Bacterial Blight of *Crambe abyssinica* in Missouri Caused by *Xanthomonas campestris*

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ABSTRACT

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Crambe abyssinica is being evaluated as an oilseed crop for Missouri. During 1990–1992, a blight affecting leaves and stems was observed on virtually all plants growing in three experimental fields in central Missouri. A yellow-pigmented, gram-negative bacterium was consistently recovered from symptomatic tissue. A combination of physiological tests and gas chromatographic analysis of cellular fatty acids identified the bacterium as *Xanthomonas campestris*. The pathogen was closely related to *X. c. armoraciae* and *X. c. campestris*. The bacterium was found to be seedborne, contaminating 50–67% of tested seed of cv. Meyer. The bacterium was detected predominantly on the silicle and the surface of the seed coat and was possibly systemic within the seed. The bacterium infected several commercially important members of the genus *Brassica* and all crambe germ plasm sources tested. Symptom development in greenhouse tests also demonstrated the ability of the pathogen to move systemically through infected plants.

Additional keywords: *Brassica campestris* subsp. *oleifera, Brassica carinata, Brassica hirta, Brassica juncea, Brassica napus* subsp. *oleifera*, fatty acid analysis, host range, rapeseed, *Xanthomonas campestris* pv. *vesicatoria*

Crambe abyssinica Hochst. ex R.E. Fr., an annual herb in the Brassicaceae, is being evaluated as a potential oilseed crop for Missouri and the Midwest. Crambe seed oil, which is predominantly erucic acid, may be useful as a lubricant in a variety of industrial processes (15). Crambe has not been widely cultivated, and thus there are few reports of diseases of crambe or its relatives. Leppik (14) compiled a bibliography of diseases for several *Crambe* spp. In the United States, fungal parasites of *C. abyssinica* include *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *conglutinans* (Wollenweb.) W.C. Snyder & H.N. Hans. (2) and *Alternaria brassicicola* (Schwein.) Wiltshire (= *A. circinans* (Berk. & M.A. Curtis) P.C. Bolle) (10,12). There are no reports of bacterial diseases of *C. abyssinica* (14), although a bacterial disease of seakale (*C. maritima* L.) caused by *Pseudomonas campestris* (Pammel) Smith (= *Xanthomonas campestris* (Pammel) Dowson) was noted in Great Britain in the 1920s (5,6,18). Crambe has been used as an experimental host for several plant viruses (8,11).

During periodic surveys of crambe growing in experimental fields in central Missouri, a blight affecting leaves and stems was observed in 1990, 1991, and 1992. In this report, we describe the disease and characterize the causal bacterium and its host range.

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MATERIALS AND METHODS

Pathogen strains 90-101 and 90-128 were recovered in 1990 from crambe cv. Meyer growing at the University of Missouri Horticulture Research Center (HRC) and Sanborn field (SF), respectively. Seven strains were recovered from crambe seeds: 92-33 from cv. Meyer; 92-34, 92-35, and 92-36 from cv. NM55; and 92-37, 92-38, and 92-39 from cv. NM61. Pathogen strains were compared with three pathovars of *Xanthomonas campestris* obtained from ICMP (Mt. Albert Research Centre, Auckland, New Zealand): *X. c. armoraciae* (McCulloch) Starr & Burkholder (ICMP 7a = NCPPB 347, pathotype strain), *X. c. campestris* (Pammel) Dowson (ICMP 13a = NCPPB 528), and *X. c. vesicatoria* (Doidge) Dye (ICMP 63a = NCPPB 422 = ATCC 35937, pathotype strain).

Pathogen isolation and characterization. Leaf and stem lesions were excised from affected plants and the tissue was ground in sterile distilled water (SDW) with a mortar and pestle to recover the pathogen in pure culture. The resulting suspension was streaked on the surface of glucose-yeast-calcium carbonate agar (GYC; 10 g of dextrose, 10 g of yeast extract, 5 g of $CaCO_3$, and 20 g of Difco Bacto agar per liter of distilled water [DW]) (19) or potato-dextrose-peptone agar (PDP; 40 g of Difco potato-dextrose agar, 10 g of peptone, 5 g of Difco Bacto agar, 5.0 ml of 1 N NaOH per liter of DW) (1). Plates were placed at 28 C for 48–72 hr of incubation.

