A Leaf Spot and Blight of *Abelmoschus moschatus* Caused by a Pathovar of *Pseudomonas syringae*

C. S. BROWN, Graduate Assistant, and S. M. McCARTER, Professor, University of Georgia, Athens 30602

ABSTRACT

Brown, C. S., and McCarter, S. M. 1991. A leaf spot and blight of *Abelmoschus moschatus* caused by a pathovar of *Pseudomonas syringae*. Plant Dis. 75:424-429.

A fluorescent bacterium that closely resembled *Pseudomonas syringae* pv. *hibisci* in morphological, biochemical, and physiological tests was consistently isolated from *Abelmoschus moschatus* with a leaf spot and blight. The bacterium also caused foliar symptoms on several *Hibiscus* species, including *H. rosa-sinensis*. Strains of *P. s. hibisci* attacked *A. moschatus* but were markedly less aggressive on that host than was the strain from *Abelmoschus*. On *A. moschatus*, disease development was favored by low temperatures (20–25 C) and succulent plant growth resulting from heavy fertilization. The bacterium was seedborne, as it was recovered from seed stored for 10 mo at 10 C.

The malvaceous plant *Abelmoschus moschatus* Medik. occurs naturally in India, southern China, the Indochina Peninsula, Indonesia, the islands of the southwestern Pacific to New Guinea, and northern Australia (1,25). It has been cultivated and naturalized elsewhere. The plant has many common names; the two most frequently used English names are musk mallow and ambrette (4,19,25). It was classified as *Hibiscus abelmoschus* until 1968, when Bates (1) considered it sufficiently different from other *Hibiscus* spp. to justify its reclassification in a new genus along with okra (*A. esculentus* (L.) Moench) and manihot (*A. manihot* (L.) Medik.). Recently, dwarf types that are 30–40 cm tall and bloom profusely have been selected in India and introduced into the United States as an annual summer ornamental bedding plant.

In January of 1988 a devastating foliage disease was observed on plants of the cultivar Oriental Red in a greenhouse in Georgia that were being propagated from seed for spring planting. Symptoms included angular leaf spots that were strongly water-soaked in their early stages, necrosis with water-soaking that advanced up the leaf midrib, and brown to black elongated lesions on the stems and petioles. These symptoms often progressed into a general shoot blight that sometimes resulted in death of the plants. Streak-plate isolations made from lesions onto KMB consistently resulted in essentially pure cultures of a fluorescent bacterium. Koch's postulates were fulfilled. The bacterium gave negative tests for oxidase (16) and arginine dihydrolase (27) and a positive reaction for tobacco hypersensitivity (15). These and other preliminary tests suggested that it was a member of the *Pseudomonas syringae* group (9,23,24,26).

No previous reports of a bacterial foliar disease on either the native or the dwarf ornamental cultivars of *A. moschatus* were found. In India, Srivastova et al (25) reported that the native plant was free of serious diseases except for occasional infection by hibiscus mosaic virus. However, pathovars of *P. syringae* have been reported on closely related species. *P. s. syringae* was isolated from a leaf spot of okra in Kenya (J. H. Young, *personal communication*). In 1923, Nakada and Takimoto (20) reported that *P. syringae* caused necrotic lesions on flowers, stems, and petioles of hibiscus in Japan. Jones et al (12) recently reported that a pathovar of *P. syringae* they designated *P. s. hibisci* caused a foliar disease of *H. rosa-sinensis* L. in Florida. They could not confirm that the bacterium was the same as the one isolated by Nakada and Takimoto because no type culture of the Japanese strain was available for comparison. The presence of foliar symptoms in Florida but not in Japan suggested the strains were different.

The objectives of the present research were to characterize the causal organism of this unreported disease of *A. moschatus* and to compare it with related strains, to determine the host range of the pathogen within the Malvaceae, to study certain factors that influence dis-

Present address of first author: Monsanto Agricultural Co., 700 Chesterfield Village Parkway, St. Louis, MO 63198.

Supported by Hatch and state funds allocated to the Georgia Agricultural Experiment Stations.

Accepted for publication 29 November 1990 (submitted for electronic processing).

ease development, and to determine whether the bacterium is seedborne. A preliminary report on portions of the work has appeared (3).

