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DISSERTATION

XANTHOMONAS LEAF BLIGHT OF ONION: BIOLOGY, EPIDEMIOLOGY, AND MANAGEMENT

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

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COLORADO STATE UNIVERSITY

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DAVID H. GENT ENTITLED XANTHOMONAS LEAF BLIGHT OF ONION: BIOLOGY, EPIDEMIOLOGY, AND MANAGEMENT BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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Committee on Graduate Work

Advisor

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ABSTRACT OF DISSERTATION

XANTHOMONAS LEAF BLIGHT OF ONION: BIOLOGY, EPIDEMIOLOGY, AND MANAGEMENT

Xanthomonas leaf blight of onion is a yield limiting disease of onion in Colorado. Sustainable management strategies have not been developed because basic elements of pathogen and disease biology are unknown. The objective of this dissertation was to develop sustainable Xanthomonas leaf blight management strategies from a basic understanding of pathogen and disease biology. The pathogenic, phenotypic, and pathogenic diversity of forty-nine onion *Xanthomonas* strains were determined. Multiplication of onion *Xanthomonas* strain R-O177 was not different than *X. axonopodis* pv. *phaseoli* in dry bean, but typical common bacterial blight disease symptoms were absent. All strains were sensitive to 100 μg/ml $CuSO_4$, $ZnSO_4$, or streptomycin sulfate. Logistic regression of fatty acid and substrate utilization profiles classified 69% of strains into their geographical region of origin. Rep-DNA fingerprinting revealed five genotype groups within onion *Xanthomonas* strains. Based upon pathogenicity to onion and genomic fingerprinting, the onion *Xanthomonas* strains examined in this study are *X. axonopodis* pv. *allii* (Xaa).

Xaa inoculum sources are unknown, but this knowledge is essential to disease management. To identify these sources, epiphytic xanthomonad populations on weed, volunteer onion, and leguminous plants were quantified at five locations in eastern Colorado over 2003 and 2004. Irrigation water and crop debris were sampled for Xaa populations in experimental plots near Fort Collins and Rocky. Xaa was recovered from irrigation water, weed, volunteer onion, and leguminous plants. The bacterium survived in unincorporated crop debris, but not in buried debris. Epiphytic populations of Xaa (10^2 to 10^3) survived on dry bean and lentil under field conditions; epiphytic *X. axonopodis* pv. *phaseoli* were recovered from onion following dry bean. Elimination of these inoculum sources should reduce Xanthomonas leaf blight losses.

Alternatives to copper bactericides are needed to reduce use of high-risk pesticides. Studies evaluated alternative treatments and determined the critical period for conventional copper applications. Acibenzolar-S-methyl was as effective as copper applications in suppressing Xathomonas leaf blight in field trials. The critical period ranged from 1 to 2 weeks before bulb initiation. Adoption of disease forecasting and novel chemical treatments such as acibenzolar-S-methyl may reduce copper bactericide use for Xanthomonas leaf blight management in Colorado.

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CHAPTER I

INTRODUCTION

Onion (*Allium cepa*) and dry bean *(Phaseolus vulgaris*) are economically important crops in Colorado and the Central High Plains region (Colorado, Wyoming, Nebraska), generating over $60 and $43 million in farm receipts annually, respectively (2). Onions are produced on 6,000 to 8,000 hectares annually in northeastern Colorado, the Arkansas Valley, and western slope production areas (15). Dry beans are grown on 175,000 to 250,000 hectares annually in the Central High plains in both irrigated (90%) and dryland (10%) production systems (18).

Xanthomonas leaf blight, caused by a *Xanthomonas* species, has emerged as a new and serious disease of onion in Colorado (16,17), as well as other western U.S. onion production areas (5,7); and is a potential threat to pulse crop production (8). This pathogen and its disease were apparently favored by unusually prolonged periods of rain, high humidity and dew that accompanied the weather patterns induced by 'El Nino' events that flowed into Colorado during recent years, especially during 1997. Storm damage, high winds, irrigation water, equipment and workers all presumably contributed to the secondary movement of the pathogen and disease severity which affected onion in Colorado production areas (16). This disease was first reported in Hawaii in 1978 (1), but has since increased its distribution in the continental United States (5,7,14,17), several East Caribbean islands (9), Reunion Island (10,12), South Africa (20), and Asia (6).

Annual losses associated with Xanthomonas leaf blight in Colorado vary depending on climatic conditions, but have been in excess of 17% in marketable yield during favorable years (16). Entire crop losses have been reported in other production areas (20). Although no management strategies have been reported in the literature (4), Colorado producers rely largely upon copper-based bactericides tank-mixed with ethylenebisdithiocarbamate (EBDC) fungicides to suppress disease (16). EBDC fungicides enhance the activity of copper bactericides, dramatically improving efficacy against onion bacterial pathogens (16). Control of onion bacterial diseases is marginal without EBDC fungicides. The loss of EBDC fungicides, although not used directly to control onion bacterial diseases, would significantly increase losses. EBDCs are important, economical, and widely-used fungicides for fungal diseases such as purple blotch (caused by *Alternaria porri*) and downy mildew (caused by *Peronospora destructor*). Growers routinely apply bactericides every 3 to10 days, with applications initiated before bulbing and continuing until harvest. Currently, the timing of initial applications is based upon crop phenology. A weather-driven forecast model for the initial appearance of Xanthomonas leaf blight was recently developed (19). The appearance of leaf blight (expressed as growing degree-days) is strongly associated with July mean daily high temperature (T_{jmax}) and August cumulative precipitation (P_a) as is described by the plane bounded by $GDD=-6153.43 +215.5T_{jmax} -0.92P_a$. This model explains 99.5% ($r^2=.995$, $p<.0001$) of the variability in initial Xanthomonas appearance in southern Colorado. Bactericides applied according to this or other forecast systems may prevent unnecessary early season sprays, but this model has not been validated under field conditions. The intelligent and judicious use of pesticides in a broader integrated pest management (IPM) context will improve grower profitability and sustainability, as well as reduce public exposure to pesticides and possible residues.

