seeds 10 mm deep. Plants were watered to field capacity daily and were fertilized by adding 50 mL per pot of a 3 g/L hydroponic nutrient solution (6.5:2.7:13 N:P:K plus micronutrients) once a week as a soil drench for the duration of the experiment. The same isolation procedures were used for greenhouse-grown plants as for field-grown plants. Five 3-mo-old plants were sampled in Jun 1997 and an additional five in Mar 1998 for leaf, petiole, and root isolations, and fourteen 4-mo-old plants were sampled in both Mar and May 1998 for stem isolations (2112 total isolation attempts).

Identification of isolates.—Isolates were identified after 1 mo after transfer to WA. Media used for identification included WA and WA with pine needles; hay agar (HA) and potato-carrot agar (PCA) (Simmons 1992); and WA with carnation leaves (CLA) and potato dextrose agar (PDA; Oxoid) (Nelson et al 1983). In the preliminary trial, conducted at site B in 1996, representative isolates were selected for microscopic examination based on cultural and morphological characteristics. Characteristics used to select the representative isolates included growth rates, colony surface texture and margin shape, hyphal pigments, and sporulating and nonsporulating structures. In 1997 and 1998, microscopic morphological characteristics were used to identify each individual isolate at all sites.

Simmons' taxonomic criteria were used to identify *Alternaria* isolates (Simmons 1990, 1995, Simmons and Roberts 1993). Spore size, observations of branching patterns, and other morphological characters were measured/recorded for representative *Alternaria* isolates grown on WA, HA, and PCA. Visual estimations of spore size and other morphological characters were made for all *Alternaria* isolates on WA.

Representative, single-conidial isolates were saved for the most common species (those occurring in 1% or more of the total collection) from all plant parts and from each site. Each isolate was obtained from a different section. These isolates are stored at 4 C on WA slants and on WA plugs in distilled water and are being maintained in the Plant Pathology Departmental Collection at the University of the Orange Free State.

Statistical analyses.—χ^2 analyses of the composition of fungal species isolated from sections in relation to plant part and geographic location/year were performed using Minitab for Windows, release 10.2 (Minitab Inc., State College, Pennsylvania).

RESULTS

Fungi were isolated most often from asymptomatic, surface-disinfested leaves, petioles, and roots of field plants (TABLE I). Frequencies of isolation were greatest from leaves, followed by petioles, and roots in 1997 for both sites. In 1996, fungal isolations from petioles were more frequent than from leaves. Isolation frequencies from asymptomatic field stems were low (6% average for both sites). No fungi were isolated from asymptomatic seeds collected in the field in 1997, and were seldom isolated in 1996 (7%).

A small percentage of the original isolates were lost before identification due to cultural contamination by mites, fungi, and/or bacteria. The percentage of isolates lost from field collected plants were: leaf 3%; petiole 1%; stems 0%; and root 2% (average for all sites in 1997). No isolates were lost due to contamination from greenhouse plants. There is no reason

TABLE I. Percentages (and number of isolation attempts) of fungi isolated from asymptomatic, surface disinfested *Amaranthus hybridus*

Plant part	Site A, 1997 Potchefstroom	Site B, 1996 Bloemfontein	Site B, 1997 Bloemfontein	Site C, 1997 Bloemfontein	Greenhouse[a] 1997–1998
Leaves	98 (2000)	150 (1000)	99 (1000)	—[b]	0.3 (1000)
Petioles	83 (1000)	200 (500)	66 (500)	—	0.6 (500)
Stems	—	—	8 (40)	5 (40)	1.8 (112)
Roots	60 (1000)	91 (500)	64 (500)	—	23 (500)
Seeds	—	7 (1000)	0 (40)	0 (40)	—

[a] Combination of 2 trials (1997 and 1998).
[b] — means no isolation attempts were made.

to believe that cultural contamination occurred more or less often with any given species.

