ETIOLOGY OF PHOMA STALK ROT OF GRAIN AMARANTH AND IDENTIFICATION OF RESISTANT GENOTYPES

by

ROLLIN M. MACHTMES

B.S., Washington State University, 1971

A REPORT

submitted in partial fulfillment of the

requirements for the degree

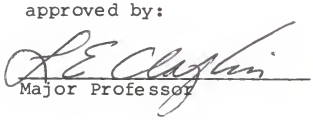
MASTER OF SCIENCE

Department of Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1989

approved by:



Major Professor

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ACKNOWLEDGMENTS

I thank my major professor, Dr. Larry Claflin for his advice on the research and review of the manuscript; and the other members of my advisory committee, Dr. Donald Stuteville, and Dr. Ned Tisserat for their suggestions on the research and on the manuscript.

I also thank Dr. Avelina Paulsen for her work, with my materials, on the Electron Microscope, Dr. Charles Kramer for his advice on my research, and Leon E. Weber of the Rodale Research Center for providing diseased plant material, and seed stocks for the resistance study.

Finally I thank my family for their love, assistance, encouragement, and support.

INTRODUCTION

Grain Amaranth (Amaranthus spp.) is an ancient food crop with apparent origins in the southern portion of North America, Central, and South America (26,15). The use of Amaranthus as a source of food dates back 5,500 years in Mexico, and evidence indicates it was one of the basic foods of the Aztec and Inca civilizations (15,17,26,27). The collapse of Indian cultures following the Spanish conquest in the early 16th century led to the decline of amaranth as a major crop when Spanish conquistadors banned the crop because of its sacred role in Aztec religious ceremonies. As a result, it survived only in small pockets of cultivation in mountain areas of Mexico and the Andes (15, 17). A hypochondriacus was introduced into Asia during the early part of the 15th century.

Grain amaranth is now grown as a commodity in India, Pakistan, Nepal, Tibet, and China, and as a minor commodity in Mexico, Central, and South America. In the United States, there is limited commercial and experimental production of grain amaranth in Kansas and other states. Amaranthus spp. are also grown as vegetables in China, Southeast Asia, Southern India, West Africa, and the Caribbean basin (9,15,18,27).

Amaranth seeds can be boiled and used as a gruel or milled to produce a flour for breads and baked goods. The seeds can also be popped and used as a snack, as breakfast cereal or mixed with molasses or honey to produce a confec-

tion or candy (15,17,26).

In recent years, an interest in the production of grain amaranth as a crop has increased due to: 1)the quality and quantity of protein in the seed, 2)apparent drought tolerance, 3)C-4 photosynthetic pathway, 4)an additional crop to broaden the food base, and 5)potential industrial uses.

The average protein content of grain amaranth is 16%, slightly higher than cereal crops (15,17,26,27). Amino acid content of amaranth protein is a very important consideration. Amaranth protein is high in lysine and sulfur amino acids but low in leucine, whereas the cereals are low in lysine and sulfur amino acids and high in leucine (5,8,9,15,17,18,22). The amino acid distribution in amaranth and the cereals make them very compatible for blending as animal feed and for flour blends to increase the nutritional quality of human food. Studies have been done on the value of amaranth in chicken feed (9), infant formulas (23), and applications to baking (22).

There are indications that, once established, grain amaranth does well under drought conditions (8,15) and would be a likely choice for a rotation crop in areas of low to intermediate rainfall or in areas where irrigation water is limited. Angus et al. (2) studied the phasic development of buckwheat and grain amaranth, and concluded that grain amaranth may have some advantages as a short season crop in difficult environments. Teutonico and Knorr (26) argue that there is little evidence in the literature

to support the claims that amaranths are drought or salt-tolerant.

Amaranthus spp. are dicots with a C-4 photosynthetic pathway, which allows for more efficient use of available carbon dioxide and is believed to increase the yield potential of grain amaranth (8). The crop currently yields from 2 to 3 metric tons/ha (2,8,26). Yields are expected to increase with more selection and varietal development (8,26,27).

The major portion of the food needs of mankind are met by 20 crops. The addition of grain amaranth as a cultivated crop would broaden the food base (8,15).

