Identification and Distribution of *Alternaria mali* on Apples in North Carolina and Susceptibility of Different Varieties of Apples to Alternaria Blotch

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ABSTRACT

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A previously unreported disease characterized by a leaf spot and defoliation of strains of Delicious apple (*Malus domestica*) occurred in the summers of 1987 and 1988 in western North Carolina. The pathogen was tentatively identified as *Alternaria mali*, the cause of Alternaria blotch. Morphological characteristics of nine North Carolina isolates were similar to the description of *A. mali* type A in the literature and a culture obtained from the American Type Culture Collection. The host-specific toxin AM-I was isolated from two North Carolina isolates and appeared identical to standards obtained from Japan. A survey of 60 orchards was conducted in the summers of 1989 and 1990 to determine the distribution of the pathogen and disease incidence in North Carolina. Disease incidence was highest in Henderson County, where it ranged from 0 to 95% in 27 orchards surveyed. Disease incidence and severity increased from 1989 to 1990. Strains of Delicious cultivars were more susceptible than strains of Golden Delicious, whereas Paulared and Idared were the most resistant of 17 varieties and strains of Delicious and Golden Delicious examined.

A leaf spot causing up to 60% defoliation of the apple (*Malus domestica* Borkh.) variety Delicious occurred during the summers of 1987 and 1988 in the apple-growing areas of North Carolina. Symptoms resembled those of frogeye leaf spot caused by *Botryosphaeria obtusa* (Schwein.) Shoemaker or captan injury. Initial symptoms appeared on the leaves about 1 mo after petal fall as small circular brown or blackish brown spots, gradually enlarging to 2–5 mm in diameter and bordered at times by dark brown to purple margins (Fig. 1). As lesions aged, they often turned grayish brown. Some spots exhibited secondary expansion, becoming irregular and darker in color. Leaves with infected petioles often turned yellow and abscised early in the season. Isolations onto potato-dextrose agar (PDA) rarely yielded *B. obtusa*; however, a species of *Alternaria* was consistently isolated from the leaf spots. Based on reports in the literature (4,5), it was tentatively identified as *A. mali* Roberts.

A. mali was first described in 1924 in the United States (4). The fungus was not considered pathogenic but was involved in enlarging areas infected by *B. obtusa* or injured by chemicals or other means. Roberts (4) reported two types of *A. mali* that had a distinctly different morphology. Type A had sparse aerial mycelium and produced abundant olivaceous conidia in a dark carpetlike mycelial mass on the surface of the medium. Type B had abundant gray aerial mycelium and produced sparse conidia. The average size of the type A conidium was 26 × 9 μm, whereas the average size of the conidium of type B was 49 × 9 μm.

In 1956, a leaf spot was noted in South Iwate Prefecture in Japan, and in a few years the disease had spread throughout apple-growing areas of Japan. In 1970 and 1972, Hoshino and Sawamura (1) and Sawamura (5) reported that the disease was caused by a pathogenic strain of *A. mali* and proposed the name Alternaria blotch. Starking Delicious and Indo were highly susceptible; Jonathan was resistant. Disease occurrence appeared to correspond to increased cultivation of susceptible varieties in Japan. Subsequently, the disease has been reported from South Korea and other countries in the Far East (6). Recently, it was reported in Zimbabwe. Three host-specific toxins (designated as AM-toxin I, AM-toxin II, and AM-toxin III) are produced by *A. mali* and are involved in pathogenicity (3,8,9). AM-toxin I is the most common.

This study was conducted to confirm the identity of the *Alternaria* sp. isolated in North Carolina as *A. mali* by examining its morphology and toxin production, to determine the distribution of *A. mali* in North Carolina, and to determine the susceptibility of some common apple varieties grown in the southeastern United States.

MATERIALS AND METHODS

Morphological characteristics of conidia. Leaves with typical Alternaria blotch symptoms were collected in the summer of 1988 from nine locations in three counties in North Carolina (Henderson, Wilkes, and Alexander). Leaves were surface-sterilized by dipping them into 0.5% NaOCl for 15–20 s. After drying the leaves with a laboratory towel, portions of lesions along with healthy leaf tissue were removed with a sterilized razor blade and placed on PDA plates. Cultures were grown under continuous light at 24 C for 5 days. The fungus was streaked onto PDA plates with a sterilized loop to separate individual conidia. After 1 day, single spores from each culture were transferred to obtain pure cultures of *A. mali*. Cultures were grown on PDA for 5 days and semipermanent slides of conidia stained with cotton blue in lactophenol were prepared, and the length and width of 50 conidia of one culture from each location were recorded. Conidial size and morphology were compared with descriptions in the literature (4) and with a standard obtained from the American Type Culture Collection (ATCC isolate 42096).