Differences in species composition occurred in the field among plant parts ($P < 0.001$) and among sites/yr ($P < 0.001$). However, the most common species isolated from field leaves at both sites and in both years were species of the *Alternaria tenuissima* group (TABLE II). This species also was most frequently isolated from petioles, but it comprised a lower percentage of the total isolates (TABLE III). The remaining non-*Alternaria* isolates from these two plant parts consisted of several species, the most common being *Epicoccum nigrum* from leaves and two species of *Phoma* from petioles. *Fusarium* species, including *Fusarium oxysporum,* were the most common species isolated from field roots (TABLE IV). Although species of *Fusarium* predominated in the roots, species of the *A. tenuissima* group also were present. Isolation frequencies from field stems (sites B and C combined) included the following numbers: one *A. tenuissima* species-group isolate, two isolates of *Penicillium,* one *Fusarium equiseti,* and one *Chaetomium* isolate. No isolates collected from seeds in 1996 were species of *Alternaria.*

Similar isolation attempts from potted *A. hybridus*

TABLE II. Species composition (percentages) of fungi in asymptomatic, surface disinfested *Amaranthus hybridus* leaves

Fungal species/genera	Site A, 1997 Potchefstroom	Site B, 1996 Bloemfontein	Site B, 1997 Bloemfontein
Alternaria tenuissima group	85	80	88
A. alternata	5	1	1
Alternaria spp.[a]	<1	<1	<1
Epicoccum nigrum	3	3	2
Phoma spp.	<1	1	8
Penicillium spp.	<1	—[b]	<1
Fusarium oxysporum	—	—	—
Fusarium spp.[c]	<1	—	—
Other identified isolates[d]	2	1	1
Unidentified isolates[e]	3	13	<1
N^f =	1959	1503	986

[a] *Alternaria* species other than *A. tenuissima* species group or *A. alternata.*
[b] — means the fungal species or genera were not isolated.
[c] *Fusarium subglutinans* and *F. equiseti.*
[d] Include, from most to least common, *Drechslera poae, Drechslera biseptata,* and *Rhizoctonia* sp.
[e] Include nonsporulating mycelial fungi. For site B in 1996, this category was used for fungi that were not examined and nonsporulating mycelial fungi.
[f] Total number of isolates.

TABLE III. Species composition (percentages) of fungi in asymptomatic, surface disinfested *Amaranthus hybridus* petioles

Fungal species/genera	Site A, 1997 Potchefstroom	Site B, 1996 Bloemfontein	Site B, 1997 Bloemfontein
Alternaria tenuissima group	68	63	78
A. alternata	3	1	2
Alternaria spp.[a]	<1	—[b]	—
Epicoccum nigrum	4	—	1
Phoma spp.	1	5	6
Penicillium spp.	2	—	3
Fusarium oxysporum	—	—	—
Fusarium spp.[c]	1	—	—
Other identified isolates[d]	6	11	3
Unidentified isolates[e]	14	20	7
N^f =	832	1002	328

[a] *Alternaria* species other than *A. tenuissima* species group or *A. alternata.*
[b] — means the fungal species or genera were not isolated.
[c] *Fusarium subglutinans* and *F. equiseti.*
[d] Include, from most to least common, *Aspergillus* sp., *Drechslera biseptata, Drechslera poae, Trichoderma* sp., and *Aureobasidium* sp.
[e] Include nonsporulating mycelial fungi. For site B in 1996, this category was used for fungi that were not examined and nonsporulating mycelial fungi.
[f] Total number of isolates.

TABLE IV. Species composition (percentages) of fungi in asymptomatic, surface disinfested *Amaranthus hybridus* roots

Fungal species/genera	Site A, 1997 Potchef-stroom	Site B, 1996 Bloem-fontein	Site B, 1997 Bloem-fontein
Alternaria tenuissima group	27	21	3
A. alternata	1	—[a]	—
Alternaria spp.[b]	<1	—	—
Epicoccum nigrum	3	1	2
Phoma spp.	26	3	2
Penicillium spp.	5	—	1
Fusarium oxysporum	13	—	61
Fusarium spp.[c]	3	20	3
Other identified isolates[d]	5	5	6
Unidentified isolates[e]	17	50	22
N^f =	602	457	318

[a] — means the fungal species or genera were not isolated.
[b] *Alternaria* species other than *A. tenuissima* species group or *A. alternata.*
[c] *Fusarium subglutinans, F. equiseti,* and other *Fusarium* spp. *Fusarium oxysporum* might be included in this group in 1996.
[d] Include, from most to least common, *Chaetomium* sp., *Drechslera biseptata, Rhizoctonia* sp., *Aureobasidium* sp., *Aspergillus* sp., Oomycete spp., *Drechslera poae,* and *Trichoderma* sp.
[e] Include nonsporulating mycelial fungi. For site B in 1996, this category was used for fungi that were not examined and nonsporulating mycelial fungi.
[f] Total number of isolates.