Industrial uses for amaranth are varied. Some varieties have a leaf area of 4000-6000 cm^2 per plant and could make an excellent biomass crop for fermentation into ethanol (27). Amaranth oil contains 8 % squalene, a complex hydrocarbon used as a raw material for manufacturing steroid drugs. Squalene from amaranth may serve as an alternate source, as squalene is currently obtained from shark livers (27). Amaranth flour contains the smallest starch granules known (average diameter 1 μm), and has high water-absorption capacity. Amaranth starch might have useful applications in food, plastics and cosmetics industries (15,27).

The Rodale Research Center, Emmaus, PA. has been a leader in developing grain amaranth as a modern viable crop. Rodale Research has been actively involved in col-

lecting germplasm, selecting and breeding for desirable traits to make crop improvements as well as conducting research on production practices and problems. One of the problems that interferes with efficient production is plant disease. Rodale Research recently found a disease problem in their nurseries that appeared to be a stalk rot.

Objectives

The objectives of this research were, 1)to isolate and identify the causative organism and complete Koch's postulates, 2)to conduct a limited host range study as an aid in identification and to gain some knowledge in the usefulness of rotations in grain amaranth production and, 3) to evaluate germplasm from the Rodale breeding project for resistance.

MATERIALS AND METHODS

The incitant was isolated from diseased grain amaranth plant material submitted by Rodale Research Center, Emmaus, PA.. Diseased plants had stalk lesions ranging from 5 - 15 cm in length, and coalescing multiple infections were often observed. The inside of the stalk, in the area of the lesion, had a shredded appearance with discolored vascular tissue only remaining at the center of the lesion. The discolored vascular tissue also bore what appeared to be small sclerotia.

Discolored vascular tissue was surface-sterilized in 10% clorox for 60 seconds, blotted dry, and plated onto potato dextrose agar (PDA) (Difco) or hay infusion agar. Hay infusion agar was prepared by boiling 20 g of dried, mature grain amaranth tissue (stems, leaves, seed heads) in distilled water, and filtering the liquid through a double-layer of cheesecloth and brought to volume (1L) with distilled water. Fifteen g of agar (Difco) was added followed by autoclaving at 115 C for 20 minutes. Subsequent transfers were made to PDA, and long term storage of cultures were on PDA slants at 4 C. To enhance formation of pycnidia, the fungus was placed on 1.5% water agar (Difco) with sterile amaranth leaf pieces on the surface. A 25 ml volume of amaranth leaf pieces (ca. 1.5 cm^2) were placed in a 100 ml screw top jar and sterilized by adding 0.5 ml of propylene oxide.

Colony morphology was studied on PDA, malt extract agar

(MEA) (Difco), and oat agar (1), as reported in numerous other studies (3,10,11,12,13,14,25,28,29). The oxidation reaction by substance E, which is a characteristic of Phoma exigua Desm. (4), was tested by adding a drop of concentrated NaOH, (prepared by dissolving 1 g NaOH in 0.9 ml of water), to the margin of several isolates of the fungus growing on MEA.

Koch's postulates and testing of pathogenicity were completed, under greenhouse conditions (20-28 C), on two cultivars of grain amaranth (K343 and K449). Seeds of each cultivar were sown in a commercial potting mix (Bacto). Upon reaching a height of 5 cm, plants were selected for uniformity and transplanted singly to 5 cm square pots filled with potting mix. Inoculations were done after the inflorescence emerged from the boot. Inoculations were completed as follows; the epidermis of the stem was removed (1.5-2.5cm) with a sterile dissecting knife, beginning 5 cm below the primary head. Agar blocks (2 mm^2) were cut from actively growing colonies on PDA, and placed on the wounded stem and held in place with transparent tape. Five plants of each cultivar were tested in a randomized complete block. To test the effect of wounding, one series of inoculations were completed with no injury to the plants. Agar blocks were placed on the surface of non-wounded stems 5 cm below the primary head and held in place with transparent tape. Five plants of each cultivar were tested in a randomized complete block. Evaluation

consisted of monitoring disease symptoms for one month.

A limited host range test was conducted to determine the potential value of crop rotation in amaranth production and as a possible aid in species determination of the fungus. Plant growth and inoculations after wounding were as stated above. Plants used in the host range study included corn (Zea mays L.), wheat (Triticum aestivum L.), alfalfa (Medicago sativa L.), sunflower (Helianthus annuus L.), soybean (Glycine max L. Merr.), lambsquarters (Chenopodium album L.), and redroot pigweed (Amaranthus retroflexus L.). Five plants of each species were tested in a randomized complete block.