Pesticides used to suppress this and other onion bacterial diseases are relatively inexpensive, but growers may experience severe economic impacts because of the 1996 Food Quality and Protection Act (FQPA) restrictions and/or elimination of currently effective

management tools. The threat of Xanthomonas leaf blight to other onion and pulse crop production regions, potential loss of EBDC fungicides and copper-based bactericides, as well as public and stakeholder concerns surrounding pesticide exposure underscore the need for new multi-tactic pest management strategies.

A more complete understanding of this pathogen's survival and ecology in relation to cropping systems and cultural practices is necessary to develop effective and durable IPM strategies that will reduce crop losses and reliance upon pesticides. The current body of knowledge is devoid of any studies on the survival and management of onion strains of *Xanthomonas* in relation to onion cultural practices including irrigation method, harvest practices, and fertility. Ecologically-based management approaches developed from a basic knowledge of pathogen biology and epidemiology should reduce or eliminate the use of copper-based bactericides and EBDC fungicides, improve grower profitability, and sustainability.

Onion strains of *Xanthomonas* have been reported to infect various pulse crops including dry bean (8), but no research has evaluated dry bean as an alternate host despite common dry bean-onion rotational schemes in many onion production regions in the western U.S., including organic production. Similarly, no study has evaluated the pathogenicity of legume isolates of *X. campestris* (i.e., *X. campestris* pv. *glycines*, *X. axonopodis* pv. *phaseoli, X. axonopodis* pv. *alfalfae*) on onion.

A more complete understanding of this pathogen's epiphytic survival and ecology in relation to cropping systems and cultural practices is necessary to develop and implement effective and durable IPM strategies that will reduce crop losses and reliance upon pesticides. The current body of knowledge is devoid of any studies on the survival and management of onion *Xanthomonas* strains in relation to onion cultural practices including irrigation method, harvest practices, and fertility.

Distribution and Description of Xanthomonas Leaf Blight

Xanthomonas leaf blight, caused by an unknown *Xanthomonas* species, was observed on the island of Molokai, Hawaii in 1975 on the sweet yellow onion cv. 'Granex 33' (1); they noted that infected leaves had lenticular water-soaked lesions which elongated into chlorotic streaks as the disease progressed. Tip dieback, stunting and premature death of infected leaves resulted in severe reductions in marketable yield of bulbs. The disease description was similar to a bacterial blight of onion reported in Colorado 25 years earlier and described as *Xanthomonas striaformans*, but the bacterium differed in 8 physiological characters (21). Paulraj and O'Garro (9) later reported *X. campestris* on onion in Barbados and suggested that it may have been responsible for an onion blast disease as early as 1971. The disease has since been reported in Texas (5), Colorado (17), South Africa (20), Reunion Island (10), Japan (6), California (7), and Georgia (14). The rapid international spread of this disease emphasizes the need to develop economical and effective IPM strategies to suppress future epidemics in other western production areas where onion is a valuable crop.

On dry bean, common bacterial blight is caused by X. *axonopodis* pv *phaseoli* (Smith) Dye. Diseased plants exhibit a scalded appearance on leaf tissue and contain water-soaked spots that enlarge to form dark brown lesions along leaflets. Pods are also infected and often cause discoloration, shriveling and bacterial contamination of seeds (13). Direct yield losses in excess of 38% have been reported (13), but reduced seed quality, marketability, and loss of certified seed status could further increase losses. Strains from Barbados are reportedly pathogenic to leguminous hosts such as snap bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*), soybean (*Glycine max*), winged bean (*Psophocarpus tetragonolobus*), moth bean (*Vigna aconitifolia*), and field pea (*Pisum sativum*), but onion has not been evaluated as a host for *X. axonopodis* pv *phaseoli*. Disease symptoms from onion isolates on legume hosts were typical of common bacterial blight, and included water-soaked lesions, necrosis and eventual defoliation. It

is unknown if isolates from infested bean can infect onion plants in adjacent fields or during succeeding seasons in the western U.S.

Disease Management

The literature is largely devoid of bacterial disease management recommendations for onion. Copper- and zinc-based bactericides are generally ineffective for managing Xanthomonas leaf blight in Barbados (9), but offer some benefit under light to moderate disease pressure as experienced recently in Colorado (16). *Pantoea agglomerans* appeared promising as a biocontrol agent in growth chamber studies, but no field level studies are reported (9). Two resistant onion genotypes were identified from commercially available cultivars, but resistance appeared to be mono- or oligogenic and may be overcome by new or current strains of the pathogen. An extensive review of reported resistance traits in commercial onion cultivars in the U.S. indicated no resistance has been identified to Xanthomonas leaf blight (Schwartz, unpublished). The lack of durable resistance in commercial onion cultivars emphasizes the need for new and effective cultural and ecologically-based practices to manage the disease. Pathogen-free seed or effective seed treatments may be essential as the pathogen can be recovered from seed (10), and contaminated seed can initiate epidemics in tropical production environments (11).

Management of common bacterial blight of dry bean emphasizes clean seed and seed treatments, crop rotation, sanitation, resistant cultivars, and cultural practices that minimize periods of canopy wetness (18). Despite these measures, copper-based bactericides are regularly applied preventatively to suppress disease. Additionally, weeds and nonhost plants can be epidemiologically important sources of epiphytic xanthomonads, allowing for pathogen survival during periods without a suitable host (3). No studies have examined dry bean as an alternate host or reservoir for epiphytic onion strains of *Xanthomonas*.

LITERATURE CITED

1. Alvarez, A. M., Buddenhagen, I. W., Buddenhagen, E. S., and Domen, H. Y. 1978. Bacterial blight of onion, a new disease caused by *Xanthomonas* sp. Phytopathol. 68:1132-1136.

