Role of Crop Debris and Weeds in the Epidemiology of Bacterial Leaf Spot of Lettuce in California

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ABSTRACT

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Bacterial leaf spot of lettuce (BLS), caused by *Xanthomonas campestris* pv. *vitians*, has increased in importance in California over the past 5 years. The pathogen can be seedborne, but it was not recovered from selected commercial lettuce seed lots planted during this time. Survival of *X. campestris* pv. *vitians* in association with plant debris was investigated in a 3-year field experiment in Salinas, CA. The initial lettuce spring crop was spray inoculated with *X. campestris* pv. *vitians*, which resulted in 100% disease incidence. Spring crops were followed by a 1-month summer fallow period, whereas fall crops were followed by a 5-month winter fallow period. High populations of *X. campestris* pv. *vitians* (up to 10^6 CFU/g) were recovered from lettuce plant debris after the 1-month summer fallow and BLS developed on all subsequent fall lettuce crops. During the winter fallow period, *X. campestris* pv. *vitians* populations associated with plant debris declined and, by 2 months after harvest, only small populations were detected. Spring crops also developed BLS, but at reduced levels. *X. campestris* pv. *vitians* was recovered from leaves of several symptomless weed species collected around commercial infested fields, but not from weeds collected around previously infested fields during fallow periods. During the course of this study, an *X. campestris* pv. *vitians*-specific polymerase chain reaction primer pair was developed.

Bacterial leaf spot (BLS) of lettuce, *Lactuca sativa* L., is caused by *Xanthomonas campestris* pv. *vitians* (Brown) Dye. BLS was first reported in 1918 on head lettuce plants in New York (4). It was subsequently reported from Illinois (30), New York (5), California (28), Florida (22), Hawaii (18), and Ohio (25). Worldwide, BLS has been reported from Australia (11), South Africa (33), New Zealand (2), Japan (21,31), Italy (36), Venezuela (7), Brazil (29), Canada (34), and Turkey (24).

The symptoms of BLS are black, water-soaked, angular lesions that are most commonly observed on mature, fully expanded leaves (16). Disease development is highly dependent on environmental conditions, with the greatest levels of disease and crop damage occurring under cool, humid conditions together with rainfall or sprinkler irrigation. Damage to lettuce crops from BLS includes reduced quality as well as increased postharvest losses.

Beginning in 1991, BLS outbreaks have occurred with increasing frequency in

commercial fields throughout San Benito, Santa Cruz, Monterey, San Luis Obispo, Santa Barbara, and Ventura counties (coastal regions) and Fresno and Imperial counties (inland areas) of California. These recurrent outbreaks suggest that BLS has become established in California. *X. campestris* pv. *vitians* can be seedborne, and may survive in association with seed for extended periods (25,32). However, *X. campestris* pv. *vitians* was not recovered from seed lots used to establish commercial fields in California (1992-96), including fields that later developed BLS (32). Thus, it may be that another inoculum source or sources play a role in BLS development in California.

In April 1997, a multiple-year field experiment was initiated in Salinas, CA, to examine (i) if and for how long *X. campestris* pv. *vitians* could survive in association with crop debris, (ii) whether tillage method influenced survival of *X. campestris* pv. *vitians* in plant debris, and (iii) whether this debris could serve as an inoculum source for BLS outbreaks. In addition, surveys were conducted to assess whether *X. campestris* pv. *vitians* could survive in association with weeds. A preliminary report has been published (1).

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. Strains were either isolated from lettuce plants with BLS symptoms; recovered from let-

tuce seed, symptomless lettuce plants, or weeds growing near lettuce fields; or were provided by other investigators. All strains were stored at -80°C in a yeast tryptone broth-80% glycerol solution and were streaked onto 523 medium (13) prior to characterization.

Pathogenicity tests. Pathogenicity tests were conducted throughout these studies to confirm the identity of putative *X. campestris* pv. *vitians* strains. Bacterial cells from a single colony of the strain to be tested were inoculated into 3 ml of yeast tryptone broth in 12-by-75-mm polystyrene culture tubes (Fisher Scientific, Pittsburgh, PA). The tubes were placed on an orbital shaker (200 rpm) and incubated at 28°C for 30 h. Carborundum was added to these cultures (approximately 10^8 CFU/ml, based upon an optical density of 0.4 at 600 nm) and the suspensions were rub inoculated with cotton swabs onto the upper (adaxial) surface of medium-sized leaves of 3-week-old lettuce plants. Leaves of negative control plants were similarly inoculated with sterile liquid media and Carborundum. A known *X. campestris* pv. *vitians* strain (Sal) was used as a positive control. Plants were maintained in a greenhouse at approximately 28 to 30°C . Inoculated plants were examined 7 to 10 days post inoculation for disease symptoms.

Polymerase chain reaction detection of *X. campestris* pv. *vitians*. To identify whether representative xanthomonad-like colonies were *X. campestris* pv. *vitians*, polymerase chain reaction (PCR) was performed with the *X. campestris* pv. *vitians*-specific primer pair, 9308B (5' TGC TCA GCA ATG ATG CGT 3' and 5' CGA GTT GCG CTT GAG TTA 3'; 14), which directs the amplification of an approximately 450-bp DNA fragment. The PCR was carried out in a 50- μl reaction volume and, unless stated otherwise, values for reagents correspond to final concentrations. The 50- μl PCR reaction included 2.5 mM MgCl_2 , deoxynucleotide triphosphate (dNTP) mixture (concentration of each dNTP was 1 mM), 2.3 ng μl^{-1} of each oligonucleotide primer, and 1.0 unit of Amplitaq DNA polymerase in $1\times$ Amplitaq PCR buffer II (PE Applied Biosystems, Foster City, CA). The PCR template was a bacterial cell extract prepared by placing a loopful of bacterial cells from the colony to be tested into 1 ml of sterile water in a 1.5-ml Eppendorf tube. The tubes were boiled for 10

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min, and 2 µl of this extract was added to the PCR reaction. PCR was carried out in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus, Emeryville, CA). The amplification profile was 35 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min. Amplified DNA fragments were examined by gel electrophoresis in 1.0% agarose gels in 0.5× Tris-borate EDTA (TBE) buffer. Gels were stained with ethidium bromide and DNA was visualized with a gel imaging system (Alpha Innotech Corporation, San Leandro, CA).

Establishment of the field plot and recovery of *X. campestris* pv. *vitians* from lettuce leaves and debris. During the field experiment, lettuce (cv. Alpha) was grown according to standard commercial practices. The lettuce seed lot used to establish the field plots was assayed for *X. campestris* pv. *vitians* prior to planting by washing 15 g of seed in 100 ml of chilled 0.85% NaCl buffer (hereafter referred to as NaCl buffer) plus 30 µl of sterile Tween 20 in a 250-ml Erlenmeyer flask. The flask was shaken at 200 rpm for 3 h at room temperature. The seed suspension was filtered through cheesecloth and the supernatant was centrifuged at 10,000 × g for 10 min. The resulting pellet was suspended in 10 ml of NaCl buffer, serial dilutions prepared, and 100-µl aliquots (2 plates/dilution) were plated onto XCS medium (35) adjusted to pH 6.6 with 5 N NaOH. Plates were incubated at 28°C for 4 days and examined for *X. campestris* pv. *vitians*-like colonies (yellow, slimy, and mucoid).