plants grown from seed in a greenhouse yielded few fungal colonies from any plant part (TABLE I). Isolations from greenhouse leaves (both years combined) consisted of two *A. tenuissima* species-group isolates and one isolate of *Penicillium.* Only three *Penicillium* isolates were obtained from greenhouse petioles (both years combined). Ten *A. tenuissima* species-group isolates and 104 isolates of nonsporulating mycelial fungi were obtained from greenhouse roots (both years combined). One isolate of *Penicillium* and one unidentified isolate were obtained from greenhouse stems (both trials combined).

Description of Alternaria *taxa.*—Special attention is given to the species of *Alternaria* since (i) *Alternaria* comprised the main component of endophytic fungi in leaves and petioles and was also found in stems and roots, (ii) this is the first study that identifies endophytic fungi in *A. hybridus,* and (iii) different authors use different criteria for identifying species of *Alternaria* that result in different species names for the same isolates. The two most common species of *Alternaria* have been identified and representative single-spore isolates were saved. Three additional species of *Alternaria* (distinct morphological forms) made up less than 1% of the total isolates collected and were not saved.

The most common *Alternaria,* originally called "*A. alternata* straight conidial chain form" (Blodgett et al 1998b), was identified as belonging to the small-spored *A. tenuissima* group (Simmons 1990, 1995, Simmons and Roberts 1993). This species has long (10–20 conidia), generally unbranched conidial chains. Branching is extremely rare, but when present consists of only 1–2 branches with 1–4 conidia/branch off the longer chain. Beaks are rare (less than 10% of the spores), but when present are short, broad to narrow but never filiform and never branched. Early sporulation (less than 7 d) might have shorter chains, but all isolates develop at least 10 conidia/chain with age. Conidia are golden brown, smooth or verruculose, with up to 8 transverse and several longitudinal or oblique septa, and 19–50 ($\bar{x}$ = 27) μm long by 8–16 ($\bar{x}$ = 11) μm wide at the broadest part. Culture numbers of representative isolates include: A2, A46, A157, A224, and A321.

The second most common *Alternaria,* originally called "*Alternaria alternata* branched conidial chain form" (Blodgett et al 1998b), was identified as belonging to the small-spored *A. alternata* group (Simmons 1990, 1995). This species has a complex (low bushy) branched chain form of at least 12 conidia/chain on the longest fork. Branching usually consists of 3–7 (or more) branches with 1–9 conidia/branch. Branching is extremely common although unbranched chains are present. Beaks are extremely rare (less than 5% of the spores). When present, beaks are short, broad and never branched. Early sporulation (less than 7 d) has shorter and unbranched chains, but all isolates develop the branching pattern with age. Dense, black clumps can be seen on plates with time. Conidia are golden brown, smooth or verruculose, with up to 7 transverse and several longitudinal or oblique septa, smaller and more consistent in size compared with the *A. tenuissima* species-group isolates, and 16–39 ($\bar{x}$ = 23) μm long by 7–12 ($\bar{x}$ = 9) μm wide at the broadest part. Culture numbers of representative isolates include: A286, 2LNB5-8, 3LG10-10, 5LU2-4, and 2PU10-2.

The third most common *Alternaria* (0.1% of the isolates) is a small-spored *Alternaria.* This species has short to moderate, generally unbranched chains of 3–8 conidia. Branching is rare, but when present, consists of only 1–2 short branches with 1–2 conidia/branch off the longer chain. Beaks are present on more than 50% of the conidia. Beaks are broad to narrow but never filiform and never branched, and

up to half the length of the conidium (usually shorter). This species never develops chains longer than 8 conidia with age. Conidia are golden brown, smooth, with 4–7 transverse and several longitudinal or oblique septa. The conidia, although small, are larger than those of our *A. tenuissima* species-group isolates (ca 60 × 17 μm). This species is potentially a species of the *A. tenuissima* group or might be *A. amaranthi* (Peck) Venkatakrishnaiah (Venkatakrishnaiah 1952).