2. Ament, D. 2004. Colorado Agricultural Statistics, Annual Report. Colorado Department of Agriculture. 146 pp.

3. Cafati, C. R., and Saettler, A. W. 1980. Role of nonhost species as alternate inoculum sources of *Xanthomonas phaseoli*. Plant Dis. 64:194-196.

4. Gitaitis, R. D., Davis, R. M., and Mohan, S. K. 1995. Diseases caused by bacteria and a yeast. Pp. 31-33 in: Compendium of Onion and Garlic Diseases. Edited by H. F. Schwartz and S. K. Mohan. APS Press, St. Paul, MN. 54 pp.

5. Isakeit, T., Miller, M. E., Barnes, L. W., Dickstein, E. R., and Jones, J. B. 2000. First report of leaf blight of onion caused by *Xanthomonas campestris* in the continental United States. Plant Dis. 84:201.

6. Kadota, I., Uehara, K., Shinohara, H., and Nishiyama, K. 2000. Bacterial blight of Welsh onion: A new disease caused by *Xanthomonas campestris* pv. *allii* pv. *nov.* J. Gen. Plant Pathol. 66:310-315.

7. Nunez, J J., Gilbertson, R. L., Meng, X., and Davis, R. M. 2002. First report of Xanthomonas leaf blight of onion in California. Plant Dis. 86:330.

8. O'Garro, L. W., and Paulraj, L. P. 1997. Onion leaf blight caused by *Xanthomonas campestris*: Alternative hosts and resistant onion genotypes. Plant Dis. 81:978-982.

9. Paulraj, L., and O'Garro, L. W. 1993. Leaf blight of onions in Barbados caused by *Xanthomonas campestris*. Plant Dis. 77:198-201.

10. Roumagnac, P., Gagnevin, L., and Pruvost, O. 2000. Detection of *Xanthomonas* sp., the causal agent of onion bacterial blight, in onion seeds using a newly developed semi-selective isolation medium. Eur. J. Plant Path. 106:867-877.

11. Roumagnac, P., Pruvost, O., Chiroleu, F., and Hughes, G. 2004. Spatial and temporal analyses of bacterial blight onion caused by *Xanthomonas axonopodis* pv. *allii*. Phytopathol. 94:138-146.

12. Roumagnac, P., Gagnevin, L., Gardan, L., Sutra, L., Manceau, C., Dickstein, E. R., Jones, J. B., Rott, P., and Pruvost, O. 2004. Polyphasic characterization of xanthomonads isolated from onion, garlic and Welsh onion (*Allium* spp.) and their relatedness to different *Xanthomonas* species. Int. J. Syst. Evol. Microbio. 54:15-24.

13. Saettler, A. W. 1989. Common bacterial blight. Pp 261-283 in: Bean Production Problems in the Tropics. Edited by H. F. Schwartz and M. A. Pastor-Coarrales. CIAT, Cali, Colombia.

14. Sanders, F. H., Langston, D. B., Brock, J. H., Gitaitis, R. D., Curry, D. E., and Torrance, R. L. 2003. First report of a leaf blight of onion caused by *Xanthomonas* spp. in Georgia. Plant Dis. 87:749.

15. Schwartz, H.F., and Bartolo, M.E. 1995. Colorado Onion Production and Integrated Pest Management. Bulletin 547A. Colo. State Univ., Ft. Collins, 39 pp.

16. Schwartz, H. F., and Otto, K. J. 1998. Onion bacterial disease management in Colorado. Pp. 214-218 in: Proc. of the 1998 National Onion (and other *Allium*) Research Conference, Dec. 10-12, 1998, Sacramento, CA.

17. Schwartz, H. F., and Otto, K. 2000. First report of a leaf blight of onion by *Xanthomonas campestris* in Colorado. Plant Dis. 84:922.

18. Schwartz, H.F., Brick, M.A., Nuland, D.S., and Franc, G.D. (Editors). 1996. Dry Bean Production and Pest Management. Reg. Bull. No. 562A, Fort Collins, CO. 106 pp.

19. Schwartz, H. F., Otto, K. L., and Gent, D. H. 2003. Relation of temperature and rainfall to development of Xanthomonas and Pantoea leaf blights of onion in Colorado. Plant Dis. 87:11-14.

20. Serfontein, J. J. 2001. Xanthomonas blight of onion in South Africa. Plant Dis. 85:442.

21. Thomas, W. D. Jr., and Weinhold, A. R. 1953. *Xanthomonas striaformans*, a new bacterial pathogen of onion. J. of the Colo.-Wyo. Academy of Science 4:22.

CHAPTER II

POLYPHASIC CHARACTERIZATION OF XANTHOMONAS STRAINS FROM ONION

INTRODUCTION

A leaf blight of onion caused by *Xanthomonas* (Pammel) Dowson strains has appeared as a major disease of onion since it was first described in Hawaii in 1978 (2). Disease symptoms are varied and include leaves with lenticular watersoaked lesions that elongate into chlorotic streaks, necrosis, tip dieback, and stunting of plants that reduces bulb size. A bulb rot has never been reported and apparently does not occur, but yield losses of 19% to 100% have been reported (26,29,36).

Since its original description by Alvarez et al. (2), the disease has been reported in Texas (14), Colorado (37), California (26), Barbados (29), South Africa (38), Reunion Island, France (33), and Georgia (35). Unreported epidemics of the disease have also been observed in Venezuela and Brazil (R. Gitaitis, *personal communication*). A similar disease caused by strains classified as *X. striaformans* was reported on onion in southern Colorado in 1953 (40), but differed in 8 of 18 physiological characteristics from the *Xanthomonas* sp. described by Alvarez et al. (2). A bacterial blight of Welsh onion (*Allium fistulosum* L.) classified as *X. campestris* pv. *allii* has also been described in Japan (16), but its relationship to other strains causing disease on *Allium cepa* L. is unknown.

