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Genus-specific host range of *Phomopsis amaranthicola* (Sphaeropsidales), a bioherbicide agent for *Amaranthus* spp.

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Abstract
Phomopsis amaranthicola, a newly described species discovered in Florida, is under development as a biological control agent for weeds belonging to the genus *Amaranthus*. Host-range testing was performed on the fungus, using the centrifugal phylogenetic scheme, with *Amaranthus hybridus* as the focal plant. Forty-five accessions of 21 species in the genus *Amaranthus* were tested for susceptibility to infection by this fungus. Fifty-six plant species outside the genus *Amaranthus* were also tested. The latter included crops in which the fungus might be used as a bioherbicide, members of genera that are closely related to *Amaranthus*, and selections of species that have been reported as hosts of other species of the genera *Phomopsis* or *Diaporthe*. *Phomopsis amaranthicola* did not infect any of the plants from outside the genus *Amaranthus* but was highly pathogenic to several species in the genus *Amaranthus*. All *Amaranthus* species tested were infected to varying degrees by *P. amaranthicola*; the resulting mortality levels were also variable. Thus, *P. amaranthicola* appears to be host-specific to the members of the genus *Amaranthus*. The generic level of specificity of this pathogen renders it not only a safe bioherbicide agent but also one that could be used against several weedy *Amaranthus* species.

Keywords: Phomopsis amaranthicola, *bioherbicide,* Amaranthus, *pigweed*

Introduction

A number of *Phomopsis* species and the associated *Diaporthe* Nitschke teleomorphs are known to cause a variety of diseases (Balducchi & McGee 1987; Yesodharan & Sharma 1987; Uecker 1988; Farr et al. 1989; Uecker & Johnson 1991; Killebrew et al. 1993). Other species of *Phomopsis* are also known to be saprophytic, endophytic, or weakly pathogenic. One example of an endophytic species is *Phomopsis oblonga* (Desmaz.) Traverso, a natural inhabitant of healthy elm trees in England. This fungus serves to limit the breeding of the bark-beetle carrying *Ophiostoma ulmi* (Buisman) Nannf. (*Ceratocystis ulmi* (Buisman) C. Moreau) and may be responsible, in part, for slowing the spread of Dutch elm disease in some areas (Webber & Gibbs 1981). Shivas

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et al. (1994) demonstrated that *P. emecis* Shivas, causal organism of stem blight in *Emex australis* Stein., was pathogenic to five closely related species in the Polygonaceae and that inoculation of other unrelated plant species resulted in infection only when the plants were wounded or were senescent and that the organism did not advance to the healthy tissues. Many species of *Phomopsis* have been important in forest pathology (Hahn 1930; Grove 1935, 1937). Symptoms associated with these pathogens include canker, seed decay, stem-end rot, root rot, fruit rot, wilt, leaf spots, and bark necrosis. Approximately 65 species of *Phomopsis*, 30 of which are found in the United States, are considered to be plant pathogenic. Those considered as plant pathogens are thought to be host specific (Uecker 1988).

The objective of this study was to determine the host range of *Phomopsis amaranthicola* Rosskopf, Charudattan, Shabana and Benny (Rosskopf et al. 2000b). This fungus was isolated from an unidentified *Amaranthus* sp., possibly *A. hybridus* L., in Florida and found to be highly pathogenic to this species in initial testing of its potential as a biological control agent. In field trials utilizing *P. amaranthicola*, conidial suspensions were found to be more efficacious than mycelium. Species controlled in the field included *A. hybridus, A. lividus, A. viridus,* and *A. spinosus*. (Rosskopf et al. 2000a). The fungus has been patented as a biological control agent for weeds in the genus *Amaranthus* (Charudattan et al. 1995, 1996).

Materials and methods

Host-range testing of *P. amaranthicola* was performed using the centrifugal phylogenetic scheme (Wapshere 1974) with *A. hybridus* as the focal plant. The first phase of testing included 45 accessions belonging to 20 known and one unknown species of *Amaranthus* obtained from the North Central Regional Plant Introduction Station in Ames, Iowa (Table I).