The second least common *Alternaria* (0.05% of the isolates) is a large-spored species. Conidia are solitary with long tapering beaks, golden or olivaceous brown, and smooth. Beaks are long, taper narrow to filiform, never branched, and approximately the same length as the body. The spore size is substantially larger than the other species of *Alternaria* isolated (ca 190 × 19 μm). This species is similar to *A. solani.*

The least common *Alternaria* (0.03% of the isolates) is a small-spored *Alternaria.* Conidia are solitary or in short chains (2–4 conidia/chain), golden brown, and beaks were never observed. It never develops chains longer than 4 conidia with age. Spores are broader than that of the *A. tenuissima* species-group isolates (ca 28 x 18 μm).

DISCUSSION

Endophytic fungi of the *A. tenuissima* species group show specificity for leaves and petioles of *A. hybridus* as indicated by the fungal species compositions observed at the two locations and two years. However, different species compositions were observed between the two sites in roots indicating less fungal specificity in colonizing roots compared with leaves and petioles. Isolation frequencies of the *A. tenuissima* group were high in leaves and petioles and preliminary results indicate that asymptomatic fungal colonization of *A. hybridus* leaves collected from the field can be extensive (Blodgett et al 1998a). This is in contrast to colonization by *A. alternata,* a common epiphyte and endophyte that occurs in low frequencies with asymptomatic host colonization often restricted to the substomatal chamber (Cabral et al 1993, O'Donnell and Dickinson 1980). The extensive colonization previously observed in this host (Blodgett et al 1998a) might be due to one or a few species and other species might be restricted to substomatal chambers as observed in annual *Juncus* (Cabral et al 1993). Petrini (1986, 1996) suggested that few endophytic fungal taxa dominate within a single plant species and these dominant fungi often are limited to one or a few taxonomically related host species. Although *A. tenuissima* is found on a number of host species it has never been identified in such high frequencies from asymptomatic host tissues. The host-specific nature of our *A. tenuissima* species-group isolates has not been tested.

The processes involved in infection, including the influence of the environment, are important aspects in the study of any plant-fungus relationship. Spores of most species of *Alternaria* are readily dispersed by wind and rain splash (Rotem 1994). Some endophytic and/or latent pathogenic fungi, including 36 species of *Alternaria,* also are carried in the seeds of their hosts (Carroll 1988, Neergaard 1977, Siegel et al 1987, Sinclair 1991). In these cases the fungi remain quiescent or dormant within the seed until seed germination. The fungi then colonize the new plant tissues. Rotem (1994) suggested that seed infection is common among *Alternaria* taxa. In the current study, however, fungi seldom were isolated from seeds and greenhouse plants grown from seeds were rarely colonized by fungi. Therefore, the endophytic fungi of *A. hybridus* are not frequently carried in asymptomatic seeds of this host.

Our results show that greenhouse plants can be produced easily and maintained virtually free of leaf and petiole endophytic fungi. Since seed transfer of endophytic fungi of *A. hybridus* are infrequent or nonexistent, the extremely low number and diversity of fungal taxa in greenhouse plants compared with field plants is likely due to reduced inoculum, reduced compatible inoculum, reduced inoculum dispersal (i.e., no wind or rain splash), and/or environment conditions incompatible for infection. The noncolonized leaves and petioles of *A. hybridus* plants grown in a greenhouse are ideal for controlled studies of the infection processes of *A. hybridus* or for other studies dealing with plant-fungal relationship of this host under controlled conditions.

Mycologists have proposed several classification schemes for separating species of *Alternaria* (Ellis 1971, 1976, Groves and Skolko 1944, Joly 1967, Neergaard 1945, Rao 1969, 1971, Simmons 1967, 1981, 1982, 1990, 1992, Wiltshire 1933). Rotem (1994) concluded that there is no generally accepted classification for *Alternaria* species, but that the taxonomic criteria used by many mycologists and pathologists is that of Ellis (1971, 1976). The references are in concise form and are cited in Rotem (1994). Ellis (1971, 1976) does not use the sporulation patterns more recently suggest by Simmons and Roberts (1993) as being a useful method for distinguishing taxa. Using Ellis' and others' taxonomic criteria, four of our five *Alternaria* groups would be classified as *A. alternata.* Using the taxonomic criteria of Simmons, the species/groups isolated from *A. hybridus* can be separated.