The host range of *Xanthomonas* isolated from onion remains uncertain. Strains from Barbados are reportedly pathogenic to leguminous hosts such as snap bean (*Phaseolus vulgaris* L.), lima bean (*Phaseolus lunatus* L.), soybean [*Glycine max* (L.) Merr.], winged bean [*Psophocarpus tetragonolobus* (L.) DC.], moth bean [*Vigna aconitifolia* (Jacq.) Marechal], and field pea (*Pisum sativum* L.) (27), as well as other *Alliums* including leek (*A. ampeloprasum* var. *porrum* L.), chives (*A. schoenoprasum* L.), garlic (*A. sativum* L.), and shallot (*A. cepa* var. *aggregatum*) (8). Strains from Hawaii, however, induced a hypersensitive response in snap bean (2). *X. campestris* pv. *allii* are nonpathogenic to chive and Chinese chive (*A. tuberosum* Rottler) (16).

The diversity among strains of *Xanthomonas* strains causing disease on onion is unknown, as is their relationship to other species and pathovars of *Xanthomonas*. Therefore, this study was initiated to characterize a broad collection of onion *Xanthomonas* strains from multiple geographic locations over many years by a polyphasic approach. A preliminary report of this work has been presented previously (12).

MATERIALS AND METHODS

Bacterial strains, culture, and DNA isolation. Forty-nine strains of *Xanthomonas* originally isolated from *Allium cepa* or *Allium fistulosum* were obtained from California, Texas, Colorado, Georgia, Hawaii, Brazil, Venezuela, Barbados, South Africa, and Japan, including the type strain of *X. campestris* pv. *allii* (MAFF 311173) (Table 1). Spontaneous rifampicin and streptomycin mutants of onion *Xanthomonas* strain O177 (ATCC 508) and *X. axonopodis* pv. *phaseoli* strain B458, respectively, were generated as previously described (44) and are referred to as R-O177 and AS-B458, respectively. All antibiotics were purchased from Sigma Chemical Co. (St. Louis, MO). Strain B458 was isolated from dry bean cultivar Bill Z in southwestern Colorado. Strain R-O177 and AS-B458 are resistant to greater than 200 μg/ml rifampicin or streptomycin,

respectively, but selection routinely was performed on nutrient agar amended with 50 μg/ml of the appropriate antibiotic. Other strains were routinely cultured on nutrient agar or broth lacking antibiotic during incubation at 26°C, and bacterial strains were preserved in 15% nutrient glycerol broth at -80°C for long term storage.

Strains were cultured in 1.5 ml of nutrient broth for 24 hours for DNA isolation procedures. The culture was adjusted to an optical density of 0.1 at 600 nm in sterile 0.02 M potassium phosphate buffer pH 7.2 (PB), and DNA was isolated using the CTAB (hexadecyltrimethylammonium bromide) method (4). DNA was stored in Tris-EDTA buffer (TE, 10 mM Tris, 1 mM EDTA, pH 8.0) at –20°C.

Pathogenicity and host range. All strains were tested for pathogenicity on *A. cepa* (cv. Blanco Duro) in growth chamber assays. Pathogenicity on *A. fistulosum* (cv. White Welsh) was confirmed with representative strains from nine geographical regions, including: O177, Calon-1, TX-3, ATCC BAA 576, Xcu 01-1, MAFF 311173, A229-1, JV 594, and JX 727. Colonies of the strain to be tested were inoculated into 3 ml of nutrient broth in 15 ml culture tubes, and were incubated at 26° C with vigorous shaking (250 oscillations/min) for 24 h. The cultures were adjusted to approximately 10^7 cfu/ml in sterile PB before spraying (Crown SpraTool, Aerovoe Industries, Inc., Gardnerville, NV) the foliage of six to eight week-old plants to runoff with the bacterial suspension. Control plants were inoculated with sterile PB. The plants were placed in a growth chamber and incubated for 7 days with a 28° C/24° C day/night temperature regime, light intensity of 350 $\mu Ms^{-1}m^{-2}$, 100% relative humidity, and daily misting with tap water to runoff. At least four plants were inoculated with each strain. Plants were observed daily for symptom development, and the pathogen was isolated from characteristic lesions by grinding leaf sections in 1 ml of PB with a sterile mortar and pestle and streaking loopfuls of the homogenate onto nutrient agar. The pathogen was confirmed as *Xanthomonas* by carbon and nitrogen substrate

utilization patterns on Biolog GN microplates (Biolog Inc., Hayward, CA) as previously described (43).

Pathogenicity on dry bean (cv. Sacramento light red kidney) was also evaluated by the spray inoculation method described for onion except plants were kept in the growth chamber for 14 days. All plants were four to five weeks old when inoculated.

Multiplication of the rifampicin resistant onion strain R-O177 and streptomycin resistant bean strain AS-B458 in inoculated dry bean, pepper (*Capsicum annuum* L., cv. Sweet California Wonder), and onion were also quantified as previously described (27). Briefly, a single colony of each pathogen was picked from a 72-hr nutrient agar plate and grown overnight in nutrient broth (26° C and 250 oscillations/min) to the mid-logarithmic phase. The culture was adjusted to approximately 10^8 cfu/ml spectrophotometrically (A600=0.12) with sterile PB, diluted to 10^4 cfu/ml in sterile PB, and pressure infiltrated into the youngest, fully expanded bean and pepper leaflets of four to five week old plants with a sterile one ml syringe. Trace levels of nutrient broth (1 µl or less per 10 ml sterile PB) from the cultivation media were also introduced into the plant during inoculation, but were assumed to not contribute to bacterial replication *in planta*. Five 20-mm^2 leaf discs were immediately removed (each from a different leaflet) with an ethanol sterilized cork-borer, and surface disinfested in 95% ethanol, followed by several rinses in sterile PB, before grinding individually in 100 µl of PB with a sterile mortar and pestle. The homogenate was serially diluted in sterile PB and 100 µl droplets were plated in duplicate onto nutrient agar with the appropriate selection antibiotic. Plants serving as controls were infiltrated with sterile PB and the homogenate was plated onto nutrient agar. Leaf discs were removed daily for seven days to generate population growth curves.

