A New Ranunculus Disease Caused by *Xanthomonas campestris*

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ABSTRACT

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A new disease of ranunculus (*Ranunculus asiaticus*) was observed on several cultivars in commercial fields in San Diego and Riverside Counties, California. Symptoms included pin-point to large irregular necrotic lesions on leaves and stems and occasionally black patches along the internal margins of leaflets in association with vein chlorosis. *Xanthomonas campestris* was consistently isolated from diseased tissues. *X. campestris* was also isolated from tubers and seeds of naturally infected plants, which suggests a means by which the pathogen is spread in the industry. One-year-old tubers of two cultivars (Picotee and Rose) were contaminated at frequencies of 4 and 7%, respectively. The frequency of seed contamination for 11 cultivars ranged from 1.1 to 16%. Symptoms appeared on inoculated ranunculus plants as early as 3 and as late as 22 days after inoculation, depending on the method of inoculation, temperature, and available moisture. Recovery of the bacterium from the tubers of plants inoculated and kept under different moisture and temperature conditions was 6.6 to 13.3%. Amplification of a DNA fragment specific for *hrp* genes by polymerase chain reaction for each strain and further analysis of the amplification product by restriction endonuclease digestion suggested that the ranunculus strains were closely related to each other and to *X. c.* pv. *campestris*; however, pathogenicity tests indicated that the ranunculus strains could be a different pathovar.

Ranunculus (*Ranunculus asiaticus* L.) is increasing in California as a field-grown bulb and cut flower crop. Ongoing variety selections are made by collecting seed from plants with desired flower color and growth habit, and the seeds are used to propagate plants for production of root tubers and for cut flowers. In January 1994, a ranunculus plant with a foliar disease was sent to our laboratory from a commercial grower in Palm Desert, California. Concurrently, an outbreak of a similar disease was observed in commercial fields in San Diego County, especially in the Carlsbad area. Symptoms of the disease from different areas included irregular necrotic lesions on leaves and stems (Fig. 1B and C). Lesions were often associated with a chlorotic halo or general leaf yellowing, but water-soaking was seldom associated with lesions. More advanced phases of the disease included coalescence of the lesions, complete necrosis, and death of leaves (Fig. 1D and E), and eventually collapse of the entire plant. Occasionally, black patches along the internal margins of leaflets in association with vein chlorosis were observed (Fig. 1F). This report describes the isolation and confirmation of *Xanthomonas campestris* as the causal agent of this new disease.

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MATERIALS AND METHODS

Isolation of causal agent. Tissue sections from lesions on original or inoculated diseased leaves were crushed in 0.5 ml of sterile distilled water (SDW). The resulting suspension was streaked, or plated in dilutions, onto MGY (8), YDC (17), Tween (11), and MS (12) media. Some naturally infected and some asymptomatic plants were collected from Carlsbad and brought into the laboratory. The root tubers (bulbs) were washed under running tap water to release soil particles. The roots were then chopped into 5-mm pieces and added to 100 ml of sterile saline containing five drops of Tween 20 in a 250-ml Erlenmeyer flask. Flasks were placed on a shaker with vigorous shaking overnight at 4°C. The liquid content of each flask was concentrated by centrifugation, and the pellet was suspended in 1 ml of SDW. Serial dilutions were plated onto YDC and Tween media. Several tubers were also cut with a razor blade, and small tissue pieces from internal necrotic areas were excised and treated as the leaf tissues described above. Dried tubers of cultivars Rose and Picotee from the previous year were ground with a mortar and pestle, soaked in saline containing Tween 20, and processed as described for the fresh tubers. To initially isolate the pathogen from seeds, 1,000 seeds from cultivar Salmon 93 were soaked in 25 ml of saline with Tween 20 overnight at 4°C. The liquid portion was concentrated and plated as described earlier for tubers. Later, 1,000 seeds (Mellano & Company, San Luis Rey, CA) from this cultivar and from 10 other cultivars were individually plated on Tween medium. Plates were incubated at 28°C for 1 week. The strains recovered from each isolation were purified by streaking single colonies on fresh media at least four times.

Identification of the bacteria. Tests for identification of the strains were performed as described by Schaad (13). The strains were also compared by their carbon source utilization profiles on Biolog GN microplates (Biolog, Inc., Hayward, CA). *X. c.* pv. *campestris* 0186-1 (from cauliflower), *X. c.* pv. *vesicatoria* 0788-2 (from tomato), and *X. c.* pv. *translucens* 0790-9 (from wheat) were used as controls.