To obtain pure cultures of the bacterium, single colonies were selected from isolation plates and streaked onto PDP. After 3 days, single colonies were again selected and transferred to test tubes containing 10 ml of nutrient broth (NB; 8 g of Difco nutrient broth per liter of DW). After 3 days of incubation at 28 C, these cultures were maintained at 3–6 C as stock cultures for all subsequent work.

The Gram reaction of bacterial strains was characterized by mixing a small amount of an actively growing culture (48–72 hr old) in a drop of 3% KOH on a clean glass microscope slide and observing the "threading" reaction described by Suslow et al (20). Starch utilization was determined by incubating cultures at 28–30 C for 3 days in petri dishes containing starch agar (Difco starch agar, 25 g/L of DW), and then flooding the plates with an iodine solution (1 g of I_2, 2 g of KI, 300 ml of DW) (9). A clear zone around the colony indicated that the strain could hydrolyze starch. Catalase production was determined by adding several drops of 3% H_2O_2 to cultures (48–72 hr old) growing on PDP. The appearance of bubbles within 5 min indicated that catalase was produced (9).

A variety of physiological tests were conducted using diagnostic kits (Table 1). The API-20E kit (API Analytab Products, Plainview, NY) is designed for identification of members of the Enterobacteriaceae. The Biolog GN Microplate kits (Biolog, Inc., Hayward, CA) are designed for detailed characterization of a wide variety of gram-negative bacteria. Each kit was prepared according to the manufacturer's specifications, and reactions were recorded after incubation at 28–30 C for 72 hr with the API-20E and for 48 hr with the Biolog GN Microplates. Both kits were used to characterize the two strains from crambe tissue and the seven strains from seeds.

The cellular fatty acid composition of strains 90-101 and 90-128 was characterized by gas chromatographic analysis with the MIDI Microbial Identification System (MIDI, Newark, DE). Fatty acid profiles of the two strains were compared with those of previously identified strains in the MIDI and University of Florida libraries, using the principal component analysis and pattern recognition algo-

Table 1. Comparison of crambe pathogens with type cultures of *Xanthomonas campestris* pv. *armoraciae*, *X. c. campestris*, and *X. c. vesicatoria*

Test[a]	Literature[b]	Type cultures: pv. *armoraciae*	Type cultures: pv. *campestris*	Type cultures: pv. *vesicatoria*	Strains from crambe cultivars[c]: Meyer	Meyer: NM55	Meyer: NM61
Gram reaction	—[d]	−	−	−	−	−	−
Starch	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+
API-20E kit							
Acetoin	−	−	−	+	+/−	−	−
Arabinose	+	−	+	−	+	+	+
Citrate	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+
Glucose	+	−	−	−	−	−	−
Indole	−	−	−	−	−	−	−
Inositol	−	−	−	−	−	−	−
Melibiose	−	−	−	−	−	−	+/−
Nitrate	−	−	−	−	−	−	−
Rhamnose	−	−	−	−	−	−	−
Sorbitol	−	−	−	−	−	−	−
Sucrose	+	−	−	−	−	−	−
Tryptophan		+	−	−	−	−	−
Biolog GN Microplate							
Adonitol	−	−	−	−	−	−	−
Alaninamide		+	−	+	+	+	+
D-Alanine		+	−	+	+	+	+
L-Alanine		+	−	+	+	+	+
L-Alanyl-glycine		+	−	+	+	+	+
L-Arabinose	+	−	−	−	+/−	+/−	+
L-Asparagine	−	−	−	−	−	−	−
L-Aspartic acid		+	−	+	+/−	+	+
Glycyl-L-aspartic acid		+	−	+	+/−	+	+
α-Hydroxybutyric acid		+	−	+	+	+	+
β-Hydroxybutyric acid		−	−	−	−	−	+/−
α-Ketobutyric acid		+	−	+	+/−	+	+
Cellobiose	+	+	+	+	+	+	+
Citric acid	+	+	+	+	+	+	+
α-Cyclodextrin		−	−	+	−	−	−
D-Fructose	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+
Gentiobiose		+	+	+	−	+	+
α-D-Glucose	+	+	+	+	+	+	+
Glucose-1-phosphate		+	−	+	−	−	−
Glycerol		+	−	+	+	+	+
DL-α-Glycerol phosphate		+	−	+	−	+/−	+/−
Glycogen		+	+	+	+/−	+	+
Inosine		−	−	−	+/−	−	−
m-Inositol	−	−	−	−	−	−	−
Itaconic acid		−	−	−	+/−	−	−
DL-Lactic acid		+	−	−	+	+	+
α-D-Lactose lactulose		+	−	+	+	+	+
L-Leucine		+	−	−	+/−	+/−	+/−
D-Mannose	+	+	+	+	+	+	+
L-Proline		+	−	+	+	+	+
Hydroxyl-L-proline		+	−	+	+/−	+	+
D-Raffinose		−	+	−	−	−	−
L-Rhamnose	−	−	−	−	−	−	−
D-Saccharic acid		+	+	−	+	+	+
D-Serine		−	−	−	−	−	+/−
D-Sorbitol	−	−	−	−	−	−	−
Sucrose	+	+	+	+	+	+	+
L-Threonine		+	−	−	+	+	+
D-Trehalose	+	+	+	+	+	+	+
Tween 80	+	+	+	+	+	+	+
Uridine		+	−	−	−	−	−