A genetic resistance study was conducted in the greenhouse on 22 lines of grain amaranth, representing four Amaranthus species. Plants were grown and inoculated as stated previously. Observations were on a weekly basis after inoculation and continued for three weeks. Measurements were taken on the length of lesion, presence of pycnidia, and girdling of the stem or death of the stem above the inoculated site.

RESULTS AND DISCUSSION

The fungus isolated from the original plant material and used to complete Koch's postulates was identified as a Phoma spp. according to the synoptic key presented by Sutton (25). A literature search revealed no previous reports of Phoma disease of grain amaranth (24). Other Amaranthus spp. reported to be parasitized by Phoma spp. include A. hybridus L. (an ornamental variety) by P. amaranthi Halst. (=P. longissima (Pers. ex Fr.) West.?), A. graecizans L. (tumblepigweed) by P. amaranthicola Brun., and A. retroflexus L., (redroot pigweed) parasitized by P. longissima (Pers. ex Fr.) West. (6). A review of these species descriptions by Persoon (19) and Saccardo (20,21) did not lead to a concurring species epithet for the fungus isolated.

Boerema and Howeler (4) report on the use of an oxidation reaction in which substance E, produced by Phoma exigua Desm., is oxidized with the addition of alkali. Production of substance E appears to be most abundant on malt agar. Addition of a small amount of concentrated NaOH, in the presence of oxygen, will oxidize substance E to pigment α (blue-green color); this color gradually passes into pigment β (red in color).

P. exigua is the causal agent of stem and leaf lesions with a wide host range and is ubiquitous in soil (4). Due to the cosmopolitan nature of P. exigua, the Phoma isolated from grain amaranth was tested for the presence of sub-

stance E to determine if the amaranth isolate belonged to P. exigua. Three isolates were tested and all were negative.

Colony Morphology in Vitro

Colony morphology of the Phoma sp. was observed on PDA, oatmeal agar was used to enhance production of pycnidial conidiomata and conidia, and MEA because it stimulates formation of chlamydospores and crystal formation (25).

Colonies on MEA were variable in appearance, with sparse aerial mycelium although sectors occasionally developed in which aerial mycelium was abundant. Colony color on MEA was dark olivaceous green to black in the center, followed by a zone of olivaceous green, which in turn was followed by a light gray outer margin of new growth. Colonies attained mean diameters of 37 mm and 80 mm after 5 and 10 days, respectively, at 20 C; 17 mm and 65 mm after 5 and 10 days, respectively, at 30 C. After 40-45 days, terminal and intercalary, chlamydospores were produced singly or in chains. Chlamydospores were smooth, thick-walled, brown, and 10-16 μm in diameter. Intercalary chlamydospores were barrel-shaped and terminal chlamydospores were subglobose. Crystal formation on MEA was negative. Colonies on PDA were generally gray with a dark olivaceous gray to black center and dark hyphae sometimes aggregating into closely appressed strands radiating out from the center. Aerial mycelium was present. Colonies attained mean diameters of 40 mm and 85 mm after 5 and 10

days, respectively, at 20 C and 19 mm and 70 mm after 5 and 10 days, respectively, at 30 C. Colonies on oatmeal agar were usually woolly with a black center and brown to reddish-brown concentric rings. The reddish-brown ring had a serrated or scalloped appearance. Mean colony diameters were 33 mm and 70 mm after 5 and 10 days, respectively, at 20 C and 16 mm and 52 mm after 5 and 10 days, respectively, at 30 C.

Pycnidia, in vitro, were globose, erumpent to embedded, medium to dark brown, with a single non papillate ostiole. The pycnidial wall normally was three cell layers thick. Pycnidia diameters varied from 250 - 400 μm but averaged 320 μm. Conidia were mostly globose, 10-15 μm in diameter, non septate, hyaline, and smooth walled. A few oblong conidia 8 - 13 μm X 13-18 μm, were observed.

Two types of hyphae were observed in culture: Type I was hyaline, septate, with irregular branching, and 1.6-3.2 μm in diameter; Type II was medium to dark brown, septate, with irregular branching, and 4 - 10 μm in diameter. Type II hyphae frequently developed single cells and short chains of swollen single cells noticeably constricted at the septa. These cells were from 10 - 32 μm long.