MATERIALS AND METHODS

Bacterial strains. The bacterial strain (hereafter designated Abel A) described and used in most of these studies was isolated originally from a necrotic leaf spot from a young *A. moschatus* Oriental Red plant obtained from a greenhouse in Athens, Georgia. Another strain was obtained from commercial seed of *A. moschatus*. Strains for comparison with the Abel A and seed strains were obtained from culture collections maintained by T. P. Denny and the second author, both at the University of Georgia; J. B. Jones, University of Florida, Bradenton; and the International Collection of Microorganisms for Plants (ICMP), New Zealand. These included three strains of *P. s. hibisci* (Nakada and Takimoto) Jones et al (434, 435, and PSH2, the latter being the type strain described by Jones et al [12]); 10 strains of *P. s. syringae* van Hall, one each from bean, corn, grapefruit, lilac, peach, soybean, tomato, and wheat and two from okra (318 and 5074 from the ICMP); one strain each of *P. s. antirrhini* (Takimoto) Young et al, *P. s. berberidis* (Thornberry and Anderson) Young et al, *P. s. coronafaciens* (Elliott) Young et al, *P. s. delphinii* (Smith) Young et al, *P. s. glycinea* (Coerper) Young et al, *P. s. lachrymans* (Smith and Bryan) Young et al, *P. s. maculicola* (McCulloch) Young et al, *P. s. phaseolicola* (Burkholder) Young et al, *P. s. pisi* (Sackett) Young et al, *P. s. tagetis* (Hellmers) Young et al, *P. s. tomato* (Okabe) Young et al, *P. cichorii* (Swingle) Stapp, *P. fluorescens* Migula, and *P. viridiflava* (Burkholder) Clara; and two strains of *P. s. tabaci* (Wolf and Foster) Young et al. Four unidentified strains isolated from *H. rosa-sinensis* were included in pathogenicity tests for comparison with the Abel A strain. Two of these (JWO 020 and JWO 026) were isolated from plants growing near Mobile, Alabama, and two (Hib I and Hib II) were collected from plants at St. Simons, Georgia. The collection was stored at −80 C in nutrient broth (400 μl) + 20% glycerol (600 μl) in 1-ml vials. Working cultures were stored on nutrient-yeast-dextrose agar (NYDA; 23 g nutrient agar, 10 g dextrose, and 5 g yeast extract, 1 L deionized water) slants at 4 C.

Inoculum production and plant inoculation. Inoculum was produced from cultures grown on plates of KMB at 25 C for 24–48 hr. Suspensions containing 10^7–10^8 cfu were prepared in sterile distilled water based on turbidity measurements with a spectrophotometer. Plants were inoculated by spraying bacterial suspensions to runoff on all plant surfaces with an airless electric paint sprayer (Model 860, Burgess Vibrocrafters, Inc., Grayslake, IL) held 30–40 cm from the plants. Except in studies in which temperature effects were studied, or where indicated otherwise, inoculated plants were covered individually with clear polyethylene bags for 36 hr and placed in growth chambers at 20–25 C with a 12-hr day length (intensity of 400 $\mu E \cdot m^{-2} \cdot s^{-1}$).

Production of experimental plants. Plants of *A. moschatus* used in all studies were grown from seed obtained from Park Seed Co. (Greenwood, SC) or Sakata Seed Corp (Yokohama, Japan). Seed were planted in trays of premoistened Pro-Mix BX (Premier Brands, Inc., New Rochelle, NY), covered with a 5- to 10-mm layer of vermiculite, and placed on a greenhouse bench. The seedlings were transplanted 5–10 days after germination into 10-cm plastic pots filled with premoistened Pro-Mix BX and placed on a greenhouse bench. Plants were fertilized with 20-20-20 water-soluble fertilizer (2.4 g/L) every 7–10 days and were inoculated after 4–6 wk (15–25 cm tall). Before inoculation, plants were produced under conditions that were not conducive for disease development.

Analysis of data. When appropriate, data were subjected to analysis of variance. Means were separated by an FLSD ($P = 0.05$).

Characterization of the Abel A strain and comparison with related organisms. The Abel A strain and other related and control strains were characterized in morphological, physiological, biochemical, and pathogenicity tests. Except where indicated otherwise, all tests were run with bacteria from 24-hr KMB cultures.

Morphological tests. All strains were compared for their Gram reaction (24) and cell morphology. Flagellation of the Abel A strain was determined by the silver-plating method of Rhodes (22). Known flagellated and nonflagellated bacteria were included for comparison purposes.

Growth temperature. The minimum, optimum, and maximum temperatures for growth of the Abel A strain were determined by placing NYDA slants inoculated with a single needle streak into incubators set at 5–40 C at 5-C intervals. Growth was rated visually after 24 hr (6).

Physiological and biochemical tests. Because the Abel A strain was found to be a fluorescent pseudomonad in preliminary tests, it and the related species and pathovars were compared for their LOPAT reactions (17) and in other tests useful for separating members of the group (9,23,26). The LOPAT tests were run as previously described (21) except that surface-sterilized whole raw potatoes were inoculated to determine soft-rotting capacity of the strains. Suspensions also were streaked onto sodium polypectate gel (pH 5.0 and 8.0) as described by Hildebrand and Schroth (9) to test for pectolytic activity.

