# An Outbreak of Bacterial Spot of Lettuce in Florida Caused by *Xanthomonas campestris* pv. *vitians*

KEN PERNEZNY and RICHARD N. RAID, University of Florida, Everglades Research and Education Center, Belle Glade 33430; ROBERT E. STALL and N. C. HODGE, University of Florida, Department of Plant Pathology, Gainesville 32611; and JANICE COLLINS, University of Florida, EREC, Belle Glade

**ABSTRACT**

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A widespread and damaging outbreak of a leaf spot disease of lettuce occurred in the 1992–93 winter vegetable season in southern Florida. Individual leaf lesions were dark brown to black, water-soaked, and greasy in appearance. A yellow-pigmented bacterium was consistently isolated. All 1992–93 lettuce strains and reference strains produced symptoms in greenhouse test plants that were identical to those seen in the field. Disease reactions generally were more severe in cos and butterhead lettuce than in crisphead. Strains were gram-negative rods, and negative for glucose fermentation, nitrate reduction, urease production, and utilization of asparagine as a sole source of carbon and nitrogen. Aesculin was hydrolyzed, gelatin was liquefied, and proteolysis occurred in litmus milk. Cellular fatty acid profiles matched well to library database strains of *Xanthomonas campestris* pv. *vitians*.

Lettuce (*Lactuca sativa* L.) is an important winter vegetable crop in southern Florida. In a recent year, the crop was valued at over $22 million (5). Production is centered on the organic soils of the Everglades Agricultural Area (EAA) south and southeast of Lake Okeechobee.

Downy mildew, caused by *Bremia lactucae* Regel, has been the major limiting foliar disease of lettuce in the EAA since about 1980 (13), causing significant losses in most winter production seasons. Few other foliar diseases have been observed. However, in the winter of 1992–93, a widespread and damaging outbreak of a leaf spot disease, with symptoms distinct from those of downy mildew, was observed throughout the EAA. All major types of lettuce were affected (crisphead, butterhead, leaf), but the disease was especially severe on cos (romaine) lettuce. Lesions on lettuce leaf blades were dark brown to black and greasy. Often there was abundant water-soaking on the abaxial surface of young lesions. Profuse bacterial streaming was evident when sections of diseased tissue were examined microscopically. These studies were undertaken to isolate and identify the organism causing leaf spot of lettuce in Florida and to compare virulence of strains on different lettuce types.

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## MATERIALS AND METHODS

**Isolation.** Leaf portions from affected lettuce grown in commercial fields in February and March 1993 were surface-sterilized for 15 s in a 0.5% aqueous solution of sodium hypochlorite and rinsed in sterile water. A flamed and cooled inoculation needle was pushed through the leaf tissue at the margin of lesions, and the wire was streaked across plates of medium B of King et al (KMB) (7) that was amended with cycloheximide at 50 μg/ml. Plates were incubated at 28 C for 3 days. Colonies characteristic of *Xanthomonas* were purified by re-streaking on yeast extract-dextrose-calcium carbonate (YDC) agar (4). Stock cultures were maintained in sterile 15% aqueous glycerol at −70 C (16); working cultures were kept up to 1 mo on YDC slants at 4 C.

Three strains each from crisphead and cos lettuce and one strain from butterhead were chosen for further study. Three *Xanthomonas campestris* (Pammel) Dowson pv. *vitians* reference strains were provided by A. Alvarez, University of Hawaii.

**Pathogen identification.** All strains were gram-stained (9) and observed for colony characteristics and pigmentation on KMB and YDC agar. Other physiological and biochemical tests were oxidative and fermentative metabolism of glucose, gelatin liquefaction, nitrate reduction, urease production, and aesculin hydrolysis using petri plate cultures solidified with 15 g of agar per liter (15). Proteolysis was determined in tubes of reconstituted Bacto litmus milk according to the manufacturer's directions. Use of asparagine as a sole source of carbon and nitrogen was determined according to the method of Dye (4).

Dye's basal medium C (4) was used to test for oxidative production of acid from various organic compounds. Filter-sterilized solutions were added to the autoclaved basal medium for a final concentration of 0.5% (w/v). The following substrates were tested: glucose, fructose, arabinose, cellobiose, galactose, mannose, rhamnose, trehalose, dulcitol, inositol, and sorbitol.

Cellular fatty acid profiles were determined for each strain, using the MIDI Microbial Identification System (14,17). Statistical matches of the strains were made to well-characterized reference strains in the MIDI library database TSBA 3-70.

**Pathogenicity.** Lettuce seeds were planted in 4-cm-diameter plastic pots in a commercial potting mix. Bacterial cultures were grown for 3 days on YDC agar plates at 28 C. Plates were flooded with sterile buffered saline solution (8), and a bent glass rod was used to suspend bacteria. Suspensions were adjusted turbidimetrically to about $2 \times 10^7$ cfu/ml. Six-week-old plants were sprayed to run-off on both adaxial and abaxial surfaces using a hand-held pump sprayer between 0900 and 1130 hr (when stomatal conductance is expected to be maximized [11]). Each strain was tested on two plants each of three lettuce types: crisphead cv. South Bay; cos cv. Tall Guzmaine; and butterhead, breeding line B-1190. Two plants of each lettuce type were sprayed with sterile buffered saline and served as controls. Plants were immediately covered with clear plastic bags and placed in an air-conditioned greenhouse with a temperature range of 23–27 C. After 4 days, bags were removed. Strains were scored for pathogenicity 2 days later using a 0–3 scale, where 0 = no disease and 3 = extensive leaf spot development with coalescence to form large blighted areas on leaves.