The field plot consisted of four tillage treatments each replicated three times in a randomized complete block design. Plots were 4.3 by 20 m and consisted of six beds each. To initiate BLS in the field plot, lettuce plants (5- to 7-leaf stage) in all treatment plots were spray inoculated with *X. campestris* pv. *vitians* (strain Sal) in June 1997. Inoculum was prepared by suspending 48-h-old cultures of *X. campestris* pv. *vitians*, grown on petri plates containing 523 medium, in sterile distilled water. The suspension was adjusted to a final concentration of 10⁸ CFU/ml. Inoculum was applied using backpack sprayers (R&D Sprayers, Opelousas, LA). The plots were sprinkler irrigated throughout the experiment according to standard commercial practices.

When lettuce plants were ready for harvest, the following harvest and tillage treatments were used: (i) lettuce was harvested (lettuce heads were removed from the field, leaving the lower 4 to 6 wrapper leaves per plant) and crop debris was incorporated with conventional tillage (debris incorporated approximately 15 cm deep with a disk); (ii) lettuce was not harvested (lettuce heads were left intact) and plants were incorporated with conventional tillage; (iii) lettuce was harvested and crop debris incorporated with minimum tillage (debris incorporated approximately 5 cm

deep with a rototiller); and (iv) lettuce was not harvested and plants were incorporated with minimum tillage. Tillage treatments were performed within 2 days after harvest.

Spring crops were planted in March or April and were harvested in June or July. Each spring crop was followed by a 1-month summer fallow period. Fall crops were planted in August and were harvested in October. Each fall crop was followed by a 5-month winter fallow period, except for the final fall crop, which was followed by a 7-month fallow period. For all crops, BLS incidence (i.e., percent of plants showing symptoms) was recorded for all plants in all treatment plots 1 day before harvest.

Recovery of *X. campestris* pv. *vitians* from lettuce leaves and debris. For each

lettuce crop, *X. campestris* pv. *vitians* populations on leaves were monitored at monthly intervals with a sonication-wash assay. Approximately 20 leaves were collected from individual plants within each plot. When plants were young (rosette stage), entire leaves were washed. For older plants, four 2.5-cm-diameter discs were arbitrarily removed from each mature leaf with a sterile cork borer. During the fallow periods, *X. campestris* pv. *vitians* populations associated with crop debris was monitored at monthly intervals by collecting debris from the soil surface and assaying 12 to 15 g of debris with the sonication-wash method. Leaf tissue or debris from each plot was placed into a 500-ml flask containing 200 ml of chilled NaCl buffer and flasks were sonicated for 20 min in an ultrasonic bath (Fisher Scientific).

Table 1. Strains of *Xanthomonas campestris* pv. *vitians* used in this study

Strain	Year isolated	Origin	Host	PCR analysis ^a	
				9308B	B162
30526	1978	Rydalmere, NSW, Australia	<i>Lactuca sativa</i>	—	+
30547	1978	Pimlico, NSW, Australia	<i>L. sativa</i>	—	+
31975	1978	Dareton, NSW, Australia	<i>L. sativa</i>	—	+
99-01	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-02	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-03	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-04	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-05	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-06	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-07	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-08	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-09	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-10	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-11	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-12	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-13	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-14	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-15	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-16	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-17	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-18	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-19	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-20	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-21	1979	Kula, HI	<i>L. sativa</i>	nt	+
99-22	1979	Kula, HI	<i>L. sativa</i>	nt	+
41335	1983	Australia	<i>L. sativa</i>	—	+
93-01	1993	Gonzales, CA	<i>L. sativa</i>	+	+
93-02	1993	Salinas, CA	<i>L. sativa</i>	+	+
93-03	1993	Gonzales, CA	<i>L. sativa</i>	+	+
93-04	1993	Chualar, CA	<i>L. sativa</i>	+	+
93-05	1993	Guadalupe, CA	<i>L. sativa</i>	+	+
93-06	1993	Salinas, CA	<i>L. sativa</i>	+	+
93-07	1993	Salinas, CA	<i>L. sativa</i>	+	+
93-08	1993	Gonzales, CA	<i>L. sativa</i>	+	+
93-09	1993	Salinas, CA	<i>L. sativa</i>	+	+
93-10	1993	Salinas, CA	<i>L. sativa</i>	+	+
94-01	1994	Salinas, CA	<i>L. sativa</i>	+	+
H+	1995	Unknown	seed	+	+
Pyb251	1995	Monterey, CA	<i>L. sativa</i>	+	+
KC	1995	King City, CA	<i>L. sativa</i>	+	+
GON	1995	Gonzales, CA	<i>L. sativa</i>	+	+
B162	1995	Guadalupe, CA	<i>L. sativa</i>	—	+
LL2	1995	Bakersfield, CA	<i>L. sativa</i>	—	+
Sal	1995	Salinas, CA	<i>L. sativa</i>	+	+
95-02	1995	Watsonville, CA	<i>L. sativa</i>	+	+
Fl-1	1995	Florida	<i>L. sativa</i>	+	+
98-62	1998	Aromas, CA	<i>Senecio vulgaris</i>	—	+

(continued on next page)

^a Polymerase chain reaction = PCR; nt = strains not tested with 9308B primer pair.

The tissue-buffer suspension was filtered through two layers of sterile cheesecloth into a 250-ml polypropylene centrifuge bottle, and bottles were centrifuged for 10 min at $10,000 \times g$. The resulting pellet was suspended in 10 ml of NaCl buffer, serial dilutions (10^0 to 10^{-4}) of this suspension were prepared, and 100- μ l aliquots (2 plates/dilution) were plated onto XCS medium. Plates were incubated at 28°C for 4 days, *X. campestris* pv. *vitians* colonies were counted, and populations were calculated based on counts from plates having 30 to 300 colonies (whenever possible).

Analysis of variance (ANOVA) was performed on *X. campestris* pv. *vitians* leaf and debris populations and disease incidence data. For the *X. campestris* pv. *vitians* population data, log (x + 1) transformations were performed and values of 0 were used when no *X. campestris* pv. *vi-*

tians colonies were detected. Means were separated using the least significant difference method.

Detection of *X. campestris* pv. *vitians* with dilution plating-PCR and colony blot hybridization. During the 1997 winter fallow period, a dilution plating-PCR method was used to detect *X. campestris* pv. *vitians* colonies recovered from debris in the presence of high populations of other bacteria. In this procedure, bacterial cells were scraped from a petri plate and a suspension of bacterial cells was prepared in 1 ml of sterile water in a 1.5-ml Eppendorf tube. The tubes were boiled for 10 min, and 2 μ l of the resulting extract was used as PCR template with the 9308B primer pair.