[a]Gram reaction, starch hydrolysis, and catalase production were determined as described in the text. API-20E and Biolog GN Microplate kits were prepared according to the manufacturer's instructions. Tests were selected for comparison of the tested strains with previously reported results or where the 12 tested strains did not react uniformly, thus providing a basis for taxonomic distinction.

[b]Reactions of *X. campestris* as reported in Buchanan and Gibbons (3) and Krieg and Holt (13).

[c]Three pathogen strains were tested for each cultivar for a total of nine pathogen strains.

[d]+ = test substrate was utilized, − = test substrate was not utilized, +/− = one or two of the three strains examined were able to use the test substrate.

rithms supplied by the manufacturer.

Pathogenicity and host range testing. Pathogenicity tests with strains 90-101 and 90-128 were conducted in the greenhouse to demonstrate the causal relationship between the bacteria and symptoms observed in the field. An aqueous inoculum suspension was prepared with 72-hr-old bacterial cultures grown on PDP. Sterile hypodermic needles were used to inject the bacterial suspension (10^6–10^8 cfu/ml) into the stems of test plants of cv. Meyer. Plants injected with SDW and unwounded plants served as controls. After inoculation, all plants were placed in plastic bags or a plastic enclosure for 24 or 48 hr to maintain high relative humidity. Pathogenicity tests were conducted between November and February in a greenhouse maintained at 20–26 C. No supplementary illumination was provided. The degree of symptom development was assessed 19 or 20 days after inoculation. Five pathogenicity tests were conducted with 10–15 plants used in each test for each of the four treatments (inoculation with two bacterial strains and two controls).

Isolations were made from all symptomatic plants by aseptically cutting the stem (at least 2 cm above the inoculation site) and gently squeezing the stem to express several drops of liquid. A sterile transfer loop was used to streak this liquid on the surface of a medium semi-selective for *X. c. campestris* (4). Culture dishes were placed at 28–30 C for 48–72 hr, then representative yellow, mucoid colonies were saved to confirm their identity by colony morphology, the Gram reaction, and starch hydrolysis as previously described.

In order to explore the potential host range of the putative crambe pathogen, a series of greenhouse experiments was conducted with members of the Brassicaceae: *Brassica campestris* L. subsp. *oleifera* (Metzg.) Sinsk. f. *annua* (rapeseed, cvs. Parkland and Tobin), *B. carinata* Braun (cv. 77-1313), *B. hirta* Moench (cv. Mustang), *B. juncea* (L.) Coss. (cv. Lethbridge), and *B. napus* L. subsp. *oleifera* (Metzg.) Sinsk. f. *annua* (rapeseed, cvs. Westar and Profit). Crambe cv. Meyer was included in each test as a positive control. Both strains from crambe tissue, 90-101 and 90-128, were used in tests 1 and 2, while only strain 90-128 was used for tests 3 and 4. Aqueous inoculum suspensions were prepared and plants were inoculated by stem injection as described previously. For tests 1 and 2, plants were placed in plastic bags for 4 days, after which bags were removed and plants were placed in a plastic enclosure for an additional 7 days. Symptoms were noted daily for the ensuing 10 days. For tests 3 and 4, plants were not placed in plastic bags or the plastic enclosure after inoculation by stem injection. In tests 3 and 4, symptoms were evaluated 22 and 26 days after