However, the forms described by Simmons for classification were not completely stable in our isolates. Morphological variation is common within *Alternaria* species (Groves and Skolko 1944, Rotem 1994). There was variation both within and between single-spore cultures of our *Alternaria* groups. For example, based on Simmons and Roberts (1993) there were several branch forms observed in a single, single-spore culture. The growth and sporulation of *Alternaria* in culture is progressive. Therefore, standard examination conditions should be used. At only 5 d, our *A. tenuissima* species-group isolates may only produce up to 4 conidia/chain and thus could be confused with other species. Also, both the robust and lean appearing conidia shapes, as defined by Simmons and Roberts (1993), were observed within a single, single-spore culture. These observations include ones made from individual isolates that were both single-spore transferred and hyphal-tip transferred, and then grown on WA, HA, and PCA. This within single-spore isolate variation can make identification difficult when using the taxonomic criteria of Simmons. However, the traditional classification criteria were not useful for separating our groups.

Although the morphological variation can result in difficulties when using Simmons' criteria, these can be used for classification if the most abundant sporulation pattern within single isolates are used. For example, the *A. tenuissima* group isolates always produced long, generally unbranched conidial chains and the *A. alternata* isolates always developed a dense, branching pattern in time. These distinct morphological differences were never observed collectively within single isolates. These isolates are morphologically distinct and we feel they represent different species. Simmons' work presents the best criteria for separating our *Alternaria* isolates in an area where additional taxonomic studies are needed (Simmons 1992). Variation among single-spore isolates of our *A. tenuissima* species group is the topic of further investigations.

This is the first study to identify the endophytic fungi in *A. hybridus*. *Alternaria* isolates collected from this host are described for the first time in the results of this paper, and this work sets the stage for further studies dealing with these fungi. Future studies will examine (i) the influence of environmental factors, such as stress, on fungal frequencies, (ii) how agricultural practices might influence these fungi and thus crop yields, (iii) the potential of these fungi to act as latent-pathogens and if so, mechanisms that trigger the pathogen to induce symptoms, (iv) whether these fungi provide protection from herbivorous insects, and (v) the genetic variation of the *A. tenuissima* group isolates to examine potential species/subspecies variation. These additional studies will take advantage of the hundreds of single spore isolates obtained thus far, will give new insight into the biology of plant-insect-fungal relationships, and will provide an opportunity to study the population of the *A. tenuissima* species group as an endophyte.

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LITERATURE CITED

Bills GF. 1996. Isolation and analysis of endophytic fungal communities from woody plants. In: Relin SC, Carris LM, eds. Endophytic fungi in grasses and woody plants. St. Paul, Minnesota: APS Press. p 31–65.

Blodgett JT, Swart WJ, Bender CM. 1998a. Infection and colonization pattern of *Amaranthus hybridus* by *Alternaria alternata*. S Afr J Sci 94:xiv.

———, ———, Louw SvdM, Weeks WJ. 1998b. Influence of soil amendments on species composition of endophytic fungi in *Amaranthus hybridus*. S Afr J Sci 94:xviii.

Cabral D, Stone JK, Carroll GC. 1993. The internal mycobiota of *Juncus* spp.: microscopic and cultural observations of infection patterns. Mycol Res 97:367–376.

Carroll GC. 1986. The biology of endophytism in plants with particular reference to woody perennials. In: Fokkema NJ, van den Heûvel J, eds. Microbiology of the phyllosphere. Cambridge, UK: Cambridge University Press. p 205–222.

———. 1988. Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. Ecology 69:2–9.

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

Dix NJ, Webster J. 1995. Fungal ecology. London: Chapman & Hall. 549 p.

Ellis MB. 1971. Dematiaceous hyphomycetes. Kew, England: Commonwealth Mycological Institute. 608 p.

———. 1976. More Dematiaceous hyphomycetes. Kew, England: Commonwealth Mycological Institute. 507 p.

Groves JW, Skolko AJ. 1944. Notes on seed-borne fungi. II. *Alternaria*. Can J Res Sect C 22:217–234.

Harlan JR. 1992. Crops and man. 2nd ed. Madison, WI: American Soc. Agronomy. 284 p.

Joly P. 1967. Key for determination of the most common species of the genus *Alternaria* (Nees) Wiltsh. emend. Joly. Pl Dis Rep 51:296–298.