During the 1998 winter fallow period, a colony blot hybridization method (26) was used to detect *X. campestris* pv. *vitians*

colonies recovered from debris. A nitrocellulose membrane (80 mm in diameter; Magna, Osmonica, MA) was placed over the bacterial colonies (usually a lawn of bacterial growth) growing on an XCS plate. A negative control consisted of plates of bacteria recovered from debris of a lettuce crop that did not have BLS. The membranes were lifted from the plates and placed, colony side up, on 3MM Whatman filter paper saturated with 10% sodium dodecyl sulfate (SDS) for 3 min. Next, membranes were placed on 3MM paper saturated with denaturing solution (0.5 N NaOH, 1.5 M NaCl) for 5 min, and then on 3MM paper saturated with neutralizing solution (1.5 M NaCl, 0.5 M Tris-Cl, pH 7.4) for 5 min. Membranes were then transferred to 3MM paper saturated with 2 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 5 min, and then allowed to dry on a sheet of 3MM paper for at least 30 min. DNA was fixed on membranes by baking in an oven at 80°C for 15 min, and crosslinked in a UV crosslinker (1,200 $\times$ 100 μ j/cm² for 1.5 min) (FB-UVXL-1000, Fisher Scientific). Membranes were hybridized with a ³²P-labeled probe in modified Church's buffer (0.36 M Na₂HPO₄, 0.14 M NaH₂PO₄, 1 mM EDTA, and 7% SDS). Hybridization was conducted overnight at 65°C in a hybridization oven (model HB-1D; Techne, Princeton, NJ). The membranes were washed once in 2 $\times$ SSC and 0.5% SDS at 65°C for 15 min, and once in 0.5 $\times$ SSC and 0.1% SDS at 65°C for 15 min. Membranes were exposed to X-ray film (Kodak, Rochester, NY) for 24 and 48 h at -80°C.

The probe used to detect *X. campestris* pv. *vitians* colonies was a cloned 450-bp random amplified polymorphic DNA (RAPD) fragment that was amplified with Operon primer AB9 (Operon, Inc., Alameda, CA). This fragment was excised from a 1% agarose gel, recovered with the QIAquick PCR purification kit (Qiagen, Valencia, CA) and cloned with the TA cloning system (Invitrogen, Carlsbad, CA). The recombinant plasmid 8B, which contains the 450-bp *X. campestris* pv. *vitians* RAPD fragment, was used as the hybridization probe. Plasmid DNA was purified from *Escherichia coli* with the QIAprep spin miniprep kit (Qiagen), and 500 ng of DNA were labeled with [α -³²P] dCTP by nick translation according to manufacturers' recommendations (Life Technologies, Grand Island, NY).

Weed survey. In April 1998, the most common weed species around commercial lettuce fields with BLS outbreaks were sampled in Aromas (fields 1 and 2), Santa Maria Valley (fields 3 and 4), and Lompoc Valley (fields 5 and 6). In January 1999, weeds were collected around fields in the Santa Maria (fields 7 and 8) and Lompoc Valleys (fields 9 and 10) at least 2 months after harvest of lettuce crops that had BLS. In November 1999, weeds were collected

Table 1. (continued from preceding page)

Strain	Year isolated	Origin	Host	PCR analysis ^a	
				9308B	B162
98-63	1998	Aromas, CA	<i>Epilobium brachycarpum</i>	+	+
98-64	1998	Aromas, CA	<i>L. sativa</i>	-	+
98-65	1998	Aromas, CA	<i>Convolvulus arvensis</i>	+	+
98-66	1998	Aromas, CA	<i>E. brachycarpum</i>	-	+
98-17	1998	Santa Maria, CA	<i>Sonchus oleraceus</i>	-	+
98-18	1998	Santa Maria, CA	<i>Capsella bursa-pastoris</i>	-	+
98-24	1998	Santa Maria, CA	<i>S. oleraceus</i>	-	+
98-26	1998	Santa Maria, CA	<i>S. vulgaris</i>	-	+
98-29	1998	Santa Maria, CA	<i>S. vulgaris</i>	-	+
98-58	1998	Santa Maria, CA	<i>Chamomilla suaveolens</i>	-	+
98-59	1998	Santa Maria, CA	<i>C. suaveolens</i>	-	+
98-13	1998	Santa Maria, CA	<i>S. vulgaris</i>	-	+
98-14	1998	Santa Maria, CA	<i>S. vulgaris</i>	-	+
98-15	1998	Santa Maria, CA	<i>Malva parviflora</i>	-	+
98-20	1998	Santa Maria, CA	<i>Polygonum arenastrum</i>	-	+
98-30	1998	Santa Maria, CA	<i>Chenopodium murale</i>	-	+
98-16	1998	Lompoc, CA	<i>C. bursa-pastoris</i>	-	+
98-19	1998	Lompoc, CA	<i>M. parviflora</i>	-	+
98-21	1998	Lompoc, CA	<i>C. bursa-pastoris</i>	-	+
98-22	1998	Lompoc, CA	<i>P. arenastrum</i>	-	+
98-23	1998	Lompoc, CA	<i>P. arenastrum</i>	-	+
98-28	1998	Lompoc, CA	<i>Lupinus formosus</i>	-	+
98-31	1998	Lompoc, CA	<i>L. formosus</i>	-	+
98-33	1998	Lompoc, CA	<i>C. murale</i>	-	+
98-53	1998	Lompoc, CA	<i>M. parviflora</i>	+	+
98-54	1998	Lompoc, CA	<i>C. arvensis</i>	+	+
98-55	1998	Lompoc, CA	<i>C. murale</i>	+	+
98-56	1998	Lompoc, CA	<i>L. sativa</i>	+	+
98-57	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-27	1998	Lompoc, CA	<i>C. suaveolens</i>	-	+
98-32	1998	Lompoc, CA	<i>Brassica rapa</i>	-	+
98-34	1998	Lompoc, CA	<i>M. parviflora</i>	-	+
98-49	1998	Lompoc, CA	<i>C. murale</i>	+	+
98-50	1998	Lompoc, CA	<i>C. murale</i>	+	+
98-51	1998	Lompoc, CA	<i>P. arenastrum</i>	+	+
98-52	1998	Lompoc, CA	<i>P. arenastrum</i>	+	+
98-01	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-02	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-03	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-04	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-05	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-06	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-07	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-08	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-09	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-10	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-11	1998	Lompoc, CA	<i>L. sativa</i>	-	+
98-12	1998	Salinas, CA	<i>L. sativa</i>	-	+

around fields in the Santa Maria Valley (fields 11 and 12) at least 1 month after harvest of lettuce crops that had BLS.

Weed leaves were assayed for *X. campestris* pv. *vitians* using the sonication-wash method previously described. Approximately 20 leaves from separate plants were bulked and washed for each weed species from each site. *X. campestris* pv. *vitians*-like colonies were identified based on colony morphology, and one putative *X. campestris* pv. *vitians* strain from each weed species from each field was tested for pathogenicity on lettuce. Pathogenicity tests were conducted on two lettuce plants per strain as described.

