Leaf Blight of Onions in Barbados Caused by *Xanthomonas campestris*

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ABSTRACT

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A leaf blight of onions occurring in Barbados is characterized by the rapid necrosis and dieback of leaves under humid conditions. A bacterium, isolated from lesions, induced water-soaked lesions on onion leaves within 3 days. This bacterium was identified as *Xanthomonas campestris* on the basis of fatty acid analysis and other biochemical and physiological tests. The severity of the disease declined by 93–99% if plants were first treated with *Erwinia herbicola*, but it increased if *Xanthomonas* preceded *E. herbicola*. This is the first known description of the causal agent of the leaf blight of onion in Barbados.

Onion (*Allium cepa* L.) was first grown on a commercial scale in Barbados in 1968, and by 1971 a leaf blight disease, locally referred to as onion "blast," affected the crop (14). The disease is the most important constraint to the expansion of the local onion industry (10). The leaf blight is characterized by the appearance of white flecks or pale spots that develop into water-soaked lesions under humid or overcast conditions. Lesions often spread and coalesce to form extended chlorotic areas, which frequently degenerate into holes on older leaves. There is also considerable dieback of leaves and a reduction in bulb size. This study was undertaken to determine the causal agent of the disease.

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MATERIALS AND METHODS

Pathogen and host culture. Media used routinely in this study for the culture of bacteria and fungi were Czapek-Dox agar (CDA, Difco), nutrient broth–yeast extract agar (NYA) (3), and nutrient–yeast extract glycerol agar (NYGA) (2). The onion cultivar Yellow Granex served as host for all test inoculations. Seedlings used in this study were grown, 100 per seed tray (22 × 16 cm), in sterilized potting compost placed in a plant growth chamber set at 26–28 C and 85–90% relative humidity, with a 12-hr photoperiod, provided by fluorescent lamps, of total light intensity of 1,020 $\mu E \cdot s^{-1} \cdot m^{-2}$.

Isolation of the pathogen. Leaf samples with symptoms of the disease were collected from onion fields at six loca-

tions on Barbados. Leaves were surface-sterilized by dipping in ethanol (95%) for 2 sec, followed by a 10-sec rinse in sodium hypochlorite (1.25%). Sections (1–2 mm) from areas of chlorosis, white flecks, or the center and margin of water-soaked lesions were crushed in 200 μl of sterile distilled water (SDW) with an ethanol-sterilized mortar and pestle. The suspension was streaked onto NYA plates amended with cycloheximide (30 μg/ml) for isolation of bacteria. Surface-sterilized intact leaf sections with symptoms also were placed on CDA for the isolation of fungi.

Identification of the pathogen. Selected standard determinative tests were performed on each presumptive bacterial pathogen and reference strains of *Erwinia herbicola* (Löhnis) Dye and *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye. Flagellation was determined by a silver stain method (13). Catalase, urease, oxidase, and nitrate reductase activity; pectate, starch, gelatin, and milk protein hydrolysis; asparagine utilization; acid production from sugars; hydrogen sulphide and indole production; Gram stain reaction; pigmentation; and anaerobic growth were tested by standard bacteriological methods (11,13).

Representative strains of each suspected bacterium were sent to ADAS Central Science Laboratory (Ministry of Agriculture, Fisheries and Food, Hatching Green, Harpenden, England) for identification on the basis of fatty acid composition. Strains were cultured on trypticase soy broth agar at 28 C for 48 hr, and fatty acid methyl esters extracted by a standard method (15). Fatty acids were analyzed with a microbial identification system (Microbial ID, Newark, DE) that comprised an automated GC 5890 Hewlett-Packard gas chromatogram fitted with a 25-m fused silica capillary coated with a 5% methylphenyl silicone (15). The test strains were compared with known bacterial strains in the National Collection of Plant Pathogenic Bacteria (NCPPB; Ministry of Agriculture, Fisheries and Food, Hatching Green, Harpenden, England). The analysis included an assessment of the degree of similarity of fatty acid composition.

Fig. 1. Symptoms of leaf blight of onions caused by *Xanthomonas campestris*. **(A)** White flecks and **(B)** water-soaked areas from which *X. campestris* was isolated.