The ability of the strains to utilize various organic substrates was tested by streaking 10^7–10^8 cfu suspensions onto the basal medium of Misaghi and Grogan (18) as modified by Jones et al (13) and amended with 0.1% (w/v) of each substrate. The strains were tested for utilization of erythritol, L(+)-tartrate, D(−)-tartrate, DL-lactate, sucrose, mannitol, sorbitol, alanine, and trehalose.

β-Glucosidase formation was tested by stab-inoculating deeps of Hugh and Leifson's arbutin-amended medium (9,11). Casein hydrolysis and gelatin liquefaction abilities were determined by standard methods (6). Production of syringomycin or a similar antifungal compound was determined with *Geotrichum candidum* Link ex Pers. as a bioassay organ as described by Devay et al (7) with modifications described previously (21). Production of an antimetabolite toxin by the strains was determined using a modification of the indicator technique of Gasson (8). Plates of NYDA were spotted (six 1-mm-diameter spots per plate) with inoculum and incubated at 25 C for 6 days. Then they were misted with a 10^7–10^8 cfu suspension of *Escherichia coli* (HB101, obtained from T. P. Denny), using a chromatograph sprayer. The plates were incubated at 30 C, and zones of inhibition were recorded after 1 and 3 days.

The strains were tested for ice-nucleation activity and for their capacity to cause lesions on green bean pods as previously described (21). The Abel A strain also was compared with *P. s. hibisci* (PSH2) using the Biolog Microplate system (2).

Pathogenicity tests. Initially, the ornamental cultivars Oriental Red and Sakatensis Pink were inoculated to determine if differences existed in cultivar susceptibility. Ten plants of each cultivar were inoculated, and two were sprayed with water as controls. This experiment was repeated once. The plants were rated for the percentage of leaf area diseased and the number of lesions on petioles 2 wk after inoculation.

Seven separate tests were conducted to determine whether strains other than the Abel A strain would attack *A. moschatus*. Strains tested two or more times included a seed isolate from *A. moschatus*, three strains of *P. s. hibisci*, four unidentified strains (JWO 020, JWO 026, Hib I, and Hib II) from *H. rosa-sinensis*, and the 10 strains of *P. s. syringae* described previously. Sakatensis Pink was used in all tests, and three to five plants were inoculated in each test. The Abel A strain was used in all tests for comparison. Symptoms were recorded after 2 wk.

Ten species in the family Malvaceae were inoculated with the Abel A strain to determine its host range. These included okra cultivar Clemson Spine-

less; Chinese hibiscus (*H. rosa-sinensis* cvs. Tennessee Red, Hot Pink, and Brilliant Red); common rose mallow (*H. moscheutos* L. cvs. Southern Belle, Frisbee, and Disco Belle); cotton (*Gossypium hirsutum* L. cv. Stoneville 213); hollyhock (*Alcea rosea* L. cvs. Powder Puff and Majorette); prickly sida (*Sida spinosa* (L.) Mill.); *Lavatera arborea* L.; *Sidalcea* sp.; *Abutilon* sp. cv. Suntense; and malva (*Malva sylvestris* var. *mauritiana* (L.) Boiss.). Seeds of all species except *H. rosa-sinensis* were obtained from Park Seed Company (Greenwood, SC) or Thompson and Morgan Seed Company (Ipswich, England). Cuttings of the *H. rosa-sinensis* cultivars were obtained from commercial growers.

All plants propagated from seed were grown in a greenhouse in trays of Pro-Mix BX. Five to 10 days after germination, plants were transplanted to 10-cm pots containing premoistened Pro-Mix BX. Plants were given weekly applications of 20-20-20 water-soluble fertilizer (2.4 g/L) and were inoculated as previously described at the two- to four-leaf stage. Rooted cuttings of *H. rosa-sinensis* were obtained, repotted, and inoculated 1–2 wk later. Prior to inoculation, the plants were subjected to a 24-hr high-moisture period (placed in mist), as described by Jones et al (12). After inoculation, they were covered with polyethylene bags for 36 hr and held in intermittent mist to keep free moisture on foliage for an additional 3 days. These tests were run at 18–20 C. Disease observations were made frequently during the 2-wk period after inoculation, and reisolations were made on plates of KMB when symptoms were observed.

Influence of temperature on disease development. In three separate tests, plants of *A. moschatus* cv. Sakatensis Pink were inoculated with the Abel A strain to determine the influence of temperature on infection and disease development. In the first two tests, 10 inoculated and two control plants were enclosed in polyethylene bags for 36 hr as previously described and held in growth chambers at 20, 25, and 30 C. In the third test, a 15-C treatment also was included. Two weeks after inoculation, the percentage of leaf area diseased and the numbers or percentages of petioles with lesions were recorded.