Plant preparation. To obtain pathogen-free ranunculus plants, individual seeds from cultivar Salmon 93 were plated on Tween medium and incubated at 28°C for 5 days. Seeds that tested negative for the presence of the pathogen were then individually planted in 10-cm (4-in) pots containing steam-treated UC soil mix (4). Pots were placed in a growth chamber with a constant temperature of 16°C, a relative humidity that fluctuated between 50% (at 2 to 5 p.m.) and 100% (at 2 to 6 a.m.), and 10 h/day artificial light. After seed germination, the temperature was raised to 20°C, and the photoperiod was increased to 12 h. Seedlings were inoculated after they reached the five- or six-leaf stage.

Plant inoculations. Bacterial colonies of ranunculus strains and other pathovars of *X. campestris* were grown on YDC agar for 48 h at 28°C and suspended in SDW. The suspensions were diluted to about 10^7 CFU/ml, based on optical density readings with a Klett-Summerson colorimeter, which were confirmed by dilution plating on YDC agar. The bacterial suspensions were infiltrated into leaves of tobacco plants (cultivar Turk) with a needleless syringe. Observations for hypersensitive reactions were recorded daily for 7 consecutive days. One-month-old cauliflower

plants of the cultivar Snow Ball, obtained from a local nursery, were inoculated by applying bacterial suspensions directly to wounds made with a 21-gauge needle in the epidermis of leaves and on the stems at the growing points.

One strain from ranunculus (0394-13) was used in a larger study with three inoculation methods and three plant growth conditions. Bacterial suspensions (10^7 CFU/ml) were either sprayed onto leaf surfaces of ranunculus plants (cultivar Salmon 93) until runoff with an aerosol-propelled spray unit or infiltrated into leaf tissues with a syringe without a needle. Ranunculus plants were also wound-inoculated as described for cauliflower above. Fifteen plants were used for each of the three methods of inoculation, for a total of 45 plants per experiment. Of the 15 plants from each inoculation method, five were maintained on a greenhouse bench with natural light, ambient temperature between 17°C (at 2 to 8 a.m.) and 22°C (at 2 to 4:30 p.m.), and relative humidity of 65% (at 10 a.m. to 4 p.m.) to 99% (at 4 to 6 a.m.). A second group of five plants from each inoculation method was placed in a mist chamber at 23 to 26°C with intermittent misting for 5 s every 10 min for 24 h/day. These plants received artificial light for 16 h/day. The third group of five plants was returned to the growth chamber used for plant preparation. The entire experiment was repeated three times. An additional control plant for each inoculation method and growth condition in each experiment was treated with water or with *X. c.* pv. *campestris* 0186-1, *X. c.* pv. *vesicatoria* 0788-2, or *X. c.* pv. *translucens* 0790-9.

Bacterial transmission. All inoculated plants were maintained for 10 weeks. The tuber from each plant was then excised, and bacteria were isolated as outlined earlier.

Amplification of *hrp*-related DNA fragments. Total bacterial genomic DNA was isolated by the hexadecyltrimethylammonium bromide (CTAB) purification method essentially as described by Ausubel et al. (2). Primers RST21 (5′-GCACGCTCCAGATCAGCATCGAGG-3′) and RST22 (5′-GGCATCTGCATGCGT-