To further confirm the identity of the strains recovered from weeds, PCR of repetitive extragenic sequences (rep-PCR) was performed using enterobacterial repetitive intergenic consensus (ERIC)-PCR primer pair 1R and 2, and REP (repetitive extragenic palindromic)-PCR primer pair 1R and 2I (23). PCR was carried out as described by Rademaker and de Bruijn (23). The template for the PCR was 20 ng of total genomic DNA extracted by the cetyltrimethylammonium bromide (CTAB) method (15) or 2 µl of a boiled cell extract. The PCR was carried out in the Thermal Cycler 480 (Perkin-Elmer Cetus). Amplified DNA fragments were examined by gel electrophoresis in 1.5% agarose gels in 0.5× TBE buffer. Gels were stained with ethidium bromide and DNA was visualized with a gel imaging system (Alpha Innotech Corporation).

Development of a new *X. campestris* pv. *vitians*-specific primer pair. RAPD analysis was performed with total genomic DNA and Operon primer AA1. The 50-µl PCR reaction included 2.5 mM MgCl₂, dNTP mixture (concentration of each dNTP was 1 mM), 2.3 ng µl⁻¹ of oligonucleotide primer, and 1.0 unit of Amplitaq DNA polymerase in 1× Amplitaq PCR buffer II (PE Applied Biosystems). The template for the PCR was 20 ng of total genomic DNA extracted by the CTAB method. The PCR was carried out in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus). The amplification profile was 45 cycles of 94°C for 1 min, 34°C for 1.5 min, and 72°C for 2 min. Amplified DNA fragments were examined by gel electrophoresis in 1.0% agarose gels in 0.5× TBE buffer. Gels were stained with ethidium bromide, and DNA was visualized with a gel imaging system (Alpha Innotech Corporation).

A RAPD fragment (approximately 2.1 kb) was excised from an ethidium bromide-stained agarose gel and purified using the QIAquick Gel Extraction Kit (Qiagen Inc.). This DNA fragment was cloned using the TOPO TA-Cloning Kit (Invitrogen) following manufacturers' recommendations. One clone (B162) having a recombinant plasmid with the expected-size insert was selected for further charac-

terization. A partial sequence of the cloned 2.1-kb RAPD fragment was determined with ABI PRISM Dye Terminator Cycle Sequencing using dRhodamine terminator chemistry. The reactions were electrophoresed on an ABI PRISM 377 DNA Sequencer (Perkin-Elmer Cetus) in a 5% Long Ranger gel. The sequence data were compiled using ABI Prism Á Sequencing 2.1.1 software (Perkin-Elmer Cetus). Primer sequences were designed using the software Primer Designer (version 2.0, Scientific and Educational Software). Primers were synthesized by the University of California-Davis Molecular Structure Facility.

The specificity of the primer pair designed from the cloned 2.1-kb *X. campestris* pv. *vitians* RAPD fragment was tested using total genomic DNA (10 ng µl⁻¹) extracted from various bacterial strains with the CTAB method or boiled cell extracts. The PCR was carried out in a 50-µl reaction volume and, unless stated otherwise, values for reagents correspond to final concentrations. The 50-µl PCR reaction included 2.5 mM MgCl₂, dNTP mixture (concentration of each dNTP was 1 mM), 2.3 ng µl⁻¹ of each oligonucleotide primer, and 1.0 unit of Amplitaq DNA polymerase in 1× Amplitaq PCR buffer II (PE Applied Biosystems). PCR was carried out in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus). The amplification profile was 35 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min. Amplified DNA fragments were examined by gel electrophoresis in 1.0% agarose gels in 0.5× TBE buffer. Gels were stained with ethidium bromide and DNA was visualized with a gel imaging system (Alpha Innotech Corporation).

RESULTS

Recovery of *X. campestris* pv. *vitians* from lettuce leaves and debris. *X. campestris* pv. *vitians* populations recovered from leaves and debris for the 1997 and 1998 lettuce crops are shown in Figure 1. Plants were spray inoculated (June 1997) and, 4 weeks later, *X. campestris* pv. *vitians* populations of approximately 10⁸ CFU per gram of leaf tissue were detected in all plots. Representative xanthomonad-like colonies recovered from leaves were confirmed as *X. campestris* pv. *vitians* based on PCR with the 9308B primer pair and pathogenicity tests on lettuce leaves. Consistent with the detection of *X. campestris* pv. *vitians* populations on leaves, BLS symptoms developed on outer leaves of lettuce plants in all treatment plots. By harvest (July 1997), the mean BLS incidence was 100% in all plots (Fig. 2) and, on some leaves, lesions had coalesced to form large black necrotic areas. Together with the fact that assays of the seed lot used to establish this field plot failed to reveal contamination with *X. campestris* pv. *vitians*, these results indicated that the *X. campestris* pv. *vitians* populations es-

tablished on lettuce plants in this field were derived from the inoculum sprayed onto plants in June 1997.

Crop 1 was harvested in July 1997, and tillage treatments were implemented within 2 days of harvesting. After a 1-month fallow period, lettuce leaves, stems, and roots were present in each treatment plot, irrespective of tillage treatment. *X. campestris* pv. *vitians* was readily recovered from this debris, with populations ranging from 10⁶ to 10⁹ CFU/g (Fig. 1). There was no significant difference ($P \leq 0.05$) among *X. campestris* pv. *vitians* populations recovered from debris from the different tillage treatment plots (Fig. 1). These results suggest that *X. campestris* pv. *vitians* can persist in debris, at high populations, for at least 1 month.

Crop 2 was planted in August 1997. By the 5- to 7-leaf stage (September 1997), *X. campestris* pv. *vitians* populations recovered from leaves ranged from 10⁴ to 10⁶ CFU per gram of leaf tissue. Prior to harvest, epiphytic *X. campestris* pv. *vitians* populations on leaves had increased to 10⁵ to 10⁷ CFU per gram of leaf tissue. There was no significant difference ($P \leq 0.05$) among *X. campestris* pv. *vitians* populations recovered from leaves of lettuce plants from the different tillage treatments. At harvest, the mean BLS incidence was approximately 70% in all treatment plots (Fig. 2).

One month after harvest of crop 2 (December 1997), debris was plentiful in each treatment plot and *X. campestris* pv. *vitians* populations recovered from this debris ranged from 10⁵ to 10⁶ CFU/g. Again, there was no significant difference ($P \leq 0.05$) among *X. campestris* pv. *vitians* populations among treatments. By 2 months after harvest, debris had decomposed considerably and high populations of saprophytic bacteria were recovered. These bacteria formed a lawn of growth on the XCS plates at the 10⁰ and 10⁻¹ dilutions, and no *X. campestris* pv. *vitians*-like colonies were detected at higher dilutions. However, *X. campestris* pv. *vitians* was detected from debris 2 months after harvest, using the dilution plating-PCR method in two of three plots of each tillage treatment. By 3 months after harvest, lettuce plant debris was scarce, consisting of small, dried leaf fragments and large root pieces. Using the dilution plating-PCR method, *X. campestris* pv. *vitians* was detected from populations of bacteria recovered from debris in one of three plots of each tillage treatment. By 4 months after harvest, plant debris was so scarce that samples from the top 5 cm of soil had to be passed through sieves to recover debris. Again, *X. campestris* pv. *vitians* was detected from one of three plots of each tillage treatment with the dilution plating-PCR method.