Colonies and isolates were variable on all three media used to evaluate morphology. Sectors, characterized by color variation or increased aerial mycelium, developed on all media. MEA was the only medium in which production of

chlamydospores was observed. Our Phoma isolates rapidly lost the ability to produce pycnidia in culture. Only cultures freshly isolated from grain amaranth produced pycnidia on hay infusion agar and on amaranth leaves in water agar. Pycnidial production was not observed on oatmeal agar.

Characteristics of the conidiogenous cells and the spore forming process are useful in identification of the genus Phoma (25) and frequently are included in Phoma descriptions in the literature (4,11,12,13,14). In an effort to identify the characteristics of the conidiogenous cells of the Phoma species pathogenic to grain amaranth, pycnicia from amaranth stems were processed and cut in cross section for electron microscopy. All sample processing and E.M. work was done by Dr. A. Paulsen, Division of Biology, Kansas State University. We were unable to identify conidiogenous cells in the electron micrograph and efforts to identify these characteristics were discontinued. A possible explanation was our pycnidia were mature and probably dormant; therefore conidogenesis was not occuring.

Pathogenicity

Pathogenicity was tested by inoculating wounded plants of grain amaranth lines K343 and K449. Symptoms were visible in 3 to 5 days as the lesions were a medium to dark brown (darker at the center) preceded by a water-soaked zone of 3 - 5 mm. After one week, lesions were generally

4-5 cm long progressing proximally and distally on the stalk from the point of inoculation. After 2 weeks, lesions were 7-15 cm long and often girdled the stalk and killed plant tissue above the diseased area. Pycnidia were found in lesions one week after inoculation and were abundant after two weeks. Infected vascular tissue inside the stalk was medium brown, in contrast to the cream color of healthy tissue and preceded the visible lesion by 1 - 2 cm.

The fungus was readily reisolated from the lesion area of infected plants. Isolations were made on both PDA and hay infusion agar.

Five plants each of lines K343 and K449 were inoculated without wounding as a test to determine if a wound is necessary for disease development. After one week, faint water-soaked lesions, 3 - 5 cm in length were seen. After two weeks, lesions were 6 - 12 cm long, medium brown with dark brown centers; numerous plants were dead and abundant pycnidia were observed in diseased tissue.

Host Range

A host range experiment was conducted to determine if crops likely to be used in rotations with Amaranthus were also susceptible to the fungus. The results of this test were to aid in possible species identification and to determine whether crop rotation in grain amaranth production would be a potential means of control.

Corn and soybean were included in the test because they

could likely be part of a crop rotation with grain amaranth, although, it is not known if they are host plants to any Phoma species. Sorghum, wheat, alfalfa, and sunflower are all host plants to Phoma (16). Lambsquarters (16) and redroot pigweed (6) are also host plants for Phoma species and are weeds common to areas of grain amaranth production. Of all plants tested for host range, only redroot pigweed proved to be susceptible.

There have been over 2000 species described in the genus Phoma, based on host substrate and slight morphological differences (25). Numerous references appear in the literature regarding the confusion that has existed over definition of species in the genus Phoma (3,12,13,14,29). Identification of Phoma species in culture is often confusing and difficult because cultural characteristics are extremely variable (25). Efforts to describe the Phoma species, isolated from grain amaranth, based on colony morphology, host substrate, oxidation test for substance E, and conidiogenesis were not successful.

The host range study did reveal a number of crops that might be successful as rotation crops for grain amaranth production. Rotation with non-host crops must include control of pigweed to be successful and specific recommendations for rotational schemes can not be recommended without additional information on the biology and epidemiology aspects of Phoma spp..

Resistance Study

A total of 20 parent lines of grain amaranth from the germplasm collection at Rodale Research, which represents a cross section of the genetic material available, and two lines developed by the Rodale breeding program were tested (Table 1).

Final evaluations were made 3 weeks after inoculation. Measurements included, length of lesion, production of pycnidia, and girdling or death of the stalk. Data on lesion length, were analyzed by analysis of variance using a completely randomized design (Table 2). Even though care was taken to inoculate plants of similar appearance within each line, some variation in resistance should be expected, as the outcrossing rate for grain amaranth varies from 10% to 50% (7). The parent lines consisted of seed from foreign sources, therefore purity is not guaranteed (Leon E. Weber, personal communication).