Effect of host nutrition on disease development. In early inoculation studies, plants growing vigorously appeared to be more susceptible than less vigorous plants. Plants grown under different nutritional conditions were inoculated to test the effect of nutrition on susceptibility. Plants grown in potting media were fertilized at different frequencies or were grown in sand with different levels of Hoagland's solution (10).

In one study, plants were grown in 10-cm pots filled with Pro-Mix BX, and in a second study they were grown in a methyl bromide-fumigated greenhouse soil mix (soil, sand, peat moss, perlite, vermiculite, 2:1:1:1:1, v/v) that was not amended with fertilizers. The composition of the raw soil used in the soil mix was 69% sand, 13% silt, and 18% clay. The pH was 5.4, and it had the equivalent of 6, 83, 579, and 115 kg/ha of P, K, Ca, and Mg, respectively. In each study the plants were given water-soluble 20-20-20 fertilizer, 2.4 g/L, at various intervals; each pot received 100 ml. In the Pro-Mix BX test, plants were grown for 3 wk; then one group was given 100 ml of water (unfertilized) and the second, third, and fourth groups were fertilized as above at 5-, 10-, and 15-day intervals, respectively. In the greenhouse soil test, fertilizer applications began 1 wk after plants were transplanted to pots. One treatment consisted of single application at 1 wk. Other treatments included applications made at 5-, 10-, or 15-day intervals, respectively. A water control was also used. Fifteen plants of each treatment were inoculated 23 days (Pro-Mix BX test) or 30 days (greenhouse soil mix test) after the nutritional treatments were started. Five plants were sprayed with sterile water as controls. Plants were held for 36 hr in high moisture after inoculation and then arranged in a randomized complete block design in a growth chamber at 23–25 C. Plants were rated for disease severity after 14 days.

Five-week-old plants with their roots washed free of growth medium were transplanted two per pot to 30.5-cm-diameter pots containing 2.5 kg of washed white play sand that had been premoistened with 500 ml per pot of deionized water. The pots were placed in holeless plastic liners that were slightly larger than the plant container itself. Plants were given 0.1, 1.0, or 3.0× strength Hoagland's solution (10). Ten pots of each treatment were placed on a greenhouse bench. A minor element solution was added to provide balanced nutrition. Initially, 100 ml of each solution was added to each plant on alternate days, but larger amounts were added more frequently to keep the sand moist as the plants grew. Pots were removed from the liners and flushed with 250 ml of deionized water weekly to prevent salt accumulation. Four weeks after the chemical treatments were started, when growth differences due to nutrition were obvious, plants were inoculated and then arranged in a randomized complete block design in a growth chamber at 22 C and given a 36-hr moisture period. Plants were rated for disease severity 14 days after inoculation. The study was repeated once.

Survival of the pathogen in association with seed. Mature seedpods were collected in 1988 from field-grown plants of *A. moschatus* infected with the Abel A strain. After the pods were dried on a greenhouse bench, the seeds were removed, cleaned, and stored in glass jars in a seed storage incubator at 10 C. After 6 mo of storage, seed were placed, 25 per plate, with alcohol-flamed forceps onto 40 plates of KMB amended with cycloheximide (100 μg/ml). The plates were incubated on a laboratory bench. Suspect colonies were streaked on KMB plates to obtain isolated colonies and later tested for oxidase and arginine dihydrolase activity.

In a second detection method, 20 g of whole seed that had been stored for 6 mo was added to a sterile 500-ml flask containing 200 ml of 0.1% peptone-phosphate buffer (21). A second sample of 20 g was crushed in a sterile mortar with a pestle and then placed in a flask containing peptone-phosphate buffer. The flasks were placed on a wrist-action shaker at medium speed for 30 min, serial dilutions were prepared, and 0.1-ml portions were spread over five separate plates of KMB amended with 100 μg/ml of cycloheximide. These tests were repeated after the seed had been stored for 10 mo. The seed-crush method was modified to include a 10-min immersion of the seed in 0.5% sodium hypochlorite. In all tests, fluorescent colonies were picked from plates and tested for oxidase and arginine dihydrolase activity. Suspect colonies that were oxidase- and arginine dihydrolase-negative were tested for pathogenicity on *A. moschatus*.