Crop 3 was planted in March 1998, 5 months after the harvest of crop 2. *X. cam-*

pestris pv. *vitians* populations initially detected on leaves of crop 3 were low (0 to 10^4 CFU per gram of leaf tissue), and *X. campestris* pv. *vitians* was not recovered from leaves of plants from every treatment plot. By harvest (June 1998), *X. campestris* pv. *vitians* populations had increased to 10^3 to 10^5 CFU per gram of leaf tissue, and the mean BLS incidence was 5% in all treatment plots (Fig. 2). In July, after a 1-month fallow period, debris was plentiful in each treatment plot and *X. campestris* pv. *vitians* populations recovered from this debris ranged from 10^4 to 10^6 CFU/g.

Crop 4 was planted in July 1998 and, 1 month after planting (August), *X. campestris* pv. *vitians* populations recovered from leaves ranged from 10^2 to 10^5 CFU per gram of leaf tissue (Fig. 1). By harvest (October 1998), *X. campestris* pv. *vitians* populations on leaves increased to 10^6 to 10^7 CFU/g, and mean BLS incidence was 57% in all treatment plots (Fig. 2). Again, there was no significant difference ($P \leq 0.05$) among *X. campestris* pv. *vitians* populations recovered from leaves of lettuce plants from the different tillage treatment plots.

After harvest of crop 4 (October 1998), tillage treatments were implemented as before and the field plot was left fallow for a 5-month period. As with the previous winter fallow period, the amount of lettuce debris recovered from soil decreased with each succeeding month of fallow and, by 4 months after harvest, debris was difficult to recover. The sonication-wash assay was used to recover *X. campestris* pv. *vitians* from debris 1 month after harvest. Thereafter, assays were done with the colony blot hybridization method. *X. campestris* pv. *vitians* populations recovered from debris 1 month after harvest ranged from 10^6 to 10^7 CFU per gram of debris. Based on colony blot hybridization results, *X. campestris* pv. *vitians* populations were detected from debris as long as 4 months after harvest and populations decreased from 10^5 to 10^7 CFU/g at 2 months after harvest to approximately 10^2 CFU/g at 4 months after harvest (Fig. 1). There was no significant difference ($P \leq 0.05$) among *X. campestris* pv. *vitians* debris populations recovered from debris from the different tillage treatment plots.

Crop 5 was planted in March 1999. However, birds damaged the emerging seedlings, and crop 5 had to be replanted in May 1999. This resulted in a fallow period of 7 months. Lettuce leaves of crop 5 were assayed for *X. campestris* pv. *vitians* populations at monthly intervals (June, July, and August) until harvest (August 1999). Epiphytic populations of *X. campestris* pv. *vitians* were not detected at any time, nor were symptoms of BLS observed.

Recovery of *X. campestris* pv. *vitians* from weeds. A weed survey was conducted in April 1998 around two lettuce fields (1 and 2) in Aromas, CA. The incidence of BLS in field 1 was 100%, whereas that in field 2 was approximately 70%. Nine weed species were collected from around each field, and leaves from representative weed species were assayed for *X. campestris* pv. *vitians* (Table 2). Populations of xanthomonad-like bacteria were recovered from leaves of three species from field 1: *Convolvulus arvensis* L. (field bindweed), *Epilobium brachycarpum* C. Presl. (panicked willoweed), and *Senecio vulgaris* L. (common groundsel), and

Fig. 1. Populations of *Xanthomonas campestris* pv. *vitians* recovered from lettuce (cv. Alpha) leaves and debris in a multiple-year field study in Salinas, CA. Standard deviations of the average *X. campestris* pv. *vitians* populations are represented by error bars. Asterisks indicate months in which populations were determined based on the colony hybridization method. The following tillage treatments were used: lettuce was harvested and crop debris were incorporated with conventional tillage (Harvest/Con. tillage); lettuce was harvested and crop debris were incorporated with minimum tillage (Harvest/Min. tillage); lettuce was not harvested and crop debris were incorporated with conventional tillage (No harvest/Con. tillage); lettuce was not harvested and crop debris were incorporated using minimum tillage (No harvest/Min. tillage). The "H" indicates when harvest was done for each crop.

from leaves of two species from field 2: *Sonchus oleraceus* L. (annual sowthistle) and panicle willoweed. With the 9308B primer pair, a 450-bp DNA fragment was amplified from strains recovered from field bindweed and panicle willoweed from field 1 and annual sowthistle from field 2, whereas no DNA fragment was amplified from the strains from common groundsel or panicle willoweed from field 1. However, all five of these xanthomonad-like bacteria were pathogenic on lettuce (Table 2). The rep-PCR fingerprints of the five pathogenic xanthomonad-like bacteria recovered from weeds were indistinguishable from those of *X. campestris* pv. *vitians* (Fig. 3). These results establish that *X. campestris* pv. *vitians* can be recovered from weeds and that the 9308B primer pair failed to direct the amplification of the 450-bp DNA fragment from some *X. campestris* pv. *vitians* strains (Table 1).

A second weed survey was conducted in April 1998 around two lettuce fields (fields 3 and 4) in the Santa Maria Valley, CA. The incidence of BLS in field 3 was approximately 75%, whereas that in field 4 was approximately 60%. A total of 5 and 10 weed species were collected from around fields 3 and 4, respectively, and leaves were assayed for *X. campestris* pv. *vitians* (Table 2). Populations of xanthomonad-like bacteria were recovered from leaves of four species from field 3: *Capsella bursa-pastoris* (L.) Medikus (shepherd's purse), *Chamomilla suaveolens* (Pursh) Rydb. (pineapple weed), common

groundsel, and annual sowthistle; and from leaves of four weed species from field 4: *Chenopodium murale* L. (nettleleaf goosefoot), *Malva parviflora* L. (little mallow), *Polygonum arenastrum* Boreau (common knotweed), and common groundsel. No 450-bp DNA fragment was amplified from strains recovered from weeds from fields 3 or 4 (Table 1). However, all these strains were pathogenic on lettuce (Table 2) and had rep-PCR DNA (ERIC) fingerprints that were indistinguishable from those of *X. campestris* pv. *vitians* (Fig. 3).

A third weed survey was conducted in April 1998 around two lettuce fields (fields 5 and 6) in the Lompoc Valley, CA. The incidence of BLS in both fields was approximately 100%. A total of 8 and 13 weed species were collected from around field 5 and 6, respectively, and leaves were assayed for *X. campestris* pv. *vitians* (Table 2). Populations of xanthomonad-like bacteria were recovered from leaves of six species from field 5: shepherd's purse, nettleleaf goosefoot, field bindweed, *Lupinus formosus* Greene var. *robustus* C. P. Sm. (summer lupine), little mallow, and common knotweed; and from leaves of six weed species from field 6: *Brassica rapa* L. (wild mustard), pineapple weed, nettleleaf goosefoot, little mallow, common knotweed, and common groundsel. A 450-bp DNA fragment was amplified from xanthomonad-like bacteria recovered from nettleleaf goosefoot (two strains), little mallow, and field bindweed from field 5; and from strains from common knotweed

(two strains) and nettleleaf goosefoot from field 6. No DNA fragment was amplified from strains recovered from common knotweed (two strains), nettleleaf goosefoot, shepherd's purse, and summer lupine (two strains) from field 5; or from little mallow, wild mustard, and pineapple weed from around field 6. However, all these xanthomonad-like bacteria were pathogenic on lettuce (Table 2) and had rep-PCR DNA (ERIC) fingerprints that were indistinguishable from those of *X. campestris* pv. *vitians*.