Resistant lines were found in all four species of Amaranthus tested. There was a highly significant line effect based on lesion length. Line susceptiblity was grouped into 3 classes. The most resistant lines had mean lesion lengths ranging from 2.9 - 6.1 cm. The moderately susceptible class had mean lesion lengths of 6.6 - 9.0 cm. The highly susceptible class had mean lesion lengths of 9.8 - 12.9 cm (Table 3). Pycnidia formation and girdling evaluations indicate that lines 449, 1036, 130, 722, should be classed as moderately susceptible rather than remaining

in the resistant class. The results of the resistance study indicate that ample resistance is present to make progress in developing resistant varieties. Future work might include a survey for distribution of the disease, field testing for resistance, studies on biology and epidemiology, and continued effort on species identification.

Table 1. Species and origin of *Amaranthus* tested for tolerance to Phoma stalk rot.

RRC#	Species	Seed Color	Origin
713	*A. caudatus*	pink	Cusco, Peru
988	*A. caudatus*	white	San Lorenzo, Bolivia
1113	*A. caudatus*	white	Ayacucho, Peru
1036	*A. caudatus*	white	Coimbatore, India
1034	*A. cruentus*	brown	Benin
622	*A. cruentus*	white	Aldea Choatalum, Guatemala
434	*A. cruentus*	white	Morelos, Mexico
1157	*A. cruentus*	white	Unknown
1023	*A. hypochondriacus*	white	Mexico
1024	*A. hypochondriacus*	gold	Mexico
412	*A. hypochondriacus*	white	Tulyehualco, Mexico
646	*A. hypochondriacus*	white	Texmelucan, Mexico
1221	*A. hypochondriacus*	white	Jumla, Nepal
718	*A. hypochondriacus*	white	Oaxaca, Mexico
722	*A. hypochondriacus*	white	Oaxaca, Mexico
674	*A. hypochondriacus*	brown	Unknown
130	*A. hypochondriacus*	white	Nepal
489	*A. hypochondriacus*	white	Unknown
1004	*A. hybridus*	brown	W. Pakistan
139	*A. hybridus*	white	Unknown
K343	1024 x 1004	white	Rodale Research Center
K449	(1024 x 1004) x 489	white	Rodale Research Center

Table 2. Mean squares from analysis of variance on length of lesion for 22 inoculated grain amaranth accessions

Source	df	Lesion Length (cm)
Rep	4	4.65
Lines	21	48.85****
Error	84	6.71
CV (%) 38.6		

**** Significant at the .0001 probability level.

Table 3. Mean lesion length and pathogenicity notes of inoculated grain amaranth accessions

line	Mean lesion length (cm)	Pathogenicity notes
343	12.9 a	*(5), +(5)
1221	12.6 a	*(5), +(5)
1024	10.8 ab	*(4), +(5)
674	10.6 ab	*(5), +(5)
1023	9.8 ab	*(5), +(5)
489	9.0 bc	*(2), +(5)
622	8.9 bcd	*(5), +(4)
646	8.2 bcde	*(1), +(5)
139	6.6 cdef	*(1), +(2)
988	6.1 cdefg	*(1), +(1)
449	5.6 defg	*(1), +(5)
1036	5.5 efg	*(2), +(4)
130	5.3 efg	*(1), +(3)
713	5.0 fg	*(0), +(1)
722	4.8 fg	*(1), +(5)
1004	4.2 fg	*(1), +(0)
434	4.1 fg	*(0), +(0)
412	4.0 fg	*(0), +(2)
1034	3.8 fg	*(0), +(2)
1113	3.5 fg	*(0), +(0)
718	3.2 g	*(0), +(0)
1157	2.9 g	*(0), +(0)

Means with the same letter not significantly different
* = girdling of stalk
+ = formation of pycnidia
( ) = number of plants out of 5 affected

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Manhattan, Kansas

1989

ABSTRACT

The causal agent of a previously unreported stalk rot disease of grain amaranth (*Amaranthus spp.*) was identified as a species of *Phoma*. Host range studies show that redroot pigweed (*Amaranthus retroflexus*) was susceptible whereas corn, wheat, alfalfa, sunflower, soybean, and lambsquarters were resistant to the pathogen. Evaluation of *Amaranthus* genotypes identified lines 1157, 718, 1113, 1034, 412, 434, 1004, and 713 as having resistance to the disease. Chlamydospores were produced singly and in chains after 40 - 45 days on malt extract agar.