Populations in onion were determined as described by O'Garro and Paulraj (27). Briefly, the youngest, fully extended leaves of 8 week old onion plants (cv. Blanco Duro) were pinpricked seven times at 1 cm intervals with a 22 gauge needle bearing strain R-O177 or AS-B458 removed from 72-hour old nutrient agar culture plates. Each pinpricked leaf area was inoculated with a bacterial matrix approximately equal in size to the needle tip. Leaf sections (5 by 1 cm in length), each having an inoculated area, were removed every two days, surface disinfested in 95% ethanol, rinsed in sterile PB, and ground aseptically in 1 ml of sterile PB with a mortar and pestle. Recoveries were done up to 14 days after inoculation to generate population growth curves. The homogenate was serially diluted and plated onto nutrient agar amended with 50 μg per ml of the appropriate selection antibiotic. Colonies were enumerated after 72 hr of incubation at 26° C, and characteristic colonies were confirmed as strain R-O177 or AS-B458 by genomic fingerprinting with BOX primers as previously described (19). Pathogenicity of recovered onion *Xanthomonas* strain R-O177 and *X. axonopodis* pv. *phaseoli* strain AS-B458 on onion cv. Blanco Duro and dry bean cv. Sacramento light red kidney, respectively, was conducted as previously described.

Copper, zinc and streptomycin resistance screening. The minimum inhibitory concentrations (MIC) of $CuSO_4$, $ZnSO_4$ and streptomycin sulfate were determined using a modification of the broth microdilution test guidelines used from the National Committee for Clinical Laboratory Standards (25). Serial dilutions (1:2) of each bactericide were made in nutrient broth in round-bottom 96-well plates to a final volume of 100 μl per well. Each well was inoculated with 5 μl of a 10^7 cfu/ml suspension of *Xanthomonas* from a 3 ml nutrient broth overnight culture. The MIC was considered the lowest concentration of each bactericide that completely inhibited visual growth after 24 hours. Xanthomonads are generally considered resistant to copper, zinc, or streptomycin if they are able to grow on artificial media amended with 100 to 200 μg/ml of a given bactericide (6,7,17,24,39). In this study, strains were considered resistant to $CuSO_4$,

$ZnSO_4$, or streptomycin sulfate if the MIC was greater than 100 μg/ml. *X. axonopodis* pv. *vesicatoria* strains 81-23 and E3 (provided by J. Jones, Univ. of Florida) were included as copper and streptomycin resistant positive controls, respectively.

Substrate utilization and fatty acid methyl ester profiles. Substrate utilization and fatty acid methyl ester profiles were generated for all 49 onion *Xanthomonas* strains presented in Table 1 by Microbe Inotech Laboratories, Inc. (St. Louis, MO) by standard procedures (42,43). Cultures were streaked onto trypticase soy broth agar at 26°C for 24 h before inoculating onto Biolog GN microplates according to Biolog protocols for Gram negative aerobic bacteria. The resulting metabolic fingerprints were read by an automated plate reader and compared to version 4.1 of the Biolog database. Fatty acid compositions were determined by gas chromatographic analysis by standard methods and compared to the Microbial Identification, Inc (MIDI, Newark, DE) Aerobe (v. 4.1) and Clinical Aerobe (v. 4.0) databases.

***hrpB6* gene amplification and sequencing.** A portion of the highly conserved putative ATPase gene *hrpB6* was amplified from extracted genomic DNA with the oligonucletoide primers RST2 (5'-AGGCCCTGGAAGGTGCCCTGGA-3') and RST3 (5'-ATCGCACTGCGTACCGCGCGCGA-3'), which direct the amplification of an approximately 840-bp fragment (11,18). DNA was extracted from 24 strains (noted in Table 1) as previously described; one to five representative strains from each geographic location were selected for the experiment. A polymerase chain reaction (PCR) product for this gene is expected from most phytopathogenic xanthomonads, but is not expected from nonpathogenic xanthomonads which lack the *hrp* gene cluster. DNA was amplified in reaction mixtures containing 12.5 μl of Promega (Madison, WI) PCR Master Mix, 1 μM of each primer, 100 ng of CTAB extracted DNA, and PCR-grade water to a total volume of 25 μl. PCR amplification cycle conditions were as described by Leite et al. (18). Amplified DNAs were separated by electrophoresis in 1.0%

agarose (Bio-Rad Laboratories, Hercules, CA) gels in Tris-acetate buffer (TAE, 40 mM Tris-acetate, 1mM EDTA, pH 8.2). Ethidium bromide (0.5 μg/ml) was added to each gel and the DNA fragments were visualized over a UV transilluminator (Fotodyne, Hartland, WI). The approximately 840-bp amplicon was gel purified (Qiagen MinElute gel extraction kit, Qiagen Inc., Valencia, CA) and sequenced (Davis Sequencing, Davis, CA). Sequence, alignment, calculation of similarity values, and cluster analysis were performed with MegAlign software (DNASTAR Inc., Madison, WI). The National Center for Biotechnology Information GenBank non-redundant database was searched for sequence similarity using the BLASTn algorithm (1).

16S and intergenic spacer region RFLP and sequencing. Polymerase chain reaction experiments were performed as previously described (22) to amplify the entire 16S and 16S-23S rDNA internally transcribed spacer region (ITS) or only the 16S-23S rDNA ITS region using the universal primer pairs 27F (5'-AGAGTTTGATCCTGGCTCAG-3')/FGPS-132(5'-CCGGGTTTCCCCATTCGG-3') or G1(5'-GAAGTCGTAACA AGG-3')/L1(5'-CAAGGCATCCACCGT-3'), respectively. Oligonucleotide primers were synthesized by Integrated DNA Technologies (Coralville, IA). One to five representative strains from each geographic location were used in each experiment. A 10 μl aliquot of the resulting DNA fragment from the 16S and ITS region amplified by primers 27F and FGPS-132 was restricted with *Eco*RI or *Hae*III as recommended by the manufacturer (Promega Corp.) and separated by electrophoresis in 1.0% agarose (Bio-Rad Laboratories) gels, visualized, and photographed as previously described. The ITS amplicon was gel purified (Qiagen MinElute gel extraction kit, Qiagen Inc.) and sequenced (Davis Sequencing). Sequence alignment, calculation of similarity values, and cluster analysis were performed in MegAlign software. The National Center for Biotechnology Information GenBank non-redundant database was searched for sequence similarity using the BLASTn algorithm (1).