A fourth weed survey was conducted in January 1999 around two lettuce fields in the Santa Maria Valley (fields 7 and 8) and two fields in the Lompoc Valley (fields 9 and 10). These surveys were conducted at least 2 months after harvest of a lettuce crop that had BLS. Three and five weed species were collected from around fields 7 and 8, respectively, and leaves were assayed for *X. campestris* pv. *vitians*. Populations of xanthomonad-like bacteria were recovered from leaves of one species from field 7, shepherd's purse, and not from leaves of any weeds from field 8. Six and five weed species were collected from around fields 9 and 10, respectively, and leaves were assayed for *X. campestris* pv. *vitians*. Populations of xanthomonad-like bacteria were recovered from leaves of one species from field 9 (shepherd's purse) and from leaves of two weed species from field 10 (little mallow and common groundsel). No DNA fragment was amplified from any of the strains recovered from any of the weed species assayed in this survey (Table 2). Furthermore, rep-PCR DNA (REP) fingerprints generated for these strains were variable and were clearly distinguishable from those of *X. campestris* pv. *vitians* (Fig. 4).

A fifth weed survey was conducted in November 1999 around two lettuce fields (fields 11 and 12) in the Santa Maria Valley, CA. This survey was conducted at least 1 month after harvest of a lettuce crop that had BLS. Five and eight weed species were collected from fields 11 and 12, respectively, and leaves were assayed for *X. campestris* pv. *vitians*. Populations of xanthomonad-like bacteria were recovered from leaves of four species from field 11: shepherd's purse, little mallow, common groundsel, and annual sowthistle; and from leaves of six species from field 12: shepherd's purse, *Cotula coronopifolia* L. (brass button), little mallow, common groundsel, *Sisymbrium irio* L. (London rocket), and annual sowthistle. PCR analysis with the B162 primer pair (described below) was performed with at least one xanthomonad-like strain recovered from each weed species from each field. No DNA fragment was amplified with the B162 primer pair from any of the strains recovered from any of the weed species assayed in this survey (Table 2). Furthermore, rep-PCR DNA (REP) fingerprints

Fig. 2. Populations of *Xanthomonas campestris* pv. *vitians* recovered from lettuce (cv. Alpha) leaves and bacterial leaf spot incidence (determined 1 day prior to harvest) in a multiple-year field study in Salinas, CA. The following tillage treatments were used: lettuce was harvested and crop debris were incorporated with conventional tillage (Harvest/Con. tillage); lettuce was harvested and crop debris incorporated with minimum tillage (Harvest/Min. tillage); lettuce was not harvested and crop debris were incorporated with conventional tillage (No harvest/Con. tillage); lettuce was not harvested and crop debris were incorporated using minimum tillage (No harvest/Min. tillage).

generated for these strains were variable and were clearly distinguishable from those of *X. campestris* pv. *vitians* (Fig. 4), and none of the strains were pathogenic on lettuce (Table 2).

RAPD analysis and primer design.

The 9308B primer pair did not direct the amplification of the 450-bp fragment from all *X. campestris* pv. *vitians* strains, particularly those recovered from weeds. Therefore, a new *X. campestris* pv. *vitians* primer pair was developed. RAPD analysis with Operon primer AA1 (previously

shown to generate RAPDs from *X. campestris* pv. *vitians*) was utilized to direct the amplification of a RAPD fragment common to selected *X. campestris* pv. *vitians* strains (strains 30547 and 41335 from Australia; strain 9911 from Hawaii; and California strains Sal and 9308 from Salinas, strain B162 from Guadelupe, strain LL2 from Bakersfield, and strain 9842 from Santa Maria). These strains were chosen because (i) they come from diverse geographical regions and (ii) the 9308B primer did not direct the amplification of

the 450-bp DNA fragment. Primer AA1 directed the amplification of an approximately 2.1-kb DNA fragment from total genomic DNA of these *X. campestris* pv. *vitians* strains (*data not shown*), and this fragment was cloned from strain B162. Using the T7 sequencing primer, approximately 800 nucleotides of one end of this RAPD fragment were determined. Based on this sequence, a new *X. campestris* pv. *vitians* primer pair (B162) was designed (B162f, 5' CGG CCG AAT AAT CAA GAG CA 3' and B162r, 5' TTG TCC GTT ACG ATT GGT GC 3').

Primer pair specificity. The B162 primer pair directed the amplification of an approximately 700-bp DNA fragment from total genomic DNA of all 130 *X. campestris* pv. *vitians* strains tested (Fig. 5), including 4 strains from Australia; 1 from Florida; 22 from Hawaii; and 103 from California (5 strains from Aromas, 31 strains from Lompoc, 40 strains from Salinas, and 27 strains from Santa Maria; Table 1). Moreover, the 700-bp fragment was amplified from all strains that did not have amplification of the 450-bp fragment by the 9308B primer pair. To evaluate the specificity of this primer pair, it was tested with total genomic DNA of various *X. campestris* pathovars (*alfalfae*, *armoraciae*, *carotae*, *glycinea*, *juglandis*, *phaseoli*, and *pelargonii*). No DNA fragments were amplified from total genomic DNA of any of these pathovars. Moreover, no DNA fragments were amplified from total genomic DNA of *X. oryzae* pv. *oryzae*, *Pseudomonas syringae* pv. *syringae*, *P. fluorescens*, *Erwinia carotovora* subsp. *carotovora*, *E. amylovora*, *Clavibacter michiganense* subsp. *michiganense*, or *Agrobacterium tumefaciens*.