Pathogenicity to plants in the genus Amaranthus

Plants were grown from seed and then transplanted to 9-cm clay pots. Six pots were planted with three plants from each accession; plants in three pots were inoculated with conidia of *P. amaranthicola* and three pots were used as non-inoculated controls (three replications per accession for each treatment). Conidia of *P. amaranthicola* were produced on V8® juice (Campbell Soup Company, Camden, NJ) agar (200 ml V8 juice, 800 ml water, and 3 g $CaCO_3$) and were harvested after 14 days incubation at 25°C (+/− 2°C) under a 12-h light-dark cycle. Conidial suspensions containing 1.0×10^6 conidia ml^{-1} were amended with psyllium mucilloid (Metamucil®, Procter & Gamble, Cincinnati, OH), as a humectant, at 0.5% (w:v). Suspensions were spray-inoculated onto plants at the four- to six-leaf stage, which was found to be a stage of intermediate susceptibility in *A. hybridus.* A hand-held, pump sprayer was used for this inoculation. Control plants were sprayed with a suspension containing psyllium mucilloid alone. Inoculated and control plants were then placed in a dark dew chamber for 24 h at 28°C (+/− 2°C). After the dew period, plants were placed in a greenhouse and observed for symptom development and mortality for 5 weeks.

Pathogenicity to plants outside the genus Amaranthus

The second phase of testing involved the inoculation of additional weed, crop, and ornamental species chosen on the basis of a close relationship to the genus

Table I. Reaction of species of *Amaranthus* L. to inoculation with *Phomopsis amaranthicola*.

Species	Origin	Percent incidence*	Percent mortality†
A. acutilobus Uline & Bray	Germany	100	100
A. albus L.	Germany	100	50
A. australis (Gray) J.D. Sauer	USA-FL	31	0
A. australis	USA-FL	39	0
A. blitoides S. Wats	Germany	100	0
A. caudatus L.	Argentina	100	28
A. caudatus	USA	94	28
A. crassipes Schlect.	Czechoslovakia	100	56
A. cruentus L.	Mexico	84	0
A. cruentus	USA-AR	100	50
A. cruentus	USA-ME	100	5
A. cruentus	Mexico	84	17
A. delflexus L.	Germany	100	67
A. dubius Mart. ex Thell.	Ghana	67	0
A. dubius	Jamaica	100	6
A. floridanus (S. Wats) Sauer	USA-FL	23	0
A. graecizans L.	USA-IA	100	50
A. hybridus L.	Argentina	100	18
A. hybridus	Ecuador	89	50
A. hybridus	USA-PA	100	26
A. hybridus	Zimbabwe	78	8
A. hybridus	USA-IN	100	56
A. hybridus	USA-AR	100	56
A. lividus L.	Hong Kong	100	100
A. lividus	India	100	86
A. palmeri S. Wats	USA-AR	100	0
A. palmeri	USA-IA	100	39
A. palmeri	Senegal	95	28
A. powelli S. Wats	Germany	100	84
A. powelli	USSR	100	100
A. quintensis H.B.K.	Ecuador	100	50
A. retroflexus L.	India	100	56
A. retroflexus	USA-CA	100	100
A. retroflexus	USA-IA	100	42
A. retroflexus	USA-IL	100	89
A. retroflexus	USA-PA	100	67
A. retroflexus	USA-WA	100	100
A. rudis Sauer	USA-IA	84	50
A. spinosus L.	Indonesia	100	44
A. spinosus	Zimbabwe	100	0
A. tricolor L.	India	78	50
A. tricolor	USA	56	28
A. tricolor	USA	100	67
A. viridis L.	Indonesia	100	84
A. viridis	Unknown	100	92

*Results were recorded five weeks after inoculation. Plants were inoculated at the four- to six-leaf stage (20 days after planting) and were inoculated by spraying with conidial suspension containing 1.0×10^6 conidia ml^{-1}.
†Data represent the average of three replicates combined from two trials.

Amaranthus, a report of an association of a plant with another species of *Phomopsis* or *Diaporthe*, or a crop plant in which *Amaranthus* spp. are a problem and therefore a crop in which *P. amaranthicola* might be utilized as a bioherbicide. Plants in this testing

phase were treated in the same way as previously described. In addition, tissue was taken from each of the treated plants and plated onto potato dextrose agar (PDA) to determine the presence of non-symptomatic, quiescent infection, as this has been known to occur with other *Phomopsis* spp. Tissues were surface-sterilized using a 10% (v:v) commercial bleach solution. The species tested and their reactions are listed in Table II. *Amaranthus hybridus* plants were included in each testing phase to ascertain the viability and infectivity of *P. amaranthicola* suspensions used for inoculation. The host range testing was performed twice.