rep-PCR genomic fingerprinting. Genomic fingerprints were determined for each strain as described by Louws et al. (19-21) using primers corresponding to prokaryotic enterobacterial repetitive intergenic consensus (ERIC) sequences, repetitive extragenic palindromic (REP) sequences, and the BOXA subunit of the BOX element. *Stenotrophomonas maltophilia* was included as a positive control. Additionally, 45 other strains of phytopathogenic xanthomonads representative of the DNA:DNA homology groups described by Vauterin et al. (41) were included to determine the relationship of onion strains of *Xanthomonas* to other species and pathovars of *Xanthomonas*. Seventeen of these strains are included in this report (Table 1).

Images were imported into GelCompar software (v. 4.1 Applied Maths, Kortrijk, Belgium), linearly combined, and similarity calculated using Pearson's correlation coefficient applied to the entire densitometric curves of the gel tracks as previously described (30). Gels were standardized with an Invitrogen Corp. (Carlsbad, CA) 1kb DNA molecular weight ladder. Cluster analysis was performed using the unweighted pair group method with arithmetic averages clustering. All PCR reactions were repeated at least twice.

Statistical analysis. Analysis of *in planta* population growth was conducted in SAS v. 8.0 (PROC MIXED, SAS Institute, Cary, NC) to generate means and standard errors of log transformed data. Factor analysis was conducted on merged Biolog and fatty acid profile data sets to generate 10 unique and uncorrelated factors (SAS PROC FACTOR, SAS Institute, Cary, NC) with orthogonal varimax rotation. These factors were subsequently used in logistic regression (PROC LOGISTIC) with stepwise selection to predict the region of origin of each strain. Cluster and principal component analysis of fatty acid composition content and substrate utilization profiles were conducted with MINITAB 13 (Minitab Inc., State College, PA) using the unweighted pair-group method with arithmetic averages clustering. The percent color change in each Biolog GN2 MicroPlate well, compared to the water control, was used in cluster and

principal component analysis. Percent compositions of each fatty acid were used in the analysis of fatty acid profiles.

RESULTS

Pathogenicity and host range. All onion strains of *Xanthomonas* used in this study were pathogenic on onion except strains A1889, A1890, and TX-2b. Strain TX-2b was identified as *X. campestris* pv. *carotae* by rep-PCR, Biolog, and ITS sequence and produced typical bacterial leaf blight symptoms on carrot (*Daucus carota* L.). Characteristic lenticular shaped, water soaked lesions also developed on *A. fistulosum* within 7 days following inoculation with representative strains from nine geographical regions. Disease symptoms were not observed with any onion strain of *Xanthomonas* on dry bean cultivar Sacramento, but typical common bacterial blight symptoms were observed on dry bean 5 days after inoculating with *X. axonopodis* pv. *phaseoli* strain AS-B458.

Populations of onion *Xanthomonas* strain R-O177 increased *in planta* in dry bean and onion, but not in pepper (Figure 1). Populations of *X. axonopodis* pv. *phaseoli* strain AS-B458 and onion *Xanthomonas* strain R-O177 increased in dry bean to greater than 10^8 cfu and 10^7 cfu per 20 mm^2 leaf disk, respectively, 7 days after inoculation. Strain R-O177 increased to greater than 10^8 cfu per 1 cm leaf section in onion during the study time course. Populations of *X. axonopodis* pv. *phaseoli* decreased to less than 10^3 cfu per sample area in onion and pepper 7 days after inoculation (data not presented).

Copper, zinc and streptomycin resistance screening. Resistance to copper, zinc, or streptomycin was not observed among onion strains of *Xanthomonas*: all were inhibited by 100

μg/ml or less of copper sulfate, zinc sulfate, or streptomycin. All strains were highly sensitive to streptomycin and were inhibited by 1.6 μg/ml or less.

Substrate utilization and fatty acid methyl ester profiles. A diversity of substrate utilization and fatty acid methyl ester profiles were observed in onion strains of *Xanthomonas*. Mean Biolog and fatty acid profile similarity indices of the 49 onion *Xanthomonas* strains tested were 0.62 and 0.78, respectively, with the *X. axonopodis* profile. Corresponding distance coefficients for Biolog and fatty acid profiles were 4.428 and 2.581, respectively, compared to the *X. axonopodis* profile. The closest pathovar matches within the Biolog v. 4.1 database were *X. axonopodis* pv. *dieffenbachiae* (57%), *X. axonopodis* pv. *begonia* (16%), *X. axonopodis* pv. *phaseoli* (4%), and *X. axonopodis* pv. *malvacearum* (4%). Nearly 12% of the strains did not have a closest match and were simply identified as *Xanthomonas axonopodis*. The MIDI Aerobe (v. 4.1) and Clinical Aerobe (v. 4.0) fatty acid databases indicated onion strains of *Xanthomonas* were most closely related to *X. arboricola* pv. *poinsettiicola* (37%), *X. axonopodis* pv. *dieffenbachiae* (18%), and *X. axonopodis* pv. *citrumelo* (16%).

Biolog substrate utilization profiles revealed diversity in carbon and nitrogen utilization patterns by onion strains of *Xanthomonas*, but this diversity was not fully explained by geographic origin (Figure 2). Euclidean distances among strains were less than 13 with the exception of strains JV 594 (Brazil), ATCC BAA 576 (South Africa), and A225-2 (Hawaii). Principal component analysis did not reveal distinct clustering of strains (data not presented).