DISCUSSION

Recent outbreaks of BLS in coastal and inland lettuce-producing areas of California, together with the inability to recover *X. campestris* pv. *vitians* from seed lots used to establish fields with these outbreaks, led to the investigation of alternate inoculum sources. We found that high populations of *X. campestris* pv. *vitians* can survive in association with crop debris for at least 1 month. Contaminated debris can serve as an inoculum source for subsequent crops, resulting in severe BLS outbreaks. Using sensitive techniques such as PCR or colony blot hybridization, small populations of *X. campestris* pv. *vitians* were detected from debris for up to 4 months after harvest. The small populations of *X. campestris* pv. *vitians* that survived over this 4-month crop-free period caused BLS outbreaks in a subsequent lettuce crop. The rapid decrease in *X. campestris* pv. *vitians* populations associated with debris was likely due to colonization and decomposition of debris by saprophytic microbes. It is likely that *X. campestris* pv. *vitians*,

Table 2. *Xanthomonas campestris* pv. *vitians* populations recovered from weed species surveyed in April 1998 (fields 1–6) around fields with a bacterial leaf spot (BLS) outbreak^a

Location	Weed species	Population	PCR ^b	Pathogenicity	rep-fingerprint ^c
Aromas					
Field 1	<i>Convolvulus arvensis</i>	10 ⁴	+	+	+
	<i>Epilobium brachycarpum</i>	10 ⁴	+	+	+
	<i>Senecio vulgaris</i>	10 ⁴	–	+	+
Field 2	<i>Sonchus oleraceus</i>	10 ²	+	+	+
	<i>Epilobium brachycarpum</i>	10 ⁴	–	+	+
Santa Maria					
Field 3	<i>Capsella bursa-pastoris</i>	10 ⁵	–	+	+
	<i>Senecio vulgaris</i>	10 ⁴	–	+	+
	<i>Sonchus oleraceus</i>	10 ⁴	–	+	+
Field 4	<i>Chamomilla suaveolens</i>	10 ⁵	+	+	+
	<i>Chenopodium murale</i>	10 ⁴	–	+	+
	<i>Malva parviflora</i>	10 ⁴	–	+	+
	<i>Polygonum arenastrum</i>	10 ⁴	+	+	+
	<i>Senecio vulgaris</i>	10 ⁵	–	+	+
Lompoc					
Field 5	<i>Capsella bursa-pastoris</i>	10 ⁴	–	+	+
	<i>Chenopodium murale</i>	10 ⁴	+	+	+
	<i>Convolvulus arvensis</i>	10 ⁴	–	+	+
	<i>Lupinus formosus</i>	10 ⁴	–	+	+
	<i>Malva parviflora</i>	10 ⁴	+	+	+
	<i>Polygonum arenastrum</i>	10 ³	+	+	+
Field 6	<i>Brassica rapa</i>	10 ⁴	–	+	+
	<i>Chamomilla suaveolens</i>	10 ⁴	–	+	+
	<i>Chenopodium murale</i>	10 ³	+	+	+
	<i>Malva parviflora</i>	10 ⁴	–	+	+
	<i>Polygonum arenastrum</i>	10 ⁴	–	+	+
	<i>Senecio vulgaris</i>	10 ⁴	–	+	+
Santa Maria					
Field 7	<i>Capsella bursa-pastoris</i>	nd	–	nt	–
Field 8	None	...	...	...	...
Lompoc					
Field 9	<i>Capsella bursa-pastoris</i>	nd	–	nt	–
Field 10	<i>Malva parviflora</i>	nd	...	nt	–
	<i>Senecio vulgaris</i>	nd	...	nt	–
Santa Maria					
Field 11	<i>Capsella bursa-pastoris</i>	nd	– ^d	–	–
	<i>Malva parviflora</i>	nd	– ^d	–	–
	<i>Senecio vulgaris</i>	nd	– ^d	–	–
	<i>Sonchus oleraceus</i>	nd	– ^d	–	–
	<i>Capsella bursa-pastoris</i>	nd	– ^d	–	–
Field 12	<i>Cotula coronopifolia</i>	nd	– ^d	–	–
	<i>Malva parviflora</i>	nd	– ^d	–	–
	<i>Senecio vulgaris</i>	nd	– ^d	–	–
	<i>Sisymbrium irio</i>	nd	– ^d	–	–
	<i>Sonchus oleraceus</i>	nd	– ^d	–	–

^a Surveys in January 1999 (fields 7–10) and November 1999 (fields 11–12) were conducted at least 2 months and 1 month, respectively, after harvest of a lettuce crop that had BLS; nd = populations of bacteria not determined; nt = strains not tested for pathogenicity.

^b Polymerase chain reaction (PCR) analysis with the 9308B primer pair, pathogenicity test, and repetitive extragenic sequences (rep)-PCR fingerprinting was performed with at least one xanthomonad-like colony from each weed species from each field.

^c DNA fingerprints generated with rep-PCR (enterobacterial repetitive intergenic consensus, fields 1–6 and repetitive extragenic palindromic, fields 7–12) were compared with the fingerprint of a known *X. campestris* pv. *vitians* strain; + = fingerprint identical to that of *X. campestris* pv. *vitians*, and – = fingerprint clearly distinguishable from that of *X. campestris* pv. *vitians*.

^d Results of PCR analyses with the B162 primer pair.

like many other leaf spot bacteria, is a poor soil inhabitant.

Other xanthomonads also can survive in association with debris, including *X. campestris* pvs. *phaseoli* (10), *campestris* (27), and *malvacearum* (3). *X. campestris* pv. *phaseoli* survived in association with debris in no-tillage but not in reduced- or deep-tillage plots over a winter fallow period in Wisconsin. Contaminated debris

served as an inoculum source for common bacterial blight in a subsequent bean crop (10). In contrast, *X. campestris* pv. *vitians* in California survived in debris regardless of the tillage practice used. This may be due to differences in winter conditions in California and Wisconsin. In both the California lettuce work and the Wisconsin bean study, colony hybridization provided useful for detection of pathovars of *X. campestris*

from among nonpathogenic xanthomonads or saprophytic bacteria (9). Schaad and White (27) reported the survival of *X. campestris* pv. *campestris* in association with cabbage debris for at least 2 years in Georgia, and that time of year had little or no influence on survival.

Our results also established that *X. campestris* pv. *vitians* could survive in debris during summer as well as winter months. The ability to survive in debris during the summer contrasts with results for other bacterial pathogens such as *X. campestris* pv. *translucens* in Arkansas (20), *P. syringae* pv. *tomato* in Georgia (19), and *X. campestris* pv. *vesicatoria* in Florida (12). This could be due to high air and soil temperatures during summer months in the southeastern United States, which may reach 45°C and average 39°C for extended periods (19). Brinkerhoff and Fink (3) showed that decomposition of cotton debris, sufficient to eliminate *X. campestris* pv. *malvacearum* as an inoculum source, took twice as long in field soil in Oklahoma in the winter (−3 to 11°C) compared with soil maintained in a greenhouse (25 to 34°C). Thus, warm, moist conditions may be unfavorable for bacterial survival in association with debris. In contrast, the summer temperatures in the coastal California lettuce-growing regions are relatively cool (10 to 25°C), possibly favoring survival of *X. campestris* pv. *vitians*.

The ability of *X. campestris* pv. *vitians* to persist in association with lettuce debris is a potential problem in double-cropping (i.e., two lettuce crops per year in a single field) or continuous lettuce cropping systems. Contaminated debris can provide inoculum that can spread to young plants, increase rapidly, and lead to BLS outbreaks. In California, *X. campestris* pv. *vitians*-contaminated lettuce debris may be a more important inoculum source for BLS than seedborne inoculum, which has not been consistently recovered from commercial seed lots (14). A similar situation exists in Florida for bacterial spot disease of tomato, caused by *X. campestris* pv. *vesicatoria*, where inoculum sources other than seed, such as volunteer tomato plants and crop residue, appear to play a more important role in the epidemiology of the disease (12).