In a third method for detection of seedborne pathogenic bacteria, the seed wash water prepared as above was vacuum-infiltrated into plants of *A. moschatus* cv. Sakatensis Pink as described by Jones et al (14). Before infiltration, the wash water was filtered through a double thickness of cheesecloth to remove seed and debris. The cleared suspensions (approximately 200 ml) were placed in 355-ml plastic drinking cups and three drops of Tween 20 added. Five 15-cm tall plants were inverted in the cups with their leaves immersed in the suspension and were subjected to a vacuum as previously described (14). Infiltrated plants were repotted in their original pots and placed in a growth chamber at 20 C (first trial) or 25 C (second trial). Sterile 0.1% peptone-phosphate buffer with Tween 20 was included as a control. If suspect lesions appeared, some were removed and crushed in sterile water, and a portion was streaked on KMB. Fluorescent bacteria that tested oxidase- and arginine dihydrolase-negative were tested further for pathogenicity on *A. moschatus*.

In a fourth detection method, 1,250 seeds stored for 10 mo were planted, 50 per pot, in 10-cm-diameter sterile pots filled with Pro-Mix BX and covered with a 5- to 10-mm layer of vermiculite. The seeded pots were placed in saucers in a growth chamber at 25 C. The saucers were filled with water as needed to provide adequate moisture. Upon emer-

gence of the seedlings, pots were covered with clear polyethylene bags for 36 hr. One week later, the bagging procedure was repeated. The seedlings were observed daily for disease development. Samples of lesions were handled as above. Presumptive evidence for recovery of the pathogen was provided by the biochemical and pathogenicity tests described above.

RESULTS

Characterization of the Abel A strain and comparison with related organisms. *Morphological tests and growth temperature.* The Abel A strain was a gram-negative rod about 2–2.5 μm long and motile with three to four lophotrichous flagella. Minimum, optimum, and maximum temperatures for growth were 10, 20–30, and 35 C, respectively.

Physiological and biochemical tests. All of the strains produced a typical fluorescent pigment on KMB. The two strains from *A. moschatus*, the three known strains of *P. s. hibisci*, and most of the 10 strains of *P. s. syringae* gave similar results in the LOPAT tests (Table 1). However, two of the 10 strains of *P. s. syringae* gave atypical results (oxidase-positive and causing potato soft rot) and were probably originally misidentified. The strains from foliage (Abel A) and seed of *A. moschatus* gave identical results and were similar to the strains of *P. s. hibisci*. However, both strains of *A. moschatus* caused a necrotic lesion on green bean pods, whereas the three strains of *P. s. hibisci* did not. Strains from *A. moschatus* and those identified as *P. s. hibisci* utilized L(+)-tartrate, whereas eight strains of *P. s. syringae* did not. Many of the strains of *P. s. syringae* utilized D(−)-tartrate and DL-lactate and produced syringomycin, or an antimetabolite against *E. coli*. The strains from *A. moschatus* and the strains of *P. s. hibisci* generally gave negative results in these tests.

In the Biolog Microplate tests, the Abel A strain and the PSH2 strain of *P. s. hibisci* behaved similarly in their substrate utilization patterns. The organisms gave similar or identical results on 88 of the 95 compounds tested. However, *P. s. hibisci* used D-galacturonic acid, D-glucuronic acid, glucuronamide, and L-pyroglutamic acid, whereas the Abel A strain did not.

Pathogenicity tests. Both Oriental Red and Sakatensis Pink were highly susceptible to the Abel A strain, and no differences in susceptibility were consistently observed. Only the three strains of *P. s. hibisci* and three of the unidentified strains (JWO 020, Hib I, and Hib II) from *H. rosa-sinensis* and the seed strain from *A. moschatus* produced symptoms on *A. moschatus* similar to those caused by the Abel A strain (Table 2). All six of these strains from *H. rosa-sinensis* were considerably less aggressive on *A. moschatus* than was the foliage (Abel A) or seed strains from *A. moschatus*. The JWO 026 strain produced symptoms similar to those caused by *P. cichorii* (5). The bacterium was oxidase-positive and has since been identified as *P. cichorii*. Symptoms on *A. moschatus* caused by the strains from *A. moschatus* included dark brown to black foliar lesions, often with halos, water-soaked lesions along veins, and brown to black elongated lesions on petioles and stems. The strains from *H. rosa-sinensis*, except JWO 026, caused smaller lesions of similar appearance with less water-soaking. Six of the 10 strains of *P. s. syringae* did not produce symptoms on *A. moschatus* (Table 2). Strains of *P. s. syringae* from bean, soybean, wheat, and okra produced foliage lesions that were either large and tan or small and raised. Dry, shriveled lesions sometimes occurred on the petioles and stems. The strain from wheat caused a severe blight in every test. This strain was oxidase-positive and is probably *P. cichorii*, although it was originally identified as *P. s. syringae*. Strain JWO 026 from *H. rosa-sinensis* produced symptoms on *A. moschatus* similar to