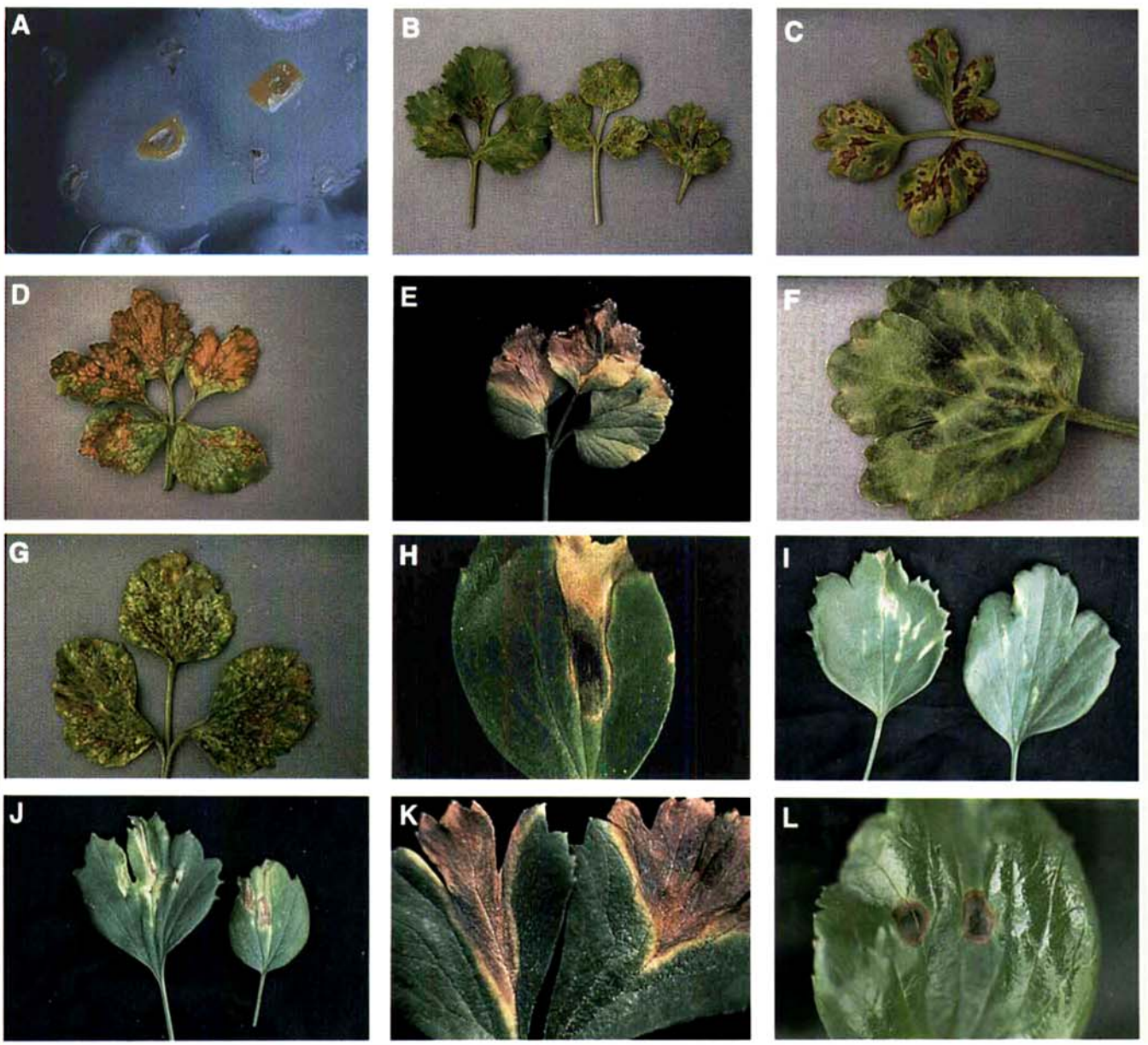

Fig. 1. *Xanthomonas campestris* and associated disease symptoms on ranunculus leaves. **(A)** *X. campestris* growing from seeds on Tween medium. **(B–F)** Symptoms from natural infections in the field. **(G)** Symptoms from spray-inoculated plants in the mist chamber. **(H)** Spread of infection from the site of bacterial infiltration. **(I)** Initial appearance of symptoms at the sites of needle inoculations. **(J)** Symptoms at the sites of needle inoculations 7 days after appearance. **(K)** Symptoms at the sites of needle inoculations 15 days after appearance. **(L)** HR reaction after infiltration of a ranunculus leaf with cell of *X. c.* pv. *campestris* 0186-1.

GCTCTCCGA-3′) were designed by Leite et al. (10) to amplify a 1,075-bp fragment of the *hrp* gene region of *X. c.* pv. *vesicatoria*. Primers were synthesized commercially (Cruachem, Inc., Sterling, VA 22170) for this study. DNA was amplified in a total volume of 100 µl of reaction mixture, which contained 10 µl of Vent DNA polymerase 10× buffer (New England BioLabs, Inc., Beverly, MA 01915), 200 µM each deoxynucleoside triphosphate (New England BioLabs), 0.1 µg of BSA, 1 mM $MgSO_4$, 5% DMSO, 1 µM of each primer, and 1 U of Vent polymerase (5). The amount of template DNA added was 25 ng of total bacterial DNA. PCR amplification was performed in an automated thermocycler (EasyCycler Series, Ericomp, Inc., San Diego, CA 92121) using the following cycles: one initial denaturation of DNA template at 95°C for 8 min; 24 cycles of denaturation at 95°C for 1 min, annealing at 57°C for 30 s, and extension at 72°C for 1 min; and a final extension at 72°C for 5 min. Detection of amplified DNAs was performed according to procedures outlined elsewhere (2,10).