Results and discussion

Results of the first phase of the host-range study are listed in Table I. All accessions of *Amaranthus* species tested were susceptible to infection by *P. amaranthicola*. Incidence of infection ranged from 23–100%, depending on species or accession. Plant mortality ranged from 0–100%. Accessions were grouped into mortality classes with 10% intervals to illustrate the frequency distribution (Figure 1). Eleven accessions from eight species fell into the mortality class 1 ($\leq$10% mortality). Another eleven accessions from nine species fell into mortality class 6 ($\geq$50% and $<$60% mortality). Accessions of *A. cruentus*, *A. palmeri*, *A. spinosus*, and *A. tricolor* fell into two or more mortality classes ranging from mortality class 1 ($<$10%) to 7 ($\geq$60% and $<$70%). There were only six accessions that fell into mortality class 10 ($>$90% mortality), these were accessions of *A. acutilobus*, *A. lividus*, *A. powelli*, *A. retroflexus*, and two accessions of *A. viridis*. The other accessions of *A. lividus*, *A. powelli*, and *A. viridis* fell into mortality class 9 ($\geq$80% and $<$90%) while other accessions of *A. retroflexus* fell into mortality class 5 ($\geq$40% and $<$50%), 6 ($\geq$50% and $<$60%), and 7 ($\geq$60% and $<$70%). Ten symptomatic plants were chosen for plating of diseased tissue to confirm the presence of *P. amaranthicola*. All symptomatic tissues produced colonies of *P. amaranthicola*. Some of the economically important, weedy *Amaranthus* spp. were highly susceptible while others were not.

The second phase of testing included members of other genera belonging to Amaranthaceae and representative species from the families Apiaceae, Apocynaceae, Asteraceae, Brassicaceae, Cactaceae, Campanulaceae, Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Liliaceae, Malvaceae, Poaceae, Solanaceae, and Verbanaceae. No plants became symptomatic and tissue from each of three plants from each inoculated species plated onto PDA produced no colonies of *P. amaranthicola*. Hence, these plants were considered to be immune to infection by *P. amaranthicola* (Table II).

Unlike other *Phomopsis* species that have been reported as pathogens of plants from more than one plant genus, *P. amaranthicola* exhibited a high degree of specificity only to the members of the genus *Amaranthus*. The *Amaranthus* species and their accessions exhibited different levels of susceptibility to *P. amaranthicola*. Previous studies have shown that the level of susceptibility or resistance to pathogens is highly variable in the genus *Amaranthus*; variability exists within cultivars or accessions of the same *Amaranthus* species and also among closely related species (i.e., grain amaranths). Sealy et al. (1988) demonstrated the differential resistance of 126 accessions of *Amaranthus* species and cultivars to *Pythium myriotylum* Drechs., causal organism of damping-off. They further attempted to place the accessions into resistance groups but the accessions did not cluster into naturally distinct groups.

Table II. Reactions of plant species to inoculation with *Phomopsis amaranthicola* in the host-range trial*.

Family Genus/species	Reaction
Apiaceae	
Daucus carrota L. subsp. *sativus* Hoffm. (cv. Long Imperator #58)	I
Amaranthaceae	
Alternanthera philoxeroides (L.) R. Brown	I
Celosia argentea L. (cv. Dwarf Fiery Feather)	I
Celosia argentea L. var. cristata (L.) Kuntze	I
Iresine rhizomatosa Standley	I
Froelichia gracilis (Hooker) Moq.	I
Gomphrena globosa L.	I
Apocynaceae	
Vinca minor L.	I
Asteraceae	
Helianthus giganteus L. (cvs. Giant Grey Stripe, Mammoth)	I
Lactuca sativa L. (cv. Iceberg)	I
Lactuca sativa L. var. *longifolia* Lam.	I
Achillea millefolium L.	I
Achillea ptarmica L.	I
Brassicaceae	
Brassica juncae (L.) Czern. (cv. Florida Broadleaf)	I
Cactaceae	
Opuntia compressa (Salisbury) Macbride	I
Campanulaceae	
Lobelia inflata L.	I
Caryophyllaceae	
Stellaria media (L.) Cyrillo	I
Lychnis alba Miller	I
Saponaria officinalis L.	I
Silene stellata (L.) Aiton F.	I
Dianthus armeria L.	I
Dianthus barbatus L.	I
Chenopodiaceae	
Chenopodium album L.	I
Atriplex patula L.	I
Beta vulgaris L. (cv. Detroit Dark Red)	I
Spinacia oleracea L. (cv. Bloomsdale Long Standing)	I
Kochia scoparia Roth	I
Cucurbitaceae	
Cucurbita pepo L. (cvs. Early Prolific Straightneck, Summer Crookneck)	I
Cucurbita maxima Duchesne (cvs. Cocozelle Bush, Dixie)	
Cucurbita moschata (Duchesne) Duchesne ex. Por.	I
Cucumis melo L. var. *cantalupensis* Naudin (cvs. Hale's Jumbo, Imperial 45)	I
Cucumis sativus L. (cvs. Marketer, Poinsett)	I
Citrullus lanatus (Thunb.) Matsum. and Nakai	I
Fabaceae	
Glycine max (L.) Merr. (cvs. Centennial, Coker 488, Hutton)	I
Senna obtusifolia (L.) H. S. Irwin and Barneby	I
Pisum sativum L. (cv. Little Marvel)	I
Pisum sativum L. var. *macrocarpon* Ser.	I