**Sensitivity to streptomycin and copper.** Sensitivity of Florida strains to streptomycin was tested by comparing growth on glucose-nutrient agar with and without an amendment of streptomycin sulfate at 200 μg/ml (Sigma Chemical, St. Louis, MO). Copper sensitivity was assessed by comparing growth on a minimal copper-complexing medium (2) with and without 0.64 mM $CuSO_4$ (12).

## RESULTS

Nearly all the commercial lettuce fields in southern Florida, which are concen-

**Table 1.** Results of pathogenicity tests for strains of *Xanthomonas campestris* pv. *vitians* (L1–L7) from leaf spots of lettuce during the epidemic of 1993 in the Everglades Agricultural Area (EAA) of southern Florida

| Strain | Source | Greenhouse test reactions[a] | | |
|---|---|---|---|---|
| | | Crisphead | Romaine | Boston |
| L1 | Cos | 1.0 | 3.0 | 2.5 |
| L2 | Cos | 2.5 | 3.0 | 3.0 |
| L3 | Cos | 2.0 | 1.5 | 3.0 |
| L4 | Crisphead | 2.0 | 3.0 | 3.0 |
| L5 | Crisphead | 3.0 | 2.5 | 3.0 |
| L6 | Crisphead | 1.5 | 2.0 | 3.0 |
| L7 | Butterhead | 1.5 | 2.0 | 3.0 |
| 55-2[b] | ... | 2.5 | 3.0 | 2.5 |
| QR71[b] | ... | 1.5 | 2.5 | 2.0 |
| GAC 167A[b] | ... | 2.0 | 3.0 | 2.0 |
| $\bar{X}$ | | 1.95 | 2.65 | 2.65 |

[a]Based on spray inoculation of $10^7$ cfu/ml suspension on 6-wk-old plants. Disease reaction based on a 0–3 scale, where 0 = no disease and 3 = severe leaf spotting. Data are averages of two replications/strain/lettuce type.
[b]*X. c. vitians* reference strains, provided by A. Alvarez.

trated around Lake Okeechobee, had some plants with bacterial leaf spot when surveyed in the spring of 1993. Disease incidence ranged from 5 to about 25%. In a few instances, entire fields were abandoned because of the high levels of infection and associated poor quality of the lettuce. Monetary losses to the industry exceeded $4 million. All bacterial strains isolated from leaf spots of lettuce fit the physiological and biochemical profile of *Xanthomonas campestris*. Strains were gram-negative rods and did not fluoresce on KMB. They were negative for fermentation of glucose, nitrate reduction, urease production, and utilization of asparagine as a sole source of carbon and nitrogen. The aesculin hydrolysis and gelatin liquefaction tests were positive, and marked proteolysis was noted in litmus milk. Acid was produced from glucose, fructose, galactose, mannose, and trehalose, but not from rhamnose, dulcitol, inositol, or sorbitol.

The first choice fatty acid-profile match for all seven Florida strains was to the *X. c. vitians* entry in the TSBA library. Similarity indices (SI) ranged from 0.583 to 0.790. Two of the reference strains matched to *X. c. vesicatoria* (SI = 0.624 and 0.787), and one matched to *X. c. malvacearum* (SI = 0.706).

All strains produced leaf spot symptoms identical to those seen in the field in all three types of lettuce tested (Table 1). Disease reactions were generally more severe on cos and butterhead lettuce than on crisphead. The pathogen was readily reisolated from lesions of test plants. No symptoms were observed on control plants.

All strains were very sensitive to both streptomycin and copper in vitro. Growth was inhibited completely by 0.64 mM copper sulfate.

## DISCUSSION

*X. c. vitians* was responsible for a severe epidemic of a leaf spot of lettuce in the EAA in the winter of 1993. This bacterium has been reported to be one of several important bacterial leaf spot pathogens of crisphead lettuce in New York State (3). *X. c. vitians* has been reported as the cause of leaf spots of several foliage ornamentals in Florida (1). However, this is the first report of the pathogen attacking the important lettuce industry in the state.

*Pseudomonas cichorii* (Swingle) Stapp also has caused losses in lettuce crops in New York (3) and has been shown to be a destructive lettuce pathogen in some years in California (6). *P. cichorii* is the most important bacterial pathogen of celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.) in the EAA (12,18), causing damage yearly in the seedbed. In the winter of 1993, it was also found to be the incitant of a widespread necrosis of celery petioles (10). We assumed that *P. cichorii* was most likely the causal agent of the concurrent epidemic of bacterial leaf spot in the lettuce fields. Indeed, initially we chose KMB for our isolation medium in anticipation of recovery of a fluorescent pseudomonad. Apparently, there was no relation between the epidemics on celery and lettuce other than that both were favored by the unusually wet and warm conditions in the 1993 season.

The Florida and reference strains produced identical leaf spot symptoms in three types of lettuce. Symptoms in plants inoculated with the reference strains were generally less severe than symptoms in those inoculated with the Florida strains. However, this may simply have been the result of the extended time the reference strains were maintained in storage. We inoculated the strains with profiles matched to *X. c. vesicatoria* as well as a few Florida lettuce strains to pepper and compared the reactions to a race one *X. c. vesicatoria* strain isolated from pepper in southern Florida. Although the lettuce strains produced many fewer lesions than the homologous *X. c. vesicatoria* strain, the lesions that were formed were typical of bacterial spot on pepper (K. Pernezny and J. Collins, *unpublished*). We are currently investigating the effect of repeated passage through pepper of these lettuce strains on their virulence in pepper.


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