Survey for incidence of disease. Sixty orchards from five major apple-growing counties in North Carolina were selected arbitrarily by county agents and surveyed for incidence of disease in early August 1989 and mid-July 1990. The same 60 orchards were surveyed each year. Ten trees of strains of Delicious were arbitrarily selected from each orchard and the percentage of leaves affected with Alternaria blotch was recorded. To distinguish among leaf spots caused by *B. obtusa*, captan injury, and *A. mali*, 20 leaves with symptoms were collected from each orchard, placed in plastic bags, and brought into the laboratory in ice chests. Isolations were made 1–5 days after sampling.

Isolation of AM-toxin I. Two pathogenic isolates of *A. mali* (1544 and 1522 from Henderson County) were used for toxin isolation. Cultures were grown on potato-dextrose broth (PDB) (Difco Laboratories, Detroit, MI) in a still culture in 500-ml flasks under continuous light at 24 C for 14 days. The culture medium (1.5 L) was filtered through a double layer of cheesecloth and extracted with an equal volume of ethyl acetate at pH 6.0. The ethyl acetate layer was

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Fig. 1. Alternaria blotch symptoms on leaves of the apple variety Delicious.

evaporated to dryness under reduced pressure and yielded a brown residue (49.73 mg). The residue was redissolved in deionized water (15 ml), washed with cyclohexane (7.5 ml × 2) to remove lipid impurities (0.88 mg), and extracted with dichloromethane (7.5 ml × 2). After evaporation of the dichloromethane, an oily residue was obtained (4.46 mg). The residue was compared by thin-layer chromatography (TLC) in three solvent systems (benzene-acetone, 2:1, v/v; ethyl acetate-hexane, 2:1, v/v; and dichloromethane-methanol, 2:1, v/v) to standards of AM-toxin I obtained from K. Kohmoto (Tottori University, Japan) and K. Sawamura (Hirosaki University, Japan). The toxin was detected on TLC fluorescence and with iodine vapor.

A bioassay on apple leaves from four varieties was conducted to confirm the presence of the phytotoxin in the enriched extract. Leaf pieces (2 × 2 cm) were excised from Classic Red Delicious, Red Delicious, Golden Delicious, and Idared, which had shown susceptible, susceptible, moderately resistant, and resistant reactions to *A. mali*, respectively, in previous tests. Four leaf pieces were placed in each petri plate containing a moist filter paper on the bottom. The adaxial surfaces of the leaf pieces were wounded with a sterilized needle, and a drop (approximately 20 µl) of crude phytotoxin solution in water (1 mg/ml) was placed on the wounded area. The crude phytotoxin (1 mg/ml) was prepared by dissolving 4.46 mg of crude phytotoxin from two *A. mali* isolates (1522 and 1544) in 1 ml of methanol, then diluting the solution into 100 ml of sterile water. This aqueous solution was concentrated in a rotary evaporator to 4.5 ml (1 mg/ml) and was then used for the bioassay. Petri plates with leaf pieces were incubated at 24 C for 24 hr under continuous light. The experiment was repeated twice.

A bioassay was also conducted with spots cut from the TLC plates. A crude phytotoxin solution in 1 ml of methanol was spotted on a TLC plate. After the TLC was developed in benzene-acetone (2:1, v/v), two spots were detected (R_f = 0.47 and 0.73) which lined up with the spots developed from the standard obtained from Sawamura under UV light (254 nm). The lower spots (R_f = 0.47) were cut from the TLC plates and placed upside down on wounded leaf pieces containing 20 µl of buffer (0.05 M sodium phosphite, pH 6.0).

Susceptibility of different varieties of apples. One single-spore isolate from each of four orchards (isolates 1522, 1531, 1444, and 1556) that showed pathogenicity in the laboratory (N. Filajdić and T. B. Sutton, *unpublished*) was allowed to grow for 7 days on PDA at 24 C under continuous light. A spore suspension was prepared by flooding cultures with distilled water, scraping them with a sterilized razor blade, straining them through a double layer of sterile cheesecloth into a 1-L flask, and adjusting the spore concentration with a hemacytometer to obtain 5×10^5 conidia per milliliter. Two to three drops of Tween 20 (ICI Americas, Inc., Richmond, CA) were added to the suspension, and 2-yr-old grafted trees of 17 varieties, including five strains of Delicious and two strains of Golden Delicious, were inoculated in the greenhouse by spraying both leaf surfaces to the drip point with an artist's air brush. Three shoots of each variety were inoculated. Plastic bags were placed over each shoot and secured with tape. One wet paper towel was placed in each bag to ensure that leaves would remain wet. Trees were kept in the greenhouse at 20–25 C, and the bags were removed after 48 hr. Five days after inoculation, the eight uppermost leaves were rated on each shoot, using a modified Horsfall-Barratt scale of 0–5, where 0 = no symptoms, 1 = 0–3% of leaf surface covered with lesions, 2 = 4–6, 3 = 7–12, 4 = 13–25, and 5 = 26–50%. The experiment was repeated twice.