Table 1. Recovery of *Xanthomonas campestris* from seeds of 10 ranunculus cultivars

Cultivar	% Contamination[a]
Yellow	1.1
Gold	2.2
Salmon white	5.6
Orange	6.9
Pink	7.6
Red	9.1
Mixed	10.4
Picotee	10.5
Rose	10.8
White	16.0

[a] One thousand seeds for each cultivar were individually plated on Tween medium and incubated at 28°C for 7 days. Percent contamination was calculated as: Number of seeds from which *X. campestris* grew × 100%/total number of seeds plated.

Restriction endonuclease analysis of *hrp*-related fragments. The DNA fragments amplified from different bacterial strains were digested with the frequently cutting endonucleases *Hha*I, *Hae*III, *Sau*3AI, and *Taq*I under conditions specified by the manufacturer (New England BioLabs). The digested fragments were separated by electrophoresis in 4% agarose gels in TBE buffer at 4 V/cm. *Pst*I, *Hin*dIII–*Eco*RI, and *Ssp*I digested phage lambda DNA fragments were used as molecular-size standards.

RESULTS

Isolation and identification of causal agent. High populations of rod-shaped, motile bacteria were observed streaming into distilled water by phase-contrast microscopy of tissue sections from the leaf lesions of field-grown plants. When suspensions from the maceration of such tissues were plated, many bacterial types were recovered on YDC and MGY. However, only one bacterial type grew on Tween medium. The most prevalent colonies on YDC, MGY, and Tween plates were yellow, mucoid colonies that resembled *Xanthomonas*. Morphological, biochemical, and physiological tests showed that the yellow, mucoid bacteria were gram-negative, catalase positive, oxidase negative, and had single polar flagella. These bacteria were obligately aerobic and negative for urease, arginine dihydrolase, and nitrate reductase activity. The bacteria were able to hydrolyze starch and liquefy gelatin, but were unable to accumulate PHB granules and did not grow on a minimal defined medium without added growth factors. The bacteria also grew on YDC plates at 35°C. Comparison of these characteristics with those reported elsewhere (9,13) and for known species of *X. campestris* used in this study as controls confirmed the identification of the bacteria as *X. campestris*. The Biolog data base was also useful for rapid confirmation of the identity of this bacterium. All 36 strains isolated and purified from diseased leaves (12 strains), necrotic fresh tubers (two strains), dried tubers (11 strains), and seed (11 strains) were identified as *X. c.* pv. *campestris* by the Biolog data base. The similarity of these strains to *X. c.* pv. *campestris* in the Biolog data base ranged from 62 to 92%.

Isolations from macerates or from excised necrotic areas of two naturally infected tubers from commercial plants showing foliar symptoms also yielded *X. campestris* strains similar to those from leaf tissues. No such bacterium was isolated from asymptomatic plants. When 100 dried tubers (1 year old) of each cultivar Rose and Picotee were individually ground and tested, *X. campestris* was isolated from seven and four tubers, respectively.

An assay of 1,000 seeds of cultivar Salmon 93 by seed washing also recovered *X. campestris*. When these seeds were plated directly onto Tween medium, 3.2% were shown to be contaminated with *X. campestris*. *X. campestris* grew from contaminated seeds after 2 to 3 days incubation at 28°C (Fig. 1A). The levels of seed contamination for other cultivars ranged from 1.1 to 16% (Table 1). Pathogenicity and Biolog identification as *X. c.* pv. *campestris* were confirmed for one isolate from each seed lot.

Plant inoculations. Infiltration of tobacco leaves with cell suspensions of *X. c.* pv. *campestris* 0186-1 and *X. c.* pv. *vesicatoria* 0788-2 resulted in typical hypersensitive reactions (HR) within 24 h. The responses to infiltration of *X. c.* pv. *translucens* 0790-9 and nine strains of *X. campestris* from ranunculus (one isolated from seed, three from diseased leaves, two from necrotic fresh tubers, and three from dried tubers) in tobacco leaves were not typical of HR and ranged from mild chlorotic to mild necrotic reactions.

The same bacterial strains that were used for tobacco inoculations were also inoculated into cauliflower plants, but only *X. c.* pv. *campestris* 0186-1 produced typical black rot symptoms. The other bacteria tested, including the nine strains from ranunculus, failed to incite a disease reaction.