Table II (*Continued*)

Family Genus/species	Reaction
Phaseolus vulgaris L. (cvs. Bush Blue Lake 274, Kentucky Wonder Roma II, Royalty Purple)	I
Vicia faba L.	I
Lamiaceae	
Salvia farinacea Benth.	I
Salvia officinalis L.	I
Salvia splendens Sellow ex Roem. and Schult.	I
Plectranthus L'Her. sp.	I
Liliaceae	
Allium cepa L. (cv. Texas Grand 502)	I
Malvaceae	
Abelmoschus esculentus (L.) Moench (cv. Clemson Spineless)	I
Poaceae	
Pennisetum glaucum (L.) R. Brown (cv. Ga-Hi)	I
Triticum aestivum L. (cv. McNair 1813, Redhart)	I
Sorgum bicolor (L.) Moench (cv. 2022 NK, UF M-35)	I
Zea mays L. (cv. Golden Cross Bantam T-51)	I
Solanaceae	
Lycopersicon esculentum Mill. (cv. Floradel)	I
Capsicum annuum L. (cvs. Early Cal Wonder, Jupiter)	I
Capsicum frutescens L.	I
Nicotianum tabacum L.	I
Solanum melongena L.	I
Verbenaceae	
Verbena brasiliensis Vellozo	I
Verbena hastata L.	I

*Reactions were recorded from 3 replicates of each inoculated plant species. Two trials were performed with each variety listed. I = immune.

Eleven cultivars of two *Amaranthus* grain species, *A. cruentus* and *A. hypochondriacus* × *A. hybridus*, also exhibited differential susceptibility to Pythium stem-canker, caused by *P. aphanidermatum* (Edson) Fitzp. (Mihail & Champaco 1993). Reddy et al. (1980) found that varieties of *A. caudatus*, *A. cruentus*, *A. hypochondriacus*, grain amaranths and *A. tricolor*, an amaranth used as a vegetable crop, showed varying degrees of susceptibility to the root-knot nematode (*Meloidogyne incognita* (Kofoid & White 1919) Chitw. 1949).

Aside from differential susceptibility to certain pathogens, *Amaranthus* accessions are also known for having variable morphological characteristics, hybrid viability, chromosome numbers, and lysine and protein content of the seed (Pal & Koshoo 1972, 1974; Greizenstein & Poggio 1994; Transue et al. 1994; Zheleznov et al. 1997). According to Transue et al. (1994), morphological variation due to genetic or environmental factors may have resulted in the improper identification or misclassification of some *Amaranthus* accessions. Accession identification may also be complicated by the possibility of introgression between species, which produces offspring that may exhibit intermediate morphological traits (Murray 1940; Sauer 1967) or offspring that exhibit the traits of just one parent (Franssen et al. 2001). Franssen et al. (2001) observed that most hybrids of *A. palmeri* and *A. rudis*