Fungi were examined microscopically and identified on the basis of spore and mycelial morphology (4).

Pathogenicity tests. Leaves of 4-wk-old onion seedlings were sprayed with a bacterial or fungal suspension (10^6 cfu/ml) or pricked with a sterile pin previously inserted in a colony of a bacterium grown on NYA or NYGA. Plants treated with SDW served as controls. All treated plants were maintained under humid chambers placed in a plant growth chamber providing a temperature of 26–28 C and light intensity of 1,020 $\mu E \cdot s^{-1} \cdot m^{-2}$ at 12-hr periods. The percentage of leaf area for 100 plants covered by lesions 5 days after inoculation was used to estimate disease severity. Thirty representatives of each suspected pathogen were tested on onion, and each test was repeated twice.

The possibility that two primary suspect bacterial pathogens might interact to cause the leaf blight of onion was investigated. Three strains each of *Xanthomonas* and *E. herbicola* were tested on onion in all combinations. In one experiment, plants were injured by pricking at 20 equidistant points along the length of each leaf with a sterile pin, sprayed with *E. herbicola* (10^6 cells per milliliter), and then spray-inoculated a second time with *Xanthomonas* (10^6 cells per milliliter) 0, 1, 2, 3, 4, or 5 days later. In another experiment, injured plants were treated with *Xanthomonas* followed by *E. herbicola* at similar concentrations and time intervals mentioned above. Injured plants sprayed with a suspension of *Xanthomonas* or *E. herbicola* followed by SDW 0, 1, 2, 3, 4, or 5 days later served as controls. Seed trays, each with 100 plants per treatment, were arranged randomly, and plants were examined for disease symptoms 5 days after their second inoculation. Both experiments were conducted at the same time, and they were repeated twice. The disease severity on individual plants was recorded, and differences due to treatments were analyzed by a one-way analysis of variance.

RESULTS

Isolation of the pathogen. *Xanthomonas* sp. was isolated consistently from water-soaked margins, white flecks, and the region of tip dieback of onion leaves (Fig. 1). *E. herbicola* was recovered from all diseased areas, and *Pseudomonas* sp. and *Clavibacter* sp. were isolated from some areas of chlorosis. Fungi known to cause leaf blights on onion were not recovered.

Identification of the pathogen. All bacterial strains that caused the leaf blight of onion were characteristic of *Xanthomonas* on the basis of biochemical and physiological tests. These strains were yellow-pigmented obligate aerobes, gram- and oxidase-negative, and catalase-positive, and they produced acid from glucose, arabinose, mannose, trehalose, and cellobiose. All strains bore a polar flagellum, and none utilized asparagine as a sole source of carbon and nitrogen. Unlike *X. campestris* pv. *vesicatoria*, the test strains hydrolyzed

Table 1. Physiological and biochemical characteristics of *Xanthomonas* from onion compared with strains of *X. campestris* pv. *vesicatoria* and *Erwinia herbicola*

Characteristics	Reaction[a]		
	Test strains	*X. c. vesicatoria*	*E. herbicola*
Number of strains tested	100	3	3
Monotrichous flagellation	+	+	−
Yellow mucoid colonies on nutrient agar + 5% glucose	+	+	+
Gram-negative rods	+	+	+
Water-insoluble yellow pigment	+	+	−
Milk protein hydrolysis	+	+	−
Starch hydrolysis	+	−	+
Pectate hydrolysis	−	−	−
Gelatin hydrolysis	+	+	+
Utilization of asparagine	−	−	+
Catalase activity	+	+	+
Urease activity	−	−	−
Oxidase activity	−	−	−
Nitrate reductase activity	−	−	+
Hydrogen sulfide production	+	+	+
Indole production	−	−	−
Acid production from			
Glucose	+	+	+
Arabinose	+	+	+
Mannose	+	+	+
Trehalose	+	+	+
Cellobiose	+	+	−
Fermentative utilization of glucose	−	−	+

[a] Plus and minus signs indicate positive and negative reactions, respectively, within 7 days at 28 C.