Ranunculus plants that were spray-inoculated with *X. campestris* 0394-13 and maintained on the greenhouse bench or in the growth chamber remained symptomless for the 10-week duration of the test. Although no attempt was made to test the epiphytic survival of the inoculated bacteria on those plants, their tubers were assayed, and no *Xanthomonas* was recovered. In contrast, disease symptoms appeared on

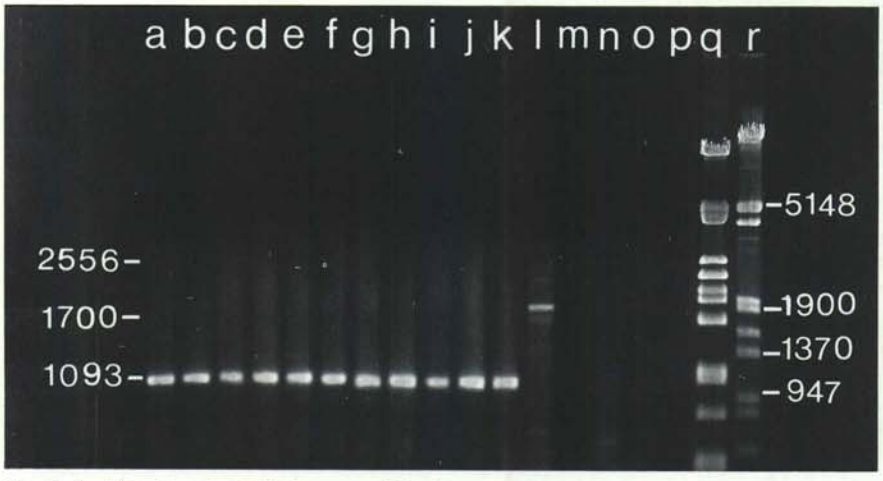

Fig. 2. Amplification of a DNA fragment of the *hrp* gene cluster from strains of *Xanthomonas campestris*. (a) *X. campestris* strain from ranunculus seed. (b–d) Three strains from ranunculus leaf with symptoms. (e–f) Two strains from fresh tubers. (g–i) Three strains from dried tubers. (j) *X. c.* pv. *campestris* 0186-1. (k) *X. c.* pv. *vesicatoria* 0788-2. (l) *X. c.* pv. *translucens* 0790-9. (m) *Erwinia carotovora* 0692-10. (n) *Agrobacterium tumefaciens* 0782-56. (o) *Pseudomonas syringae* pv. *syringae* 0584-6. (p) *P. s.* pv. *tomato* 0683-23. (q–r) Molecular size markers.

plants placed in the mist chamber for 13 days after spray-inoculation. Leaves developed water-soaked, chlorotic spots in interveinal and marginal areas. The lesions expanded over a period of 10 days to form large chlorotic–necrotic areas (Fig. 1G), which caused the collapse of the entire leaf within 10 to 15 days after lesion development. *X. campestris* was isolated from the diseased leaves but not from the tuber tissues. Pathogenicity and identification with Biolog was confirmed with a representative isolate from the diseased leaves.

When bacteria were applied to the leaves by infiltration, water-soaking occurred within 24 h, and lesions turned into brown-black necrotic areas after 48 h. Chlorotic halos started to appear around the necrotic lesions after 3 to 4 days (Fig. 1H). Further progress of the disease depended mainly on the availability of moisture. In the mist chamber, the entire leaf became necrotic within 10 to 15 days; whereas in the greenhouse, necrosis occurred in 3 to 4 weeks. Necrosis developed in 4 to 5 weeks in the growth chamber. The tuber tissues from six of the 45 plants inoculated with 0394-13 by infiltration tested positive for the presence of *X. campestris*; these included three of the 15 plants from the first experiment, one from the second, and two from the third. Pathogenicity and identification by Biolog was confirmed for each of these six isolates from tubers and three isolates from leaf symptoms. This infiltration method was also used to confirm pathogenicity of each of the 36 strains of *X. campestris* isolated from naturally infected ranunculus leaves and contaminated tubers and seed lots in this study.

Application of strain 0394-13 directly to needle-induced wounds produced reactions similar to those obtained from infiltration. Symptoms appeared as small (0.5 to 1.0 mm diameter) necrotic specks with halos (Fig. 1I) after 6 days incubation in the mist chamber and after 22 days incubation in the greenhouse or in the growth chamber. Lesions expanded over time, developing large necrotic sectors surrounded by chlorotic halos (Fig. 1J and K). Recovery of *X. campestris* from the tubers of plants in this test was 13.3, 6.6, and 13.3% for plants in the mist chamber, growth chamber, and greenhouse, respectively. Pathogenicity and identification by Biolog was confirmed for each of the five isolates from tubers and three isolates from diseased leaves.