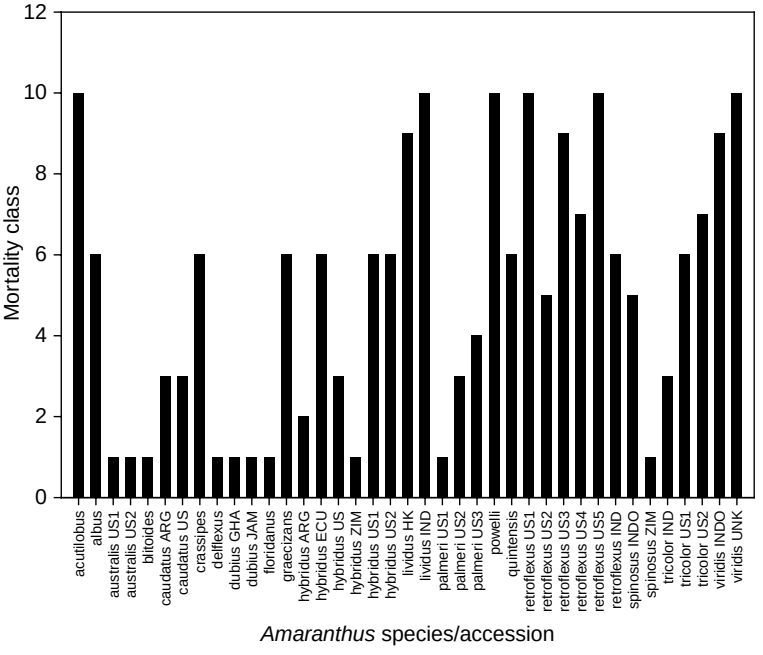

Figure 1. Mortality classes of the various *Amaranthus* species and accessions tested for susceptibility to *Phomposis amaranthicola*. The mortality classes are: 1 = <10% mortality; 2 = ≥10% and <20%; 3 = ≥20% and <30%; 4 = ≥30% and <40%; 5 = ≥40% and <50%; 6 = ≥50% and <60%; 7 = ≥60% and <70%; 8 = ≥70% and <80%; 9 = ≥80% and <90%; and 10 = ≥90% mortality. For some of the species where more than one accession were tested, the country of origin is indicated. The abbreviations used are: ARG (Argentina); ECU (Ecuador); GHA (Ghana); HK (Hong Kong); IND (India); INDO (Indonesia); JAM (Jamaica); UNK (unknown origin); US (United States; numbers are used to differentiate the accessions from the USA) and ZIM (Zimbabwe).

resembled *A. palmeri*. Hence, it is possible that in the past, some accessions may have been assigned to the wrong species because of their morphological characteristics. This may explain, in part, why accessions of the same species respond differently to the same pathogen.

In recent years, seed protein analysis, random amplified DNA analysis (RAPD), isozyme analysis, restriction enzyme analysis of amplified ribosomal DNA (rDNA), and analysis of restriction site variation in nuclear DNA and chloroplast DNA regions have been employed to establish phylogenetic relationships between cultivated and wild *Amaranthus* species (Transue et al. 1994; Chan & Sun 1997; Zheleznov et al. 1997). Restriction enzyme analysis of the ribosomal gene cluster internal transcribed spaces (ITS) regions allowed Wetzel et al. (1999) to identify some *Amaranthus* samples that were collected in the United States. With the use of isozyme and RAPD markers, Chan and Sun (1997) determined that there was a high level of genetic diversity between accessions of the same species and that genetic uniformity exists within most accessions. Clearly, the differential reaction of the some *Amaranthus* accessions to plant pathogens, or in this particular case, to *P. amaranthicola*, is a manifestation of the genetic differences among the accessions.

This host-range study establishes the specificity of *P. amaranthicola* to the genus *Amaranthus*. While the susceptibility of some economically important, edible species

of *Amaranthus* is noteworthy, it is of no significant concern because if this fungus will be used as a bioherbicide it is to be applied under a set of label specifications which would restrict its use in non-target sites and non-target crops. Moreover, as a native pathogen, *P. amaranthicola* is already naturalized in the USA and is therefore unlikely to spread in an uncontrollable manner.

The natural heterogeneity for susceptibility among *Amaranthus* species that was demonstrated in this study and the capacity of *Amaranthus* species for introgression pose two possible scenarios that may complicate the use of *P. amaranthicola* as a bioherbicide for *Amaranthus* control; 1) variable or inconsistent level of control in the field; and 2) selection for disease-resistant biotypes. While the potential for resistance development and adverse effects on beneficial *Amaranthus* spp. provide a cautionary note, it is not a basis to discredit the potential of this fungus as a bioherbicide agent. The need to control these weeds also fuels continued interest in the pathogen. In addition to the intensely competitive nature of the pigweeds, many of the pathogens that are found to be problems in the cultivated amaranths are also significant problems associated with vegetable production. *Pythium aphanidermatum* and *P. myriotylum* are serious pests in bell pepper production (Chellemi et al. 2000) and have been isolated from several pigweed species collected from within bell pepper production fields (Rosskopf, unpublished). Similarly, root-knot nematode is considered to be a limiting factor in the production of both solanaceous and cucurbit vegetables and galling is commonly found on *Amaranthus* species within vegetable production fields (Noling & Gilreath 2002). Based on the high degree of virulence of *P. amaranthicola*, coupled with the safety afforded by the narrow, genus-specific host range, there is justification for its continued development. Accordingly, research is underway to further study and register *P. amaranthicola* as a bioherbicide for *Amaranthus* spp.

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