inoculation, respectively. All tests were conducted between March and July in a greenhouse maintained at 23–28 C. No supplementary illumination was provided. Because of variation in seed viability, the number of plants included in each test differed (Table 2). For all host range tests, isolations were made from all symptomatic plants using the procedure described previously.

Seed assays. For each of five assays, seeds (cv. Meyer provided by K. Larsen, Department of Agronomy, Iowa State University) were individually placed in test tubes containing 5 ml of SDW. Racks of test tubes were placed on an orbital shaker to agitate the seeds in solution. After 18–24 hr, the solution was streaked on the surface of the semiselective medium. After 72 hr of incubation at 28–30 C, plates were examined for the presence of yellow, mucoid colonies. Representative colonies were purified and their identity as *Xanthomonas* was confirmed by the Gram reaction and starch hydrolysis as described above.

Seeds from 12 additional germ plasm sources were tested to determine if the pathogen was seedborne. Three seed assays were conducted as described previously for cv. Meyer. Initially, 30 seeds were individually tested for each of the 12 germ plasm sources. Where the bacterium was detected, representative single colonies were maintained on PDP and identity as *Xanthomonas* was confirmed by the Gram reaction, starch hydrolysis, and the API-20E diagnostic kit as described previously. For those germ plasm sources where seedborne *Xanthomonas* was detected, 30 and 50 seeds were individually assayed in the second and third tests, respectively, to confirm the proportion of infected seeds. Where no evidence of seedborne inoculum was found in the first assay, 100 seeds were tested in each of the second and third tests, with 10 seeds placed in each test tube.

Germ plasm evaluation. In addition to cv. Meyer (the cultivar grown in all observed field plantings), two cultivars (Indy and Prophet) and 10 germ plasm accessions from New Mexico (NM1, NM2, NM41, NM55, NM61, NM65, NM89, NM97, NM98, and NM100, provided by K. Lessman, New Mexico State University) were tested for susceptibility to the putative pathogen in two greenhouse tests. Because the pathogen was found to be seedborne in cv. Meyer, all seeds used in these tests were first treated to reduce seedborne inoculum. Seeds were treated by removal of the silicle, followed by immersion in 70% ethanol for 4 min to remove bacteria on the seed surface.

Two suspect pathogen strains from crambe tissue (90-101 and 90-128) were used in each test, and an aqueous inoculum suspension was prepared. Plants were inoculated by injuring the stem with a sterile needle and then spraying the injured stem with inoculum suspension until runoff. Plants injured and sprayed with SDW served as controls. Immediately after inoculation, each pot was placed in a plastic bag to maintain high relative humidity. After 24 hr, plastic bags were removed and symptom development was assessed 16 or 17 days after inoculation. The experiment was arranged as a randomized complete block with three replications. Because of uneven germination, the cultivars and germ plasm accessions were represented in each treatment by two to 11 plants per replicate. The test was conducted twice during January–April in a greenhouse maintained at 21–26 C. Supplemental illumination was provided to maintain a 12-hr photoperiod.

RESULTS AND DISCUSSION

Crambe cv. Meyer plants growing in research plots in Missouri—at the Agronomy Research Center (ARC), HRC, and SF—were examined weekly during the 1990–1992 growing seasons for disease symptoms. On 5 July 1990, marginal leaf necrosis was observed on crambe growing at HRC. Affected tissues were light tan, dry, necrotic, and often bordered by a halo of chlorotic tissue. As the disease progressed, necrotic lesions expanded to cover as much as 35% of the leaf area. By 19 July 1990, 80% of the crambe plants growing at HRC were affected by the disease. At this time, leaf symptoms were accompanied by stems with black streaks, particularly affecting the vascular tissue. Crambe was planted at SF in late July 1990; the first symptoms of blight were observed on 22 August 1990, and most plants were affected by 11 September 1990.