In general, disease symptoms on inoculated plants were similar to those of naturally infected plants in commercial fields (Fig. 1B, C, D, E, G, I, J, and K). However, the severity of the disease in our study was less than what we had observed in commercial fields. When bacteria were applied directly to wounds or infiltrated into leaves, a few plants also developed black patches along the margins of their leaves, which became associated with vein yellowing, a symptom observed occasionally on infected plants from the field. *X. campestris* was readily isolated from these yellow veins and black spots. No symptoms were observed on control plants, and no *X. campestris* was isolated from leaves or tuber tissues of those plants.

Inoculation of ranunculus leaves with *X. c.* pv. *campestris* 0186-1 by infiltration resulted in an apparent hypersensitive response within 48 h. A dry, brown-black necrosis occurred, which did not spread beyond the initial infiltrated area during the test period (Fig. 1L). HR did not occur with infiltration of *X. c.* pv. *vesicatoria* 0788-2 or *X. c.* pv. *translucens* 0790-9 into ranunculus leaves.

Amplification of *hrp*-related DNA fragments. Primer pairs RST21 and RST22 were used to amplify DNA sequences from strains recovered from ranunculus leaves, tubers, and seeds, as well as a few known bacteria. Fragments of the same size (1.0 kb) were amplified from all strains of *X. campestris* from ranunculus, as well as from *X. c.* pv. *campestris* 0186-1 and *X. c.* pv. *vesicatoria* 0788-2 (Fig. 2). No amplification occurred with purified total genomic DNA from strains of *Agrobacterium tumefaciens* 0782-56, *Erwinia carotovora* 0692-10, *Pseudomonas syringae* pv. *syringae* 0584-6, or *P. s.* pv. *tomato* 0683-23. A fragment of much lower yield and larger size (1.9 kb) was produced from DNA of *X. c.* pv. *translucens* 0790-9 (Fig. 2). Leite et al. (10) reported that no DNA amplification occurred with genomic DNA from *X. c.* pv. *translucens*. When we used the methods of Leite et al. (10), no amplification was observed for *X. c.* pv. *translucens* 0790-9 DNA. In addition, DNA fragment amplification from ranunculus strains was weaker using the procedures described by Leite et al. (10).

Restriction endonuclease analysis of *hrp*-related DNA fragments. To examine relatedness of different strains of *X. campestris* from ranunculus, the 1-kb *hrp*-related DNA fragments amplified from ranunculus strains and two *X. campestris*