Onion strains of *Xanthomonas* contained high proportions of 15:0 ISO (26.91%) and 15:0 ISO 2OH/16:1ω7c (18.87%) fatty acids. The 10:0, 10:0 2OH, 11:0 3OH, 12:0, 13:0 ISO, 13:0 2OH, 17:1 ω6c, 17:1 10methyl, and 17:0 ISO 3OH fatty acids comprised less than 1.22% of the total fatty acid profile or were absent in all strains. Table 2 presents fatty acid composition profiles for

five representative strains that characterize the diversity of fatty acid profiles in the 49 onion strains of *Xanthomonas* included in this study. Cluster analysis of fatty acid profiles revealed phenotypic diversity in onion strains of *Xanthomonas* that was partially explained by geographic origin (Figure 3). Euclidean distances among strains were generally less than 5, except for 6 strains from Hawaii and strains O177, TX-2b, Xcu 01-1, and F2:22, suggesting little diversity in fatty acid composition. Strains from California formed a distinct group that also included Georgia strain Xcu 200-2. Japanese strains formed two distinct but highly related subgroups (Euclidean distance less than 4), but other strains were not grouped solely by geographic origin. Strains A1889 and A1890, which were not pathogenic to onion, had a nearly identical fatty acid profile, but were dissimilar from all other evaluated strains. Additionally, principal component analysis did not reveal distinct clustering of strains (data not presented). Several fatty acids were only occasionally recovered from strains or were present at low levels and may have obscured the variability among geographical regions, reducing the power of principal component analysis to separate strains by geography. Fatty acid methyl ester profiles alone were not useful for determining geographic origin of all strains.

Factor analysis of combined Biolog substrate utilization and fatty acid profile data and subsequent logistic regression yielded a model with two factors (Factors 1 and 9) that correctly placed 69.3% into their geographical region; 23.5% were misclassified and 7.2% were tied. The likelihood equations for each equation were computed $Z= \beta_0 + 0.782(\text{Factor 1}) + 0.865(\text{Factor 2})$, where β_0 is the cumulative logit intercept for each region given in Table 3. The probability of group membership of a strain in a region (Z_i) is described by $p(R_i) = e^{Zi}/(1 + e^{Zi})$, where e is the natural logarithm. The probability of group membership in Venezuela is given by $p(R_{Venezuela})=1-p(R_{Texas})$

***hrp B6* gene amplification and sequencing.** A single DNA fragment was amplified with all onion *Xanthomonas* strains examined in this study. Sequence similarity of 97 to 98% was observed with onion *Xanthomonas* strains compared to the partial *hrp* gene sequence of *X. axonopodis* pv. *vesicatoria* accession U33548, which was previously deposited in GenBank (10). Cluster analysis revealed five distinct groups which generally grouped by geographical region of origin (data not presented). Strains from Texas, South Africa, and Colorado did not group solely by geographical region of origin.

16S and internally transcribed spacer region RFLP and sequencing. All onion strains of *Xanthomonas* analyzed contained a single 16S-23S internally transcribed spacer region. Sequencing of the PCR amplified ITS region yielded an approximately 600-bp amplicon with greater than 99% sequence similarity to the ITS region of *X. axonopodis* pv. *citrumelo* strain F1 (accession AF442741), which was previously deposited in GenBank (9). All strains differed from *X. axonopodis* pv. *citrumelo* strain F1 by 5 bp or less except for strains A299-1 (9-bp) and TX-2b (18-bp). The ITS sequence of strain TX-2b was 100% similar to accession AF279428 from *X. campestris* pv. *carotae* (5). Onion strains of *Xanthomonas* also appear to contain the transfer RNA (tRNA) genes $tRNA^{Ala}$ and $tRNA^{Ile}$.

Variability within the ITS region was limited among onion strains of *Xanthomonas*. Greater than 98% sequence similarity was observed among strains evaluated, but strains from Japan, California, Brazil, Venezuela, Texas, Hawaii, and South Africa generally grouped by geographical region of origin in cluster analysis (data not presented). Strains from Colorado and Georgia did not group by region.

No polymorphisms were detected among onion strains of *Xanthomonas* evaluated when the entire 16S + ITS region was amplified and subsequently restricted with *Eco*RI or *Hae*III. However, *X.*

axonopodis pv. *phaseoli* strain B458 did exhibit a polymorphic band, approximately 150-bp in size, compared to onion strains of *Xanthomonas* when restricted with *Hae*III (data not presented), suggesting onions strains of *Xanthomonas* are genetically distinct from *X. axonopodis* pv. *allii*.

rep-PCR genomic fingerprinting. Complex DNA fingerprints were generated from genomic DNA extracted from 49 onion *Xanthomonas*, 45 other *Xanthomonas* species and pathovars, and the *Stenotrophomonas maltophilia* positive control (Figure 4). Amplified DNA fragments ranged in size from approximately 200-bp to greater than 4-kb and revealed a high degree of genetic diversity among onion strains of *Xanthomonas*. DNA fingerprint similarity among onion *Xanthomonas* strains ranged from greater than 98% to less than 50%. Onion *Xanthomonas* strains formed five distinct genotypes that were largely but not entirely explained by geographical origin. The majority of strains (30%) belonged to genotype 2, which all originated from Hawaii except for Colorado strain O177. Genotype 4 strains comprised 25% of the strains analyzed and represented strains originating from California, Georgia, Colorado, and Venezuela. Seven strains originally isolated from *A. fistulosum* in Japan, including the type strain of *X. campestris* pv. *allii*, comprised genotype 4. Thirteen percent of the strains comprised the heterogeneous genotype 5 and included strains from Brazil, South Africa, Barbados and Texas strain TX-2c. Genotype 1 comprised three strains (6%) from Texas. TX-2b had a nearly identical DNA fingerprint as the *X. campestris* pv. *carotae* type strain, and produced typical bacterial leaf blight symptoms on carrot, but was not pathogenic to onion. Strains A1890, A1889, and A91-1a did not cluster with the five other onion *Xanthomonas* genotypes.