In 1991, crambe was planted in early April at SF and HRC. The first leaf symptoms of bacterial blight appeared on 28 May (SF) and 13 June (HRC), with dark streaks on stems appearing by 18 June (SF) and 27 June (HRC). By the time the plants were mature, all were showing disease symptoms. In 1992, crambe was planted in late April at SF, HRC, and ARC, and the first disease symptoms were observed on 9 June at ARC and SF.

Pathogen identification. A bacterium forming yellow, mucoid colonies on PDP was consistently recovered from symptomatic crambe tissue. The bacterium was negative for Gram reaction and hydrolyzed starch and was capable of catalase production (Table 1). In addition to the results reported in Table 1, the 12 tested strains (nine pathogens and three type cultures) were all positive for the ONPG test in the API-20E kit. The 12 strains were unable to utilize the remaining substrates included in the API-20E kits: amygdalin, arginine, lysine, mannitol, ornithine, sodium thiosulfate, and urea.

In addition to the results reported in Table 1, the 12 tested strains were able to utilize the following substrates in the Biolog GN Microplate kits: acetic acid, dextrin, L-fucose, *N*-acetyl-D-glucosamine, L-glutamic acid, glycyl-L-glutamic acid, α-ketoglutaric acid, malonic acid, maltose, D-melibiose, propionic acid, psicose, methyl pyruvate, L-serine, succinamic acid, succinic acid, bromosuccinic acid, monomethyl succinate, and Tween 40. The 12 strains were unable to use the remaining substrates in the Biolog GN Microplate kits, which included: *p*-hydroxyphenylacetic acid, *cis*-aconitic acid, D-arabitol, 2,3-butanediol, γ-aminobutyric acid, γ-hydroxybutyric acid, DL-carnitine, phenylethylamine, i-erythritol, 2-aminoethanol, formic acid, D-galactonic acid lactone, D-galacturonic acid, *N*-acetyl-D-galactosamine, glucuronamide, D-gluconic acid, D-glucosaminic acid, glucose-6-phosphate, β-methyl-D-glucoside, D-glucuronic acid, L-pyroglutamic acid, L-histidine, α-lactose, D-mannitol, L-ornithine L-phenylalanine, putrescine, quinic acid, sebacic acid, D-serine, thymidine, turanose, urocanic acid, α-ketovaleric acid, and xylitol.

Table 2. Determination of the host range within the genus *Brassica* of *Xanthomonas campestris* isolated from *Crambe abyssinica*

Host	Cultivar	Genome[a]	Symptom expression[b] Test 1	Test 2	Test 3	Test 4
B. campestris	Parkland	AA (10)	0.71 (14)	0.57 (7)	0.67 (6)	0.88 (8)
subsp. *oleifera*	Tobin		0.63 (16)	0.10 (10)	0.88 (8)	0.83 (6)
B. carinata	77-1313	BBCC (17)	0.17 (12)	0 (6)	0 (8)	0 (4)
B. hirta	Mustang	DD (12)	1.00 (12)	0.55 (11)	1.00 (6)	1.00 (7)
B. juncea	Lethbridge	AABB (18)	0.87 (15)	0.38 (8)	0.75 (8)	0.63 (8)
B. napus	Profit	AACC (19)	0.13 (5)	0 (11)	0 (8)	0.17 (6)
subsp. *oleifera*	Westar		0.07 (14)	0 (12)	0 (8)	0.20 (5)
C. abyssinica	Meyer	(45)	0.85 (13)	0.42 (12)	1.00 (7)	1.00 (7)
Inoculum			10^7–10^8[c]	10^7	10^8	$<10^6$

[a] Letters represent genomic formula of Downey et al (7), with chromosome number in parentheses.
[b] Proportion of symptomatic plants. For tests 1 and 2, each value is combined result of inoculations with crambe strain 90-101 or 90-128; only strain 90-128 was used for tests 3 and 4. Parenthetical values are total number of plants inoculated. Symptoms were not observed on any control plants except for one crambe plant in test 3 and one in test 4.
[c] Colony-forming units per milliliter of the aqueous inoculum suspension.