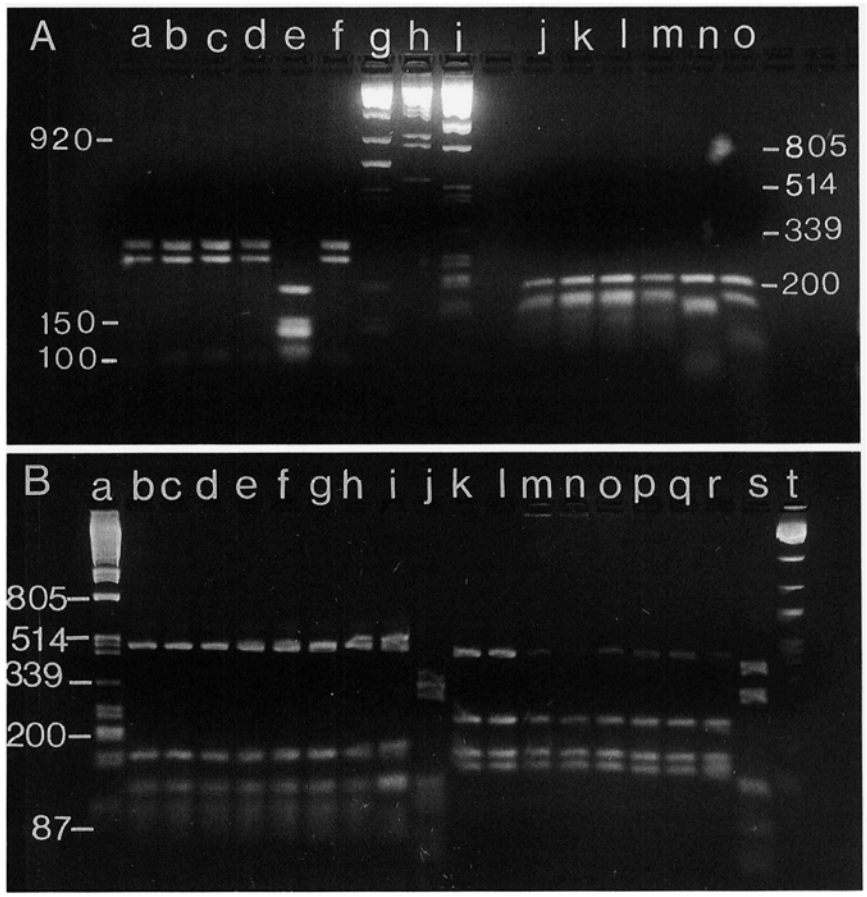

Fig. 3. Restriction endonuclease analysis of a 1-kb DNA fragment of the *hrp* gene cluster amplified from different strains of *Xanthomonas campestris* and digested with *Hae*III and *Hha*I **(A)** and *Sau*3AI and *Taq*I **(B)**. **(A)** Lanes a–f (*Hae*III digests) and lanes j–o (*Hha*I digests) of fragments amplified from strains of *X. campestris* from ranunculus seed (a and j), dried tuber (b and k), leaf (c and l), fresh tuber (d and m), and fragments amplified from *X. c.* pv. *vesicatoria* 0788-2 (lanes e and n) and *X. c.* pv. *campestris* 0186-1 (lanes f and o). Lanes g–i, molecular size markers. **(B)** Lanes b–j (*Sau*3AI digests) and lanes k–s (*Taq*I digests) of fragments amplified from strains of *X. campestris* from ranunculus seed (lanes b and k), leaves (c–e and l–n), fresh tuber (f and o), dried tubers (g–h and p–q), and fragments amplified from *X. c.* pv. *campestris* 0186-1 (i and r) and *X. c.* pv. *vesicatoria* 0788-2 (j and s). Lanes a and t, molecular size markers.

pathovars were digested with restriction endonucleases *Hha*I, *Hae*III, *Sau*3AI, and *Taq*I. Restriction fragment length polymorphisms (RFLPs) were apparent between *X. c.* pv. *vesicatoria* 0788-2 and ranunculus strains, but the banding patterns were more similar between *X. c. pv. campestris* 0186-1 and ranunculus strains (Fig. 3A and B). The digested fragments from *X. c.* pv. *campestris* 0186-1 and ranunculus strains had the same pattern of four, five, and four major fragments, ranging in size from 90 bp to 600 bp, when digested with *Hae*III, *Sau*3AI, and *Taq*I, respectively. In contrast, there were slight differences in banding patterns for fragments smaller than 150 bp when enzyme *Hha*I was used with fragments from *X. c.* pv. *campestris* 0186-1 versus the ranunculus strains.

DISCUSSION

A bacterial leaf spot of ranunculus, characterized by the appearance of necrotic lesions that expand and lead to the death of leaves and the collapse of the entire plant, was caused by *X. campestris*. To our knowledge, these disease symptoms have not been previously reported on ranunculus, and ranunculus has not been previously reported as a host of *X. campestris*.

Since an extensive host range study was not conducted, we do not know if the strains from ranunculus represent a new pathovar of *X. campestris*. The identification of strains of *X. campestris* at the pathovar level is difficult. Different techniques, such as serology (1), metabolic profile (6,15), fatty acid analysis (6,7), SDS–polyacryamide gel electrophoresis of proteins (16), and various nucleic acid analyses (14), have been used for this purpose. Recently, Leite et al. (10) showed that restriction endonuclease analysis of amplified *hrp*-related fragments may be a valuable tool for identification of subgroups of phytopathogenic strains and pathovars of *X. campestris*. We used a pair of oligonucleotide primers specific for the *hrp* region of *X. c.* pv. *vesicatoria* to amplify *hrp*-related DNA fragments from the ranunculus strains. The amplified fragments were identical in size for each strain from ranunculus, and for *X. c.* pv. *vesicatoria* and *X. c.* pv. *campestris*. This supported the observation that ranunculus strains are pathogenic, because these primers had failed to amplify DNA from nonphytopathogenic xanthomonads (10). Restriction endonuclease analysis of amplified fragments showed that *X. c.* pv. *campestris* 0186-1 and ranunculus strains had similar RFLP patterns and therefore may be closely related. However, the failure of ranunculus strains to cause disease in cauliflower and a positive hypersensitive response observed with infiltration of *X. c.* pv. *campestris* 0186-1 cells into ranunculus plants indicate that they are different pathovars.

Negative or atypical HR obtained with infiltration of ranunculus strains into tobacco indicates a lack of relationship between pathogenicity and tobacco hypersensitivity. This is not unique to this pathogen and has been previously reported (3).