Table 1. Comparison of unidentified *Pseudomonas* strains from *Abelmoschus moschatus* with known *Pseudomonas* species and *Pseudomonas syringae* pathovars in various physiological, biochemical, and pathogenicity tests

Diagnostic tests	*Abelmoschus* strains (2)[a]	*P.s. syringae* (10)	*P.s hibisci* (3)	*P.s. glycinea* (1)	*P.s. antirrhini* (1)	*P.s.. berberidis* (1)	*P.s. delphinii* (1)	*P.s. phaseolicola* (1)	*P.s. lachrymans* (1)	*P.s. coronafaciens* (1)	*P.s. tagetis* (1)	*P.s. maculicola* (1)	*P.s. tomato* (1)	*P.s. tabaci* (2)	*P.s. pisi* (1)	*P. viridiflava* (1)	*P. cichorii* (1)	*P. fluorescens* (1)
Levan	2[b]	7	3	1	1	1	0	1	1	1	1	1	1	2	1	1	1	0
Oxidase	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Potato soft rot	0	2	0	1	1	0	0	0	0	0	0	0	0	1	0	1	1	0
Arginine dihydrolase	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Tobacco hypersensitivity	2	9	3	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Fluorescence	2	10	3	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1
Casein hydrolysis	0	5	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1
Gelatin liquefaction	0	7	0	1	0	0	0	0	0	0	0	0	1	1	0	1	1	1
Pathogenicity on green bean pod	2	9	0	0	1	1	1	1	1	0	0	1	1	2	0	1	0	1
Sodium polypectate degradation																		
pH 5.0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
pH 8.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Production of:																		
β-Glucosidase	2	10	3	0	1	0	1	0	0	1	1	1	1	2	0	0	1	0
Syringomycin	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Antimetabolite against *Escherichia coli*	0	3	1	1	0	0	1	1	1	0	0	0	0	0	0	0	1	1
Ice-nucleation activity	2	6	2	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0
Utilization of:																		
Erythritol	2	8	2	1	0	0	1	0	0	1	1	0	0	2	0	1	1	1
L (+)-Tartrate	2	2	2	1	0	0	0	0	0	0	1	0	0	2	0	0	0	1
D(−)-Tartrate	0	3	0	v[c]	v	v	0	0	0	0	0	v	v	0	0	v	1	v
DL-Lactate	0	9	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1
Sucrose	2	10	3	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1
Mannitol	2	9	2	1	1	1	1	0	0	1	1	1	1	2	1	1	1	1
Sorbitol	2	7	2	1	1	1	1	0	0	1	1	1	1	2	1	1	1	1
β-Alanine	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Trehalose	0	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	1	1

[a] Number of strains tested. The strains of *Abelmoschus* were from a diseased seedling and infested seed.
[b] Number of strains testing positive.
[c] v = Strain variability among tests.

the wheat strain.

The host range of the Abel A strain was mostly limited to *Hibiscus* and *Abelmoschus* species. The strain caused a severe foliage disease on the Tennessee Red, Hot Pink, and Brilliant Red cultivars of *H. rosa-sinensis*, provided the plants were given preinoculation and postinoculation moisture periods and were held at low temperatures (18–20 C). In other tests where the preinoculation and postinoculation moisture periods were not used, no disease developed on any of the cultivars (*unpublished*). Under optimum temperature and moisture conditions, the disease on *H. rosa-sinensis* began as water-soaking. Later, large angular lesions with halos developed, and leaves sometimes dropped. No lesions were observed on the stems or petioles. The Abel A strain also produced symptoms on the *H. moscheutos* cultivars Disco Belle, Southern Belle, and Frisbee, causing lesions on the leaves, stems, and petioles, with leaf deformation around the lesions. Three of the strains of *P. s. hibisci* tested and three of the four unknown strains (JWO 020, Hib I, and Hib II) isolated from *H. rosa-sinensis* produced symptoms similar to the Abel A strain on plants of *H. rosa-sinensis* cultivars Hot Pink and Brilliant Red.

In one test, the Abel A strain produced a few scattered lesions on cotton and okra leaves, mostly along the edges. The bacterium could be reisolated from these lesions. However, no disease was produced when these hosts were inoculated in two other similar tests. No symptoms occurred on the seven other malvaceous hosts tested.