By characterizing the nine crambe strains using two diagnostic kits (API-20E and Biolog GN Microplates) and comparing the results with the published descriptions of other phytopathogenic bacteria (3,13), the crambe strains were identified as *X. campestris* (Table 1). Nine physiological tests are nominally provided by both kits; the tested strains responded differently to the tests for arabinose, glucose, melibiose, and sucrose utilization.

In order to identify the crambe pathogens to the pathovar level, the reactions of the nine crambe strains were compared with type cultures representing three pathovars: *X. c. armoraciae, X. c. campestris*, and *X. c. vesicatoria*. The 12 strains had the same reactions in 87 of 119 physiological tests conducted, confirming that the crambe strains were indeed *X. campestris*. The identification software supplied by the manufacturer of the Biolog GN Microplates compares the reactions of the "unknown" strain with the reactions of a wide range of gram-negative bacteria, indicating the most probable identity of the "unknown" by a similarity index (SI). The SI ranges from 0 to 1.0, with a value of 0.5 or greater considered sufficient for a positive identification of an unknown strain. The type cultures and crambe strains were each tested with Biolog GN Microplates. *X. c. vesicatoria* was correctly identified in both tests (SI = 0.83 and 0.40). *X. c. armoraciae* was identified correctly once (SI = 0.93) and incorrectly (as *X. c. campestris*) once (SI = 0.61). *X. c. campestris*, however, was misidentified as *X. c. vesicatoria* in both tests (SI = 0.65), suggesting that while the Biolog GN Microplates were reliable for determining species, they were less certain for pathovar determination. Of the nine crambe strains, four were identified as *X. c. campestris* (SI = 0.38–0.74), one was identified as *X. c. armoraciae* (SI = 0.44), two were identified as *X. c.* pv. *fici* (Cavara) Dye (SI = 0.37–0.39), and two were identified as *Xanthomonas* sp. (SI = 0.27–0.47).

Among the 24 physiological tests (Table 1) in which the reactions differed among the three type cultures, the crambe strains had common reactions with *X. c. armoraciae, X. c. campestris*, and *X. c. vesicatoria* as follows: strain 90-101 with 18, 7, and 16 tests, respectively; strain 90-128 with 16, 9, and 14 tests; strain 92-33 with 15, 12, and 13 tests; strain 92-34 with 20, 7, and 16 tests; strain 92-35 with 18, 9, and 16 tests; strain 92-36 with 18, 9, and 14 tests; strain 92-37 with 19, 8, and 15 tests; strain 92-38 with 20, 7, and 16 tests; and strain 92-39 with 18, 9, and 16 tests, respectively. These results suggest that the crambe strains might best be tentatively identified as either *X. c. armoraciae* or *X. c. vesicatoria*.

Gas chromatographic analysis of the cellular fatty acid methyl esters of the crambe strains suggested that these bacteria were closely related to *X. c. armoraciae* and *X. c. campestris*. Affinities of unknown strains with the library of previously characterized bacteria were again indicated by an SI ranging from 0 to 1.0. Fatty acid SI values of 0.6–1.0 are considered excellent matches. For strain 90-101, the SIs for *X. c. armoraciae* and *X. c. campestris* were 0.64–0.68 and 0.60–0.63, respectively, for four replicates. For strain 90-128, the SIs for *X. c. armoraciae* and *X. c. campestris* were 0.64–0.65 and 0.58–0.61, respectively, for eight replicates. These results indicated that the crambe pathogen was closely related to *X. c. armoraciae* and *X. c. campestris*, with the similarity to *X. c. armoraciae* being slightly greater.

In the original description of *X. c. armoraciae* (17) and in a subsequent report (16), observers noted that leaf lesions were bordered by a chlorotic halo, in contrast to leaf lesions caused by *X. c. campestris*. They further noted that *X. c. armoraciae* did not cause a systemic infection, whereas systemic infection is a hallmark of infection by *X. c. campestris* (16,17). The crambe pathogen consistently produced systemic infections and leaf lesions bordered by chlorotic halos, indicating similarities with both *X. c. armoraciae* and *X. c. campestris*.