In fields with a history of BLS, crop rotation or fallow periods of at least 5 months may be necessary to minimize the probability of BLS outbreaks in subsequent lettuce crops. Moreover, in the present study, *X. campestris* pv. *vitians* populations were eliminated or at least reduced to levels incapable of colonizing lettuce plants after a 7-month fallow period. Rotation with rapidly maturing crops such as spinach (40 days to harvest) may not be desirable for management of BLS, because *X. campestris* pv. *vitians* can survive in debris for up to 120 days. Better choices for rotation include broccoli, cauliflower, and

Fig. 3. Agarose gel showing polymerase chain reaction (PCR) fingerprint patterns generated from total genomic DNA from strains of *Xanthomonas campestris* pv. *vitians* and xanthomonad-like bacteria recovered from leaves of weeds with primers corresponding to enterobacterial repetitive intergenic consensus (ERIC) sequences. Lane M contains DNA size markers (1-kb ladder, Gibco-BRL). Lane 1, strain 9839; lane 2, strain 9851; lane 3, strain Sal; lane 4, strain 9812; lane 5, strain 9813; lane 6, strain 9815; lane 7, strain 9824; lane 8, strain 9828; lane 9, strain 9834; lane 10, strain 9844; and lane 11, strain 9862.

Fig. 4. Agarose gel showing polymerase chain reaction (PCR) fingerprint patterns generated from genomic DNA (boiled cell extracts) from strains of xanthomonad-like bacteria with primers corresponding to repetitive extragenic palindromic (REP) sequences. Lane M contains DNA size markers (1-kb ladder, Gibco-BRL). Lanes 1–9, xanthomonad-like bacteria recovered from *Capsella bursa-pastoris* (shepherd's purse); lanes 10–12, xanthomonad-like bacteria recovered from *Malva parviflora* (little mallow); lanes 13–14, xanthomonad-like bacteria recovered from *Senecio vulgaris* (common groundsel); and lane 15, *X. campestris* pv. *vitians* (strain Sal).

celery, which are in the field for considerably longer periods of time.

Weeds can harbor plant pathogenic bacteria (6,8,17). *X. campestris* pv. *vitians* can be recovered from leaves of a number of weed species in close proximity to lettuce having BLS. Because no symptoms were observed on these weeds, *X. campestris* pv. *vitians* was probably surviving as an epiphyte. Schroth et al. (28) showed that, under greenhouse conditions, *X. campestris* pv. *vitians* caused BLS symptoms on a number of weed species, including *Lactuca scariola* L. (prickly lettuce), *L. canadensis* L. (trumpet fireweed), *L. ludoviciana* (Nutt.) Riddell, *L. pulchella* (Pursh.) DC., and annual sowthistle. Tsuchiya et al. (31) infected dandelion (*Taraxacum officinale* Wiggers), groundsel, and tall goldenrod (*Solidago altissima* L.) by spray inoculation with *X. campestris* pv. *vitians*, and kitsuneazami (*Hemistepta lyrata* Bunge), wall spread (*Veronica arvensis* L.), henbit (*Lamium amplexicaule* L.), common lespedeza (*Kummerowia striata* (Thunb.) Schindler), small leaf sowbane (*Chenopodium serotinum* L.), and white-goose-foot (*C. album* L.) by prick-spray inoculation. On the basis of these studies, it was concluded that weeds might be important inoculum sources for BLS. We recovered *X. campestris* pv. *vitians* from symptomless leaves of annual sowthistle in Aromas and Santa Maria Valley. However, in our study, *X. campestris* pv. *vitians* was not recovered from weeds that were not in close proximity to lettuce plants with BLS. Thus, weeds may not be an important long-term *X. campestris* pv. *vitians* source, possibly due to the lack of stable *X. campestris*

pv. *vitians* epiphytic populations on these plants. Nonetheless, the rigorous weed control program in lettuce-growing areas of Monterey County that is practiced for management of *Lettuce mosaic virus* results in few weeds being available as potential inoculum sources of *X. campestris* pv. *vitians*. Thus, the role of weeds in the epidemiology of *X. campestris* pv. *vitians* in California remains unclear.

During the course of this study, we found that the 9308B primer pair failed to direct the amplification of the 450-bp DNA fragment from a number of *X. campestris* pv. *vitians* strains recovered from weeds and lettuce. The 9308B primer pair was designed from a RAPD fragment cloned from *X. campestris* pv. *vitians* strain 9308, collected in California in 1994 (14). It appears that genetic diversity may exist in the sequence of the RAPD fragment used to design the 9308B primer pair and that a sufficient diversity of *X. campestris* pv. *vitians* strains was not tested with the 9308B primer pair to reveal this diversity (i.e., the failure to direct the amplification of the 450-bp fragment) from all strains. The B162 primer pair, also developed based on the sequence of a cloned RAPD fragment, directed the amplification of an approximately 700-bp fragment from all *X. campestris* pv. *vitians* strains tested. These included strains from two continents and three states collected over a 7-year period. In addition, the B162 primer pair appears to be *X. campestris* pv. *vitians*-specific because no DNA fragment was amplified from other *X. campestris* pathovars or other bacteria. Thus, this primer pair should be useful in the development of

PCR-based assays for the rapid detection of *X. campestris* pv. *vitians* recovered from seed, debris, or other sources.

In summary, *X. campestris* pv. *vitians* can colonize and survive in lettuce debris for at least 5 months, and this debris can serve as an inoculum source for subsequent crops. Hence, lettuce debris infested with *X. campestris* pv. *vitians* may be an important inoculum source, particularly in regions where multiple crops are produced in the same field. *X. campestris* pv. *vitians* can also survive on weeds around lettuce fields with BLS outbreaks, but long-term survival on weeds was not detected. Therefore, an integrated management program for BLS in lettuce should include (i) planting seed tested negative for *X. campestris* pv. *vitians* contamination, (ii) practicing crop rotations or maintaining fallow periods of at least five months in fields with a history of BLS outbreaks, and (iii) practicing good weed control in and around fields.

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Fig. 5. Agarose gel showing polymerase chain reaction (PCR) with total genomic DNA from strains of *Xanthomonas campestris* pv. *vitians* and the B162 primer pair, which directs the amplification of an approximately 700-bp fragment. Lanes M contain DNA size markers (1-kb ladder, Gibco-BRL). Lane 1, strain 9801; lane 2, strain 9802; lane 3, strain 9803; lane 4, strain 9804; lane 5, strain 9805; lane 6, strain 9806; lane 7, strain 9807; lane 8, strain 9808; lane 9, strain 9809; lane 10, strain 9810; lane 11, strain 9811; lane 12, strain 9812; lane 13, strain 9813; lane 14, strain 9814; lane 15, strain 9815; lane 16, strain 9816; lane 17, strain 9817; lane 18, strain 9818; lane 19, strain 9819; lane 20, strain 9820; lane 21, strain 9821; lane 22, strain 9822; lane 23, strain 9823; lane 24, strain 9824; lane 25, strain 31; lane 26, strain 9826; lane 27, strain 9828; lane 28, strain 9829; lane 29, strain 9830; and lane 30, strain Sal.

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