Table 2. Selected fatty acid[a] profiles of xanthomonads recovered from diseased onion

Strain	Fatty acids (%)													
	C11:0 *iso*	C11:0 *iso* 30H	C12:0 30H	C13:0 *iso* 30H	C14:0	C15:0 *iso*	C15:0 *ante-iso*	C15:0	C16:0 *iso*	C16:1 *CIS9*	C16:0	C17:1 *isoF*	C17:0 *iso*	C17:0 *ante-iso*
F58A	3.15	1.36	2.95	3.90	1.90	27.25	7.55	2.14	1.72	22.66	5.45	4.86	5.67	0.45
F58B	3.63	1.27	2.70	3.84	2.23	28.95	8.06	1.97	1.76	21.38	4.76	4.27	4.22	0.32
G6	3.04	1.15	2.61	3.57	1.96	30.47	6.03	2.06	1.73	23.68	4.40	4.70	4.39	0.29
W4	3.38	1.31	2.97	3.85	2.02	29.07	7.10	1.89	1.85	23.49	1.58	4.73	4.75	0.35

[a] Analysis of the fatty acid composition of the strains was performed by gas chromatography at the ADAS Central Science Laboratory, Ministry of Agriculture, Fisheries and Food, Harpenden, England.

Table 3. Similarity indices[a] of *Xanthomonas* from onion relative to known pathovars of *X. campestris* in the National Collection of Plant Pathogenic Bacteria (NCPPB), Harpenden, England

X. campestris strain or pathovar	Similarity index
Onion strain F58A	0.915
X. c. phaseoli	0.757
X. c. ricini	0.741
X. c. manihotis	0.726
Onion strain F58B	0.740
X. c. glycines	0.786
X. c. phaseoli	0.700
X. c. ricini	0.672
Onion strain G6	0.914
X. c. vignicola	0.560
X. c. glycines	0.526
X. c. ricini	0.511
Onion strain W4	0.966
X. c. phaseoli	0.669
X. c. vignicola	0.667
X. c. ricini	0.653

[a] The test strains were included in the NCPPB library, and their fatty acid profiles recompared with the expanded library. The degree of fatty acid profile similarity is assessed in the form of a similarity index (0 = no match, 1 = 100% match).

starch. Other characteristics of test strains are shown in Table 1.

Four representative strains contained the fatty acid methyl esters C11:0 *iso* 30H, C12:0 30H, C13:0 *iso* 30H, C15:0 *iso*, and C15:0 *anteiso* in their fatty acid profiles. Moreover, the ratio of C15:0 *iso* to C15:0 *anteiso* was greater than 1 but less than 10 (Table 2). When the test strains were included in the NCPPB library, only one test strain was incorrectly identified. Pathovars of *X. campestris* most similar to the test strains in fatty acid profiles are shown in Table 3, and they include the pathovars *phaseoli, vignicola*, and *glycines*.

Pathogenicity tests. A total of 28 strains of *Xanthomonas* and 20 strains of *E. herbicola* induced water-soaked lesions on injured onion plants. Lesions occupied mean percent leaf areas of 24.7 and 12.6 of plants treated with *Xanthomonas* and *E. herbicola*, respectively. In contrast, when uninjured plants were sprayed with a bacterial suspension, only *Xanthomonas* was pathogenic, but lesions were confined to 6.8% (mean) of the leaf surface.

Table 4. Severity of leaf blight of onion treated with *Erwinia herbicola* at different times after inoculation with *Xanthomonas campestris*

Time of *Erwinia* treatment after *Xanthomonas* inoculation[a] (days)	Disease severity[b] Treatment			
	X. campestris strain F58A and *E. herbicola* strain 1	*X. campestris* strain F58A[c]	*E. herbicola* strain 1[c]	LSD (5%)
0	35.1	36.5	9.8	5.9
1	36.4	55.3	11.5	5.6
2	42.9	41.4	10.9	6.2
3	52.6	44.6	11.0	6.4
4	100	50.5	12.0	6.7
5	100	57.9	13.6	5.8
LSD (5%)	5.1	9.1	2.2	...

[a] Plants were injured by pricking the leaves with a sterile pin and spraying them with a suspension of *Xanthomonas*, followed by *Erwinia* 0–5 days later.
[b] Assessed as the percent leaf area covered by lesions 5 days after treatment with *Erwinia*.
[c] Control.