Vauterin et al (21) have recently described the challenges of delineating the species of *Xanthomonas* and the pathovars of *X. campestris*. They noted that SDS-polyacrylamide gel electrophoretic analysis of cellular proteins revealed *X. c. campestris* to be a homogenous pathovar. Further, analysis of cellular fatty acids and DNA-DNA hybridization both placed pathovars *X. c. armoraciae* and *X. c. campestris* within the same subgroup of *X. campestris*. However, the authors also noted that *X. campestris* strains from the same host were frequently shown by DNA-DNA hybridization analysis to be distantly related. Thus, they cautioned against the use of host selectivity as a primary taxonomic criterion.

Pathogenicity and host range testing. In one pathogenicity test, 22-day-old cv. Meyer plants were inoculated by stem injection with an aqueous suspension of the putative pathogens (10^8 cfu/ml). Within 19 days of inoculation, 22 of 28 plants inoculated with strain 90-101 and 25 of 29 plants inoculated with strain 90-128 showed typical disease symptoms. These included black discoloration of vascular tissue above the inoculation site accompanied by acropetal development of typical foliar lesions, both of which suggested systemic movement of the pathogen through the plant. The successful reisolation of the pathogen from symptomatic plants completed Koch's postulates. Typical disease symptoms developed in three of 30 plants injected with SDW, whereas no symptoms were observed on unwounded plants. In the five additional pathogenicity tests, 30–100% of inoculated crambe plants developed typical field symptoms within 7–20 days after inoculation. Further, in four of five tests, at least one uninoculated plant developed typical disease symptoms. This observation strongly suggested the possibility of seedborne inoculum, and this was explored in some detail, as described below.

A series of greenhouse experiments was conducted to examine the susceptibility of several *Brassica* spp. to the *X. campestris* strains from crambe (Table 2). The genomes of *Brassica* spp. have been well characterized and the relationships among the species are denoted using the genomic formulas of Downey et al (7). From Table 2 it is apparent that *B. carinata* and *B. n. oleifera* had a much lower level of symptom development than the other species included in the tests. These two species share the "CC" portion of their genomes (7). These data also illustrate the susceptibility of the remaining *Brassica* spp. to the crambe pathogen. For all species, symptom development included a combination of leaf necrosis and black streaks on stems, as described above for crambe. These results together with the evidence that the pathogen is seedborne in crambe (described subsequently) suggest that if crambe is planted in proximity to other *Brassica* spp., the potential for disease spread between the crops is substantial. Further, these results suggest that the "CC" portion of the *Brassica* genome offers the potential for development of resistant cultivars.

The type cultures—*X. c. armoraciae, X. c. campestris*, and *X. c. vesicatoria*—were included in host range studies but were only weakly pathogenic. Thus, it was not possible to use evidence from greenhouse host range tests to identify the crambe strains as *X. c. armoraciae* or *X. c. campestris*.

Seed testing. During the course of completing Koch's postulates, uninoculated control plants occasionally developed typical bacterial blight symptoms, suggesting the possibility of seedborne inoculum. When intact seeds (silicle present) were assayed, *X. campestris* was recovered from 50–67% of the seeds assayed (Table 3). When the silicle was removed, the pathogen was recovered from 15–18% of the seeds tested; surface-disinfestation treatments reduced the level of contamination to 10% or less (Table 3, protocol 1). Even with the aseptic removal of the seed coat and surface-disinfestation treatment, it was still possible to detect the pathogen in crambe seeds (Table 3, protocol 2). These results demonstrated that the bacterium was seedborne and most often located on the silicle or surface of the seed coat. However, the failure of the surface-

Table 3. Assays to determine the presence of *Xanthomonas campestris* within or on the surface of crambe seed

Silicle[a]	Seed coat[b]	Disinfestation treatment[c]	Protocol 1[d]		Protocol 2[e]		
			Test 1	Test 2	Test 1	Test 2	Test 3
+	+	None	21	20	20	19	20
−	+	None	6	7	5	3	2
−	+	NaOCl, 1 min	4	2			
−	+	NaOCl, 3 min	1	1			
−	+	NaOCl, 5 min	1	2			
−	+	Ethanol, 1 min	1	0			
−	+	Ethanol, 3 min	4	0			
−	+	Ethanol, 5 min	3	2	1	2	1
−	+	Ethanol, 10 min	0	1			
−	−	Ethanol, 5 min			0	1	1