Table 1. Dimensions of conidia (mean + SD) of *Alternaria mali* obtained from nine isolates from four apple orchards in western North Carolina

Isolate	Length[w] (µm)	Width[w] (µm)
Henderson 1[x]	20.36 + 7.71 d–f[y]	10.09 + 2.22
Henderson 2	24.17 + 7.05 cd	10.05 + 1.89
Henderson 3	30.80 + 9.39 b	12.14 + 2.04
Henderson 4	25.72 + 4.85 c	10.75 + 1.58
McKay 1	18.36 + 5.53 ef	9.69 + 1.86
McKay 2	18.80 + 4.84 f	9.35 + 1.54
Justice 1	22.31 + 7.32 cd	9.23 + 1.71
Justice 2	21.05 + 6.67 ed	11.69 + 1.37
Wilkes	20.20 + 4.42 ef	10.00 + 1.36
A mali type A[z]	26.00	9.00
A. mali type B	49.00	9.00
A mali from ATCC	26.50	10.90

[w] Values are means of 50 conidia of each isolate of *A. maili.*

[x] Henderson, McKay, and Justice orchards are in Henderson County; Wilkes orchard is in Wilkes County.

[y] Means within column followed by a different letter are significantly different (P = 0.05), according to the Waller-Duncan k-ratio t test.

[z] Conidial sizes of *A. mali* types A and B were obtained from literature (4).

Table 2. Survey for Alternaria blotch incidence in apple orchards in western North Carolina in August 1989 and July 1990

County	Orchards surveyed (no.)	Year	Incidence (%) 0	0–1	2–9	10–24	25–49	>50
Henderson	27	1989	3	12	2	4	2	4
	27	1990	0	6	6	3	2	10
Wilkes	10	1989	1	7	0	1	0	1
	10	1990	4	4	0	0	0	2
Cleveland-Lincoln	14	1989	4	5	3	2	0	0
	14	1990	2	9	2	0	0	1
Haywood	9	1989	1	6	2	0	0	0
	9	1990	2	7	0	0	0	0

RESULTS

Morphological characteristics of conidia. The average conidial dimensions of the nine isolates from western North Carolina were 22.2 × 10.3 µm, which was similar to *A. mali* type A as described

by Roberts (26 × 9 μm) (4). The average conidial size of *A. mali* obtained from American Type Culture Collection (isolate 42096) was 26.5 × 10.9 μm (Table 1). Morphological characteristics such as conidium shape and number of septations were also similar.

Survey for incidence of disease. Disease incidence in 60 apple orchards in western North Carolina ranged from 0 to 95% in 1989 and 1990. Disease severity increased from 1989 to 1990. There were 14 orchards (23.3% of all orchards surveyed) with 10% or greater disease incidence in the summer of 1989 and 18 (30%) in the summer of 1990 (Table 2). The disease was most severe in Henderson County where there were 10 orchards (37% of all orchards surveyed in Henderson County) in 1989 and 15 (55.6%) in 1990 with 10% or greater incidence. In all the other counties, there was no change from 1989 to 1990 in the number of orchards with 10% incidence or greater.

Production of the phytotoxin AM-I. TLC of crude phytotoxin prepared from the two *A. mali* isolates from North Carolina contained a substance comigrating with AM-toxin I obtained from Sawamura. Two spots were detected in our crude phytotoxin and the sample of AM-toxin I from Sawamura after TLC (R_f = 0.47 and 0.73). These two samples were of approximately the same purity. However, the standard supplied by Kohmoto had a higher purity and showed only one spot on the TLC plate (R_f = 0.50).

Lesions similar to those observed under natural conditions developed on all excised leaf pieces of Classic Red and Red Delicious after placing the crude phytotoxin solution on the wounded leaf surface. Lesions formed on one of eight leaf pieces of Golden Delicious and none of the leaf pieces from the variety Idared. Results of lesion formation were similar when a bioassay was performed after cutting the lower (R_f = 0.47) spots from TLC plates with our sample and placing them on leaf pieces. Lesions were observed on six of eight leaf pieces of Red Delicious; no lesions were observed on leaf pieces from Golden Delicious or Idared.