Table 5. Severity of leaf blight of onion inoculated with *Xanthomonas campestris* at different times after treatment with *Erwinia herbicola*

Time of *Xanthomonas* inoculation after *Erwinia* treatment[a] (days)	Disease severity[b] Treatment			
	X. campestris strain G6 and *E. herbicola* strain 2	*X. campestris* strain G6[c]	*E. herbicola* strain 2[c]	LSD (5%)
0	17.4	39.0	8.2	6.1
1	15.4	42.6	8.0	5.4
2	14.4	49.5	8.4	7.8
3	10.1	52.8	10.7	6.4
4	4.3	54.4	12.1	6.1
5	1.0	100	15.5	4.1
LSD (5%)	4.1	10.7	2.5	...

[a] Plants were injured by pricking the leaves with a sterile pin and spraying them with a suspension of *E. herbicola*, followed by *X. campestris* 0–5 days later.
[b] Assessed as the percent leaf area covered by lesions 5 days after inoculation with *Xanthomonas*.
[c] Control.

Table 4 shows the effect of inoculating plants with an onion strain of *Xanthomonas* before they were treated with a strain of *E. herbicola*. These plants were as severely affected or significantly more severely affected than control plants infected with *Xanthomonas* only. The later that *E. herbicola* was introduced, the greater the disease severity. All other treatments in which *Xanthomonas* preceded *Erwinia* gave similar results (data not shown). In contrast, plants sprayed with a strain of *E. herbicola* prior to inoculation with a strain of *Xanthomonas* were less affected than control plants infected with *Xanthomonas* (Table 5). Generally, the later that plants were infected with *Xanthomonas*, the lower the disease severity. Lesions accounted for 1% of the average leaf area of plants treated 5 days with *Xanthomonas*, and this value represents a 15-fold reduction in disease severity relative to control plants sprayed with *E. herbicola* only. Other treatments in which plants were sprayed with *E. herbicola* before *Xanthomonas* resulted in similar reductions in disease severity (data not shown).

DISCUSSION

A complex interaction of agents,

including microbial pathogen and high soil and air temperature, was suggested as a possible cause of a leaf blight of onion in Barbados (5,10). In the present study, a *Xanthomonas* sp. was consistently associated with the typical symptoms of the disease. Moreover, only inoculations with *Xanthomonas* strains caused symptoms of the leaf blight.

Strains of *Xanthomonas* from onion were characteristic of the *X. campestris* group described in *Bergey's Manual of Systematic Bacteriology* (11) (Table 1). Four representative strains were confirmed as belonging to the genus *Xanthomonas* on the basis of the presence of C11:0 *iso* 30H, C13:0 *iso* 30H, and C12:0 30H in the fatty acid profiles (Table 2). The ratio of C15:0 *iso* to C15:0 *anteiso* was in the range expected for *X. campestris*, indicating that the strains probably belong to this species (7). This is the first known report of the isolation and identification of the causal agent of the leaf blight of onion in Barbados. A leaf blight of onions also caused by *Xanthomonas* and reported in Hawaii in 1978 (1) seems identical to the one described in the present study.

Fatty acid analysis was also used in efforts to identify the test strains to the pathovar level. The NCPPB library contains data on numerous bacteria, including a large collection of *X. campestris*. The similarity index calculated for each strain in Table 3 is therefore based on a composite of profiles in this library. Generally, the test strains appeared different from *X. campestris* pathovars in the NCPPB library, and they may therefore represent a new pathovar.

E. herbicola has been reported as the causal agent of stalk and leaf necrosis of onions (8). In the present study, *E. herbicola* induced water-soaked lesions on injured plants and contributed to the severity of the disease once initiated by *Xanthomonas* (Table 4). In contrast, the severity of the disease was reduced if onions were treated with *E. herbicola* before *Xanthomonas* (Table 5). This reduction was greatest if *E. herbicola* was applied 5 days before *Xanthomonas*. This behavior of *E. herbicola* as an opportunistic pathogen and a supressor of diseases has been reported (6,16). The basis of the reduction in disease severity caused by *E. herbicola* in this study is not known, but inhibition of *Xanthomonas* by organic acids, hydroquinones, antibiotics, or bacteriocins may be involved (9,12).

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