[a]+ = Present and − = removed aseptically.
[b]+ = Present and − = absent; seed coats were aseptically removed after seeds were soaked for 3 hr in sterile distilled water.
[c]Seeds were treated with 0.525% NaOCl or 70% ethanol for 1–10 min, then rinsed in sterile distilled water to remove traces of disinfestant.
[d]Number of seeds of 40 assayed from which *Xanthomonas* was recovered and identity confirmed by colony morphology, Gram reaction, and starch utilization.
[e]Number of seeds of 30 assayed from which *Xanthomonas* was recovered and identity confirmed by colony morphology, Gram reaction, and starch utilization.

Table 4. Susceptibility of selected crambe gram plasm to *Xanthomonas campestris* and results of seed assays for presence of the pathogen

Germ plasm	Proportion with symptoms[a]				Proportion with infested seed[b]
	Test 1		Test 2		
	90-101	90-128	90-101	90-128	
Indy	1.00	0.81	0.72	0.00	0.00 (230)
Prophet	0.72	0.80	0.84	0.40	0.00 (230)
NM1	0.96	0.80	0.74	0.18	0.08 (110)
NM2	0.90	0.84	0.63	0.34	0.00 (230)
NM41	0.96	0.95	0.65	0.46	0.00 (230)
NM55	0.88	0.96	0.60	0.32	0.06 (110)
NM61	0.96	0.75	0.72	0.43	0.07 (110)
NM65	0.93	0.82	0.84	0.33	0.00 (230)
NM89	0.90	0.84	0.61	0.23	0.00 (230)
NM97	0.92	0.82	0.70	0.14	0.00 (230)
NM98	1.00	0.93	0.71	0.10	0.00 (230)
NM100	0.88	0.85	0.65	0.33	0.04 (110)
Meyer	0.89	0.69	0.73	0.43	Not included

[a]Average of three replicates for inoculations with pathogen strain 90-101 or 90-128. For strain 90-101, inoculum concentration was 1.8×10^7 for test 1 and 3.8×10^5 for test 2; for strain 90-128, inoculum concentration was 1.0×10^7 for test 1 and 1.2×10^4 for test 2. Within each test and inoculum source, means were not statistically different ($P = 0.05$) as determined by analysis of variance using data modified by the arcsine transformation.
[b]Average of three tests, with total number of seeds tested in parentheses.

disinfestation treatments demonstrated that the bacterium may be carried systemically within the seeds.

Germ plasm evaluation. Three cultivars and 10 germ plasm accessions were evaluated in two greenhouse experiments for their susceptibility to two pathogen strains, 90-101 and 90-128. No statistical differences in susceptibility were detected among the 13 germ plasm sources in either test (Table 4), with symptom expression ranging from 69 to 100% in test 1. In test 2, symptoms were observed in 61–84% and 0–46% of plants inoculated with strains 90-101 and 90-128, respectively (Table 4). The initial inoculum concentrations were 3.8×10^5 cfu/ml and 1.2×10^4 cfu/ml for strains 90-101 and 90-128, respectively, which may account for the lower level of symptom development for plants inoculated with 90-128 (Table 4). The development of typical foliar symptoms on leaves above the inoculation site again demonstrated that the pathogen moved systemically within infected plants. The pathogen was detected in 4–8% of the seeds of germ plasm accessions NM1, NM55, NM61, and NM100 (Table 4), as compared with a level of 50–67% for cv. Meyer (Table 3).

Bacterial blight of crambe has been observed to affect virtually all plants grown in experimental fields between 1990 and 1992. On the basis of physiological tests and analysis of cellular fatty acids, the bacterium most closely resembled *X. c. armoraciae*. However, the crambe pathogen was able to move systemically through infected plants, which was more characteristic of *X. c. campestris* than of *X. c. armoraciae* (16,17). Because the bacterium was externally, and possibly internally, seedborne, production of pathogen-free seed should be an integral component of crambe commercialization. Subsequent research should focus on the impact of the disease on seed yield and oil quality.

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