Kauffman CS, Haas PW. 1983. Grain amaranth: a crop with low water requirements and high nutritional value. In: Lockeretz W, ed. Environmentally sound agriculture, selected papers, 4th conference, International Federation of Organic Agriculture Movements, Cambridge,

Massachusetts, August 18–20, 1982. New York: Praeger. p 299–313.

———, Weber LE. 1990. Grain amaranth. In: Janick J, Simon JE, eds. Advances in new crops: proceedings of the First National Symposium on New Crops, Research, Development, Economics, Indianapolis, Indiana, Oct. 23–26 1988. Portland, Oregon: Timber Press. p 127–139.

Latch GCM, Hunt WF, Musgrave RR. 1985. Endophyte fungi affect growth of perennial ryegrass. New Zealand J Agric Res 28:165–168.

Neergaard P. 1945. Danish species of *Alternaria* and *Stemphylium*. London: Oxford University Press. 562 p.

———. 1977. Seed pathology. London: Macmillan. 839 p.

Nelson PE, Toussoun TA, Marasas WFO. 1983. *Fusarium* species: an illustrated manual for identification. University Park, Pennsylvania: The Pennsylvania State University Press. 193 p.

O'Donnell J, Dickinson CH. 1980. Pathology of *Alternaria* and *Cladosporium* isolates on *Phaseolus*. Trans Br Mycol Soc 74:335–342.

Petrini O. 1986. Taxonomy of endophytic fungi of aerial plant tissues. In: Fokkema NJ, van den Heuvel J, eds. Microbiology of the phyllosphere. Cambridge, UK: Cambridge University Press. p 175–187.

———. 1996. Ecological and physiological aspects of host specificity in endophytic fungi. In: Redlin SC, Carris LM, eds. Endophytic fungi in grasses and woody plants: systematics, ecology, and evolution. St. Paul, Minnesota: APS Press. p 87–100.

Rao VG. 1969. The genus *Alternaria*—from India. Nova Hedwigia 17:219–258.

———. 1971. An account of the fungus genus *Alternaria* Nees from India. Mycopathol Mycol Appl 43:361–374.

Rawate PD. 1983. Amaranth (pigweed): a crop to help solve the world protein shortage. In: Lockeretz W, ed. Environmentally sound agriculture, selected papers, 4th conference, International Federation of Organic Agriculture Movements, Cambridge, Massachusetts, August 18–20, 1982. New York: Praeger. p 287–298.

Redlin SC, Carris LM. 1996. Endophytic fungi in grasses and woody plants: systematics, ecology, and evolution. St. Paul, Minnesota: APS Press. 223 p.

Rotem J. 1994. The genus *Alternaria*: biology, epidemiology, and pathogenicity. St. Paul, Minnesota: APS Press. 326 p.

Siegel MR, Latch GCM, Johnson MC. 1987. Fungal endophytes of grasses. Annu Rev Phytopathol 25:293–315.

Simmons EG. 1967. Typification of *Alternaria*, *Stemphylium* and *Ulocladium*. Mycologia 59:67–92.

———. 1981. *Alternaria* themes and variation. Mycotaxon 13:16–34.

———. 1982. *Alternaria* themes and variation (7–10). Mycotaxon 14:17–43.

———. 1990. *Alternaria* themes and variation (27–53). Mycotaxon 37:79–119.

———. 1992. *Alternaria* taxonomy: current status, viewpoint, challenge. In: Chelkoski J, Visconti A, eds. *Alternaria* biology, plant disease and metabolites. Amsterdam: Elsevier. p 1–35.

———. 1995. *Alternaria* themes and variation (112–144). Mycotaxon 55:55–163.

———, Roberts RG. 1993. *Alternaria* themes and variation 73. Mycotaxon 48:109–140.

Sinclair JB. 1991. Latent infection of soybean plants and seeds by fungi. Pl Dis 75:220–224.

———, Cerkauskas RF. 1996. Latent infection vs. endophytic colonization by fungi. In: Redlin SC, Carris LM, eds. Endophytic fungi in grasses and woody plants: systematics, ecology, and evolution. St. Paul, Minnesota: APS Press. p 3–29.

Venkatakrishnaiah NS. 1952. Blight of *Amaranthus paniculatus* (L) caused by *Alternaria*. Phytopathology 42:668–669.

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.

Wiltshire SP. 1933. The foundation species of *Alternaria* and *Macrosporium*. Trans Br Mycol Soc 18:135–160.