When ranunculus plants were spray-inoculated with bacterial suspensions, infection occurred only on misted plants, where free moisture existed. Free moisture also frequently develops along the Pacific coast in southern California where most of the disease outbreaks occurred. Failure to isolate *Xanthomonas* from the roots of infected plants in the mist chamber may have been due to development of the disease and death of the plants in such a short time that the pathogen did not have a chance to move down to the roots. The importance of moisture in infection and disease progress was also evident when inoculation was done by infiltration or by application of bacteria to wounds. Although disease symptoms on inoculated plants were similar to those of natural infections in the field, severity of the disease was less in the greenhouse. The severity of the disease may depend on combinations of humidity and temperature, conditions that we were not able to duplicate in the greenhouse.

Isolation of *Xanthomonas* from seeds and tubers of naturally infected ranunculus plants and from tubers of inoculated plants suggests a means by which the pathogen is spread in the industry. Therefore, we speculate that the appearance of this pathogen in commercial fields is most likely to have occurred by introduction of infected seeds and tubers. Seed contamination among different varieties suggests that the pathogen is not restricted to a particular variety. This is supported by observation of the disease on different varieties in the field. We are currently evaluating the effectiveness of several seed treatment methods for controlling the seedborne spread of the ranunculus strains of *X. campestris* in commercial plantings.

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LITERATURE CITED

1. Alvarez, A. M., Benedict, A. A., Mizumoto, C. Y., Pollard, L. W., and Civerolo, E. L. 1991. Analysis of *Xanthomonas campestris* pv. *citri* and *X. c. citrumelo* with monoclonal antibodies. Phytopathology 81:857-865.
2. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds. 1987. Current Protocols in Molecular Biology. John Wiley & Sons, New York.
3. Azad, H. R., and Kado, C. I. 1984. Relation of tobacco hypersensitivity to pathogenicity of *Erwinia rubrifaciens*. Phytopathology 74:61-64.
4. Baker, K. F., ed. 1957. The U. C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Man. 23.
5. Cease, K. B., Potcova, C. A., Lohff, C. J., and Zeigler, M. E. 1994. Optimized PCR using Vent polymerase. PCR Meth. Appl. 3:298-300.
6. Chase, A. R., Stall, R. E., Hodge, N. C., and Jones, J. B. 1992. Characterization of *Xanthomonas campestris* stains from aroids using physiological, pathological, and fatty acid analyses. Phytopathology 82:754-759.
7. Graham, J. H., Hartung, J. S., Stall, R. E., and Chase, A. R. 1990. Pathological, restriction-fragment length polymorphism, and fatty acid profile relationships between *Xanthomonas campestris* from citrus and noncitrus hosts. Phytopathology 80:829-836.
8. Keane, P. J., Kerr, A., and New, P. B. 1970. Crown gall of stone fruit. II. Identification and nomenclature of *Agrobacterium* isolates. Aust. J. Biol. Sci. 23:585-595.
9. Krieg, N. R., and Holt, J. G., ed. 1984. Bergey's Manual of Systematic Bacteriology. Vol. 1. Williams and Wilkins, Baltimore, MD.
10. Leite, R. P., Jr., Minsavage, G. V., Bonas, U., and Stall, R. E. 1994. Detection and identification of phytopathogenic *Xanthomonas* strains by amplification of DNA sequences related to the *hrp* genes of *Xanthomonas campestris* pv. *vesicatoria*. Appl. Environ. Microbiol. 60:1068-1077.
11. McGuire, R. G., Jones, J. B., and Sasser, M. 1986. Tween media for semiselective isolation of *Xanthomonas campestris* pv. *vesicatoria* from soil and plant material. Plant Dis. 70:887-891.
12. Miller, T. D., and Schroth, M. N. 1972. Monitoring the epiphytic population of *Erwinia amylovora* on pear with a selective medium. Phytopathology 62:1175-1182.
13. Schaad, N. W., ed. 1988. Laboratory Guide for Identification of Plant Pathogenic Bacteria. American Phytopathological Society, St. Paul, MN.
14. Stall, R. E., and Civerolo, E. L. 1991. Research relating to the recent outbreak of citrus canker in Florida. Annu. Rev. Phytopathol. 29:399-420.
15. Van den Mooter, M., and Swings, L. 1990. Numerical analysis of 295 phenotypic features of 266 *Xanthomonas* strains and an improved taxonomy of the genus. Int. J. Syst. Bacteriol. 40:348-369.
16. Vauterin, L., Swings, J., and Kersters, K. 1991. Grouping of *Xanthomonas campestris* pathovars by SDS-PAGE of proteins. J. Gen. Microbiol. 137:1677-1687.
17. Wilson, E. E., Zeitoun, F. M., and Fredrickson, D. L. 1967. Bacterial phloem canker, a new disease of Persian walnut trees. Phytopathology 57:618-621.