Influence of temperature on disease development. Disease was more severe at 20 and 25 C than at 15 and 30 C. In the first two tests, readings for percent leaf area infection were 13.5, 13.8, and 2.7 (FLSD [$P = 0.05$] = 3.1) and 5.8, 4.6, and 0.8 (FLSD [$P = 0.05$] = 2.5) at 20, 25, and 30 C, respectively. Mean numbers of petiole lesions were 8.1, 11.2, and 2.0 (FLSD [$P = 0.05$] = 5.8) and 6.6, 3.0, and 0.0 (FLSD [$P = 0.05$] = 2.9) at 20, 25, and 30 C in the two tests. In the third test, percent leaf area infection readings were 1.7, 9.4, 4.0, and 0.2 (FLSD [$P = 0.05$] = 1.4) at 15, 20, 25, and 30 C. Percentages of petioles infected were 55, 51, 34, and 0 (FLSD [$P = 0.05$] = 10.0) at the four temperatures. At 30 C, lesions showed little water-soaking and were small and nonspreading. Lesions were also small at 15 C.

Influence of host nutrition on disease development. All disease components (percent leaf area infected, percent dead leaves, and percent infected petioles) were higher on plants receiving additional nutrient solution applications than on those grown without additional fertilization or those that received only an initial treatment (Table 3). In the sand culture experiments, the leaf area infection readings were 1.9, 3.8, and 4.8% (FLSD [$P = 0.05$] = 1.2) for the 0.1, 1.0, and 3.0× strength Hoagland's solution treatments, respectively. Equivalent petiole infection readings were 32, 54, and 57% (FLSD [$P = 0.05$] = 15), respectively.

Survival of the pathogen in association with seed. The Abel A strain was not recovered when seed from diseased plants were stored and plated on KMB amended with cycloheximide. The seed were heavily infested with fluorescent saprophytes, and these rapidly multiplied around each plated seed. Bacteria picked from the seed were consistently oxidase- and arginine dihydrolase-positive.

High populations of fluorescent bacteria were isolated from seed washings of whole and macerated seed. Populations on whole seed were 1.2×10^7 and 2.0×10^6 cfu/g of seed after 6 and 10 mo of storage, respectively. Populations from macerated seed after 6 and 10 mo were 3.4×10^6 and 1.6×10^6 cfu/g. After 10 mo, the population count from surface-sterilized macerated seed was 7.0×10^5 cfu/g. Most of these bacteria were saprophytes that were oxidase- and arginine dihydrolase-positive. Twenty colonies picked at random from plates prepared for the 6-mo assay were all oxidase- and arginine dihydrolase-positive. However, seven of 20 suspect colonies picked from plates prepared in the 10-mo assay were oxidase- and arginine dihydrolase-negative and were confirmed to be identical to the Abel A strain in laboratory and pathogenicity tests.

When seed washings were infiltrated into young *A. moschatus* plants, numerous water-soaked lesions appeared on leaves 2–4 days after infiltration. Many of these enlarged and were typical of

Table 2. Pathogenicity of various strains and pathovars of *Pseudomonas syringae* on *Abelmoschus moschatus* cv. Sakatensis Pink

Test organism	Original host	Times tested[a]	Times positive[b]
Abel A	*A. moschatus*	7	7
Seed isolate	*A. moschatus*	3	3
P. s. hibisci (PSH2)	*Hibiscus rosa-sinensis*	5	3
P. s. hibisci (434)	*H. rosa-sinensis*	4	3
P. s. hibisci (435)	*H. rosa-sinensis*	4	3
JWO 020	*H. rosa-sinensis*	3	3
JWO 026	*H. rosa-sinensis*	3	3
Hib I	*H. rosa-sinensis*	2	2
Hib II	*H. rosa-sinensis*	2	2
P. s. syringae (318)	Okra	4	1
P. s. syringae (5074)	Okra	5	0
P. s. syringae	Corn	3	0
P. s. syringae	Tomato	3	0
P. s. syringae	Grapefruit	3	0
P. s. syringae	Bean	4	1
P. s. syringae	Soybean	4	1
P. s. syringae	Wheat	6	6
P. s. syringae	Lilac	5	0
P. s. syringae	Peach	3	0

[a] Pathogenicity of various strains was determined in a series of seven tests in which three to five plants were inoculated with the test strains, covered with polyethylene bags for 36 hr, and placed in growth chambers at 18–25 C.
[b] Number of times each strain produced symptoms on *A. moschatus*.

Table 3. Effect of fertilization on susceptibility of *Abelmoschus moschatus* grown in a commercial potting mix (CM) and a greenhouse soil mix (GSM) to a pathovar of *Pseudomonas syringae*[a]

	Leaf area diseased (%)[c]		Dead leaves (%)		Diseased petioles (%)	
Fertilization frequency[b]	CM	GSM	CM	GSM	CM	GSM
No fertilization	1.5	1.6	2	3	14	30
Initial fertilization only	···	1.6	···	5	···	24
Intervals of 15 days	6.8	14.3	19	33	80	45
Intervals of 10 days	9.2	14.5	19	41	90	42
Intervals of 5 days	9.0	17.2	22	39	89	52
FLSD ($P = 0.05$)	0.8	2.2	8	7	8	9