Susceptibility of different varieties of apples. Strains of Delicious (Classic Red, Red Delicious, Silver Spur, Oregon II, and Red Chief) were more susceptible than any other varieties tested, except Empire. Super Gold, Stayman, Golden Delicious, Smoothee, Firm Gold, Law Rome, Gala, Jonathan, and McIntosh were intermediate in susceptibility. Idared and Paulared showed the least susceptible reaction (Table 3).

Table 3. Relative susceptibility of 17 apple varieties, including five strains of Delicious and two strains of Golden Delicious, to *Alternaria mali*

Cultivar	Mean disease severity (0–5 scale)[x]	Lesion area[y]
Classic Red	2.79 a[z]	11.61 a
Red Delicious	2.47 a	9.33 b
Silver Spur	1.94 b	5.77 c
Oregon II	1.88 b	5.10 c
Empire	1.77 b	4.95 c
Red Chief	1.78 b	4.37 c
Super Gold	1.01 c	1.73 d
Stayman	0.88 cd	1.67 d
Golden Delicious	0.74 c–e	1.13 d
Smoothee	0.72 c–e	1.08 d
Firm Gold	0.63 c–f	1.06 d
Law Rome	0.61 d–f	1.04 d
Gala	0.56 d–f	0.91 d
Jonathan	0.49 d–f	0.75 d
McIntosh	0.48 ef	0.72 d
Idared	0.26 fg	0.40 d
Paulared	0.08 g	0.12 d

[x] Modified Horsfall-Barratt scale was used to assess disease severity on the eight uppermost leaves on each plant where 0 = no symptoms, 1 = 0–3% of leaf surface covered with lesions, 2 = 4–6, 3 = 7–12, 4 = 13–25, and 5 = 26–50%.

[y] Percent leaf surface covered with lesions.

[z] Means within column followed by a different letters are significantly different (P = 0.05), according to Waller-Duncan k-ratio t test.

DISCUSSION

Based on morphological characteristics and phytotoxin production, we conclude that the fungus isolated from apple leaf lesions in North Carolina is *A. mali* type A. This is the first report of Alternaria blotch of apple in the United States. Previous reports of *A. mali* in the United States suggested that it was primarily a secondary organism (4,7). Since our initial observations, the disease has also been reported on Delicious in Georgia (Floyd Hendrix, *personal communication*), South Carolina, and Virginia (Keith Yoder, *personal communication*).

A toxic extract of North Carolina *A. mali* isolates contained a substance indistinguishable from authentic AM-toxin in three chromatographic solvents using detection by fluorescence and reaction with iodine vapor. As reported by Kohmoto et al (2), the activity of AM-toxin I is specific for apple varieties susceptible to *A. mali*. We demonstrated this using four apple varieties with different reactions to *A. mali*. Further purification and spectroscopy of the phytotoxin produced by North Carolina isolates needs to be done to confirm that it is AM-toxin I.

Disease incidence has dramatically increased since the first time Alternaria blotch was noted in North Carolina. The primary reason for this outbreak of Alternaria blotch in the summer of 1987 is not known. It is possible that the fungus was always present in North Carolina orchards but never reached a certain population density threshold, until recently, from which the disease progressed rapidly. Alternatively, the disease may have been present for some years but misidentified as frogeye leaf spot or captan injury. It is also possible that the increase in incidence and severity of Alternaria blotch is attributable to reduced spray schedules used by many growers because of light crops after freeze injuries in the 1980s. However, disease incidence and severity was as high in some well-sprayed blocks as in those poorly sprayed.

Strains of Delicious were more susceptible than any other variety tested with exception of Empire. Empire was derived from a cross of Delicious and McIntosh. Golden Delicious, Smoothee (a russet-free strain of Golden Delicious), Super Gold, and Firm Gold (Golden Delicious seedlings) were moderately resistant. Cultivation of more resistant varieties would aid in control of Alternaria blotch. However, the susceptibility of new varieties needs to be determined. A strain of *A. mali* has been reported recently on Jonathan, which was considered resistant (6).

The appearance of Alternaria blotch necessitates additional work to develop a management strategy compatible with current strategies employed for summer disease control. Fungicides currently employed for summer disease control (e.g., captan and benomyl) do not provide satisfactory control (N. Filajdić and T. B. Sutton, *unpublished*). Thus, it is expected that Alternaria blotch will increase in the future because susceptible strains of Delicious are the most common apples grown in North Carolina and satisfactory chemical means of control are not yet available.

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