[a] The commercial mix was Pro-Mix BX and the greenhouse soil mix consisted of soil, sand, peat moss, perlite, and vermiculite (3:1:1:1:1, v/v) that was not fertilized but was fumigated with methyl bromide. The raw soil consisted of 69.2, 13.2, and 17.6% sand, silt, and clay, respectively, and had the equivalent of 6, 83, 579, and 115 kg/ha of P, K, Ca, and Mg.
[b] Sakatensis Pink plants (15 per treatment) were grown in 10-cm pots and either were not fertilized or were fertilized as indicated with 100 ml per pot of a 20-20-20 water-soluble fertilizer (2 g/L) as indicated.
[c] Infection calculated on the basis of total leaf area of infected leaves, not including dead leaves.

lesions caused by the Abel A strain. A plant-pathogenic bacterium was readily isolated and was found to be identical to the Abel A strain in biochemical and pathogenicity tests.

Three seedlings from 1,250 seed planted in Pro-Mix BX developed symptoms typical of the Abel A strain. Isolations from these diseased seedlings yielded essentially pure cultures of fluorescent bacteria that were oxidase- and arginine dihydrolase-negative. Two cultures were tested in laboratory and pathogenicity tests and were identical to the Abel A strain.

DISCUSSION

The bacterial leaf spot and blight described here is a potentially serious disease on the ornamental cultivars of *A. moschatus*. The disease will probably cause more losses in propagation greenhouses maintained at suboptimum temperatures for growth or on outside plantings during cool, wet periods. Indeed, the disease was first observed during late winter and early spring when greenhouse temperatures were low. In field plantings, the disease became severe when heavy rains were followed by daily mean temperatures of 22–23 C (*unpublished*). However, the disease should not become a field problem under normal midsummer conditions in the southern United States, where mean daily temperatures may be 27–28 C.

The bacterium can survive periods of high temperatures and low rainfall as an epiphyte on leaves (*unpublished*). It becomes active with the onset of environmental conditions conducive for disease development. In the field, lesions appear dry and purple as temperature and moisture become less than optimal for disease development.

Plant succulence favors the development of disease. Excessive fertilization will likely increase disease severity in the greenhouse and field.

The bacterium from *A. moschatus* appears to be similar to or the same as *P. s. hibisci* described by Jones et al (12) in 1986. Like *P. s. hibisci*, it utilized L(+)-tartrate but did not use D(−)-tartrate or DL-lactate or produce syringomycin or a compound inhibitory to *E. coli* (12). In contrast, the strains of *P. s. syringae* tested consistently used DL-lactate but not L(+)-tartrate. In the Biolog Microplate system (2), a strong similarity existed between the strain from *A. moschatus* and the type strain of *P. s. hibisci*. The two bacteria were similar in 92.6% of the 95 tests. Attempts were made to use fatty acid analysis to compare the strains for *Abelmoschus* and *P. s. hibisci* with each other and with several typical strains of *P. s. syringae* (*unpublished*). The results were inconclusive.

The strain from *A. moschatus* and strains of *P. s. hibisci* also behaved similarly in pathogenicity tests, although some differences were observed. The former strain produced symptoms on *H. rosa-sinensis* similar to those produced by the known strains of *P. s. hibisci* and three of the then unknown strains (JWO 020, Hib I, and Hib II) isolated from *H. rosa-sinensis*. The latter three strains were recently identified as *P. s. hibisci*. Jones et al (12) reported that preinoculation and postinoculation mist periods were required for the infection of *H. rosa-sinensis* by *P. s. hibisci*. These findings were confirmed in our work; the same was true for the strain from *A. moschatus*.

The three known strains and three recently identified strains (JWO 020, Hib I, and Hib II) of *P. s. hibisci* were also pathogenic on *A. moschatus*. However, the symptoms caused by these six strains were less severe than those caused by the strain from *A. moschatus*. The latter bacterium may have become pathologically specialized because of its association with *A. moschatus*. Additional strains of the bacterium must be tested before this belief can be confirmed.

The strain from *A. moschatus* apparently is seedborne. When the disease was observed initially in the greenhouse, the initial inoculum appeared to originate from seed. Second, in our tests the bacterium was recovered from seed that had been stored for extended periods.

The strain from *A. moschatus* has a limited host range in the Malvaceae, being restricted to certain *Hibiscus* and *Abelmoschus* species. This is not surprising, as *A. moschatus* was once classified in the genus *Hibiscus* (1). Symptoms were produced on several cultivars of *H. moscheutos* and *H. rosa-sinensis*, but symptoms were severe only on the latter host. A few lesions sometimes occurred on cotton and okra. Additional work is needed to determine whether these are hosts under field conditions.

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