Eight rep-PCR DNA fragments were common to the five genotype groups of onion *Xanthomonas* strains (Figure 5) and appear to be signature bands of these strains. Strains pathogenic on onion share these DNA fragments, including *X. campestris* pv. *allii* strains, independent of geographical region of origin. All eight of these bands are present in the *X. axonopodis* pv. *citrumelo* type

strain, but seven bands are also present in *X. axonopodis* pv. *alfalfae* and *X. axonopodis* pv. *betlicola*. The DNA fingerprint of *X. axonopodis* pv. *citrumelo* was nearly identical to Colorado strain O177 and grouped within the genotype 2 group (Figures 4 and 6). The *X. axonopodis* pv. *betlicola* type strain DNA fingerprint was also highly similar to genotype 2 strains. *X. axonopodis* pv. *alfalfae* grouped with genotype 4 strains from Japan, and *X. axonopodis* pv. *vesicatoria* grouped with the heterogeneous genotype 5 strains. The other pathovars of *Xanthomonas* which grouped with onion *Xanthomonas* strains all belong to DNA homology group 9-2 (41), providing strong evidence that onion *Xanthomonas* strains also belong within DNA homology group 9-2.

DISCUSSION

In this paper we have described the phenotypic and genetic diversity among strains of *Xanthomonas* causing Xanthomonas leaf blight of onion and the relationship of these strains to other described *Xanthomonas* pathovars and species. Of particular importance is the relationship of onion *Xanthomonas* to the described pathovar *X. campestris* pv. *allii*. We conclude the onion *Xanthomonas* strains examined in this study are pathovar *X. campestris* pv. *allii* based upon pathogenicity to onion, as well as phenotypic (carbon substrate utilization, fatty acid profiles) and genetic (*hrpb6* gene sequence, 16S rDNA RFLP profile, ITS sequence, rep-PCR-mediated DNA fingerprints) similarities. A pathovar is designated solely on the basis of distinctive pathogenicity to one or more plant hosts (10). The *X. campestris* pv. *allii* and onion *Xanthomonas* strains included in this study were indistinguishable based upon pathogenicity to *A. cepa* and *A. fistulosum*, and therefore satisfy the basic requirement for pathovar designation. DNA fingerprinting by rep-PCR demonstrated that although considerable genetic diversity is present within onion *Xanthomonas*, the strains included in this study clearly group with and belong to the described pathovar *X. campestris* pv. *allii*. No onion strains of *Xanthomonas*, including the type

strain of *X. campestris* pv. *allii*, grouped with the *X. campestris* DNA homology group 15 strains. However, they all grouped within the *X. axonopodis* DNA homology group 9-2, indicating the correct species epithet should be *X. axonopodis* and not *X. campestris*. Therefore, we propose onion strains of *Xanthomonas* be classified as *X. axonopodis* pv. *allii* to correctly represent their genotypic and phylogenetic relationship to other *X. axonopodis* species within DNA homology group 9-2. Onion strains of *Xanthomonas* are hereafter referred to as *X. axonopodis* pv. *allii*. The results of this polyphasic characterization and transfer of *X. campestris* pv. *allii* strains to *X. axonopodis* pv. *allii* are in agreement with similar research independently conducted by Rougmanac et al. (34).

Additionally, the high ITS sequence similarity and 16S RFLP profiles among strains suggest a close phylogenetic relationship exists within *X. axonopodis* pv. *allii*. The limited genetic diversity within the 16S rDNA region is consistent with other findings (13), and may limit utility of the ITS region for differentiating *X. axonopodis* pv. *allii* from other xanthomonads. However, the rDNA spacer region is useful for identifying other bacteria (15) and could provide presumptive identification of *X. axonopodis* pv. *allii*. Describing this relationship among these strains and between other *Xanthomonas* species and pathovars should clarify the taxonomic position of *X. axonopodis* pv. *allii*, and elucidation of this relationship should aid in identification and future epidemiological studies of *X. axonopodis* pv. *allii*.

Pathogenicity on onion and multiplication in dry bean were clearly demonstrated, but the absence of disease symptoms on dry bean suggests most *X. axonopodis* pv. *allii* strains may be only weakly virulent on dry bean and perhaps other pulse crops. Alternatively, dry bean may be a nonsymptomatic host of some *X. axonopodis* pv. *allii* strains. Pathogenicity and/or virulence on alternate hosts (i.e., pulse crops) appears to be highly variable among populations of *X. axonopodis* pv. *allii*. In this study, bean common bacterial blight disease symptoms were absent

with all *X. axonopodis* pv. *allii* strains evaluated, but the Barbados population is reportedly highly virulent on dry bean and other pulse crops (27). Although most *X. axonopodis* pv. *allii* strains appear weakly virulent, dry beans may serve as a reservoir of *X. axonopodis* pv. *allii* surviving as weak pathogens or epiphytically. Greenhouse and field studies are in progress to quantitate the survival of *X. axonopodis* pv. *allii* between onion crops on dry bean and other hosts.

Resistance to commonly applied bactericides did not appear within the collection of strains included in this study. This is consistent with Paulraj and O'Garro's (28) findings from Barbados. Resistance to copper, zinc, and streptomycin has been widely reported in phytopathogenic bacteria (6,7,17,24,39), and insensitivity is often conferred by resistance genes located on self-transmissible plasmids (6,7,24,39). Xanthomonads are generally considered resistant to copper, zinc, or streptomycin if they are able to grow on artificial media amended with 100 to 200 μg/ml of the bactericide, but resistance to streptomycin concentrations of 800 μg/ml or greater have been reported (24). Growers in Colorado and other onion producing regions rely heavily on copper-based bactericides for disease suppression and resistance may emerge with continued use (36), underscoring the need for an integrated pest management program to manage Xanthomonas leaf blight. The MIC of zinc was equal to or less than copper for all *X. axonopodis* pv. *allii* included in this study (data not presented), and zinc may be an effective bactericide to manage *X. axonopodis* pv. *allii* and delay copper resistance development. However, the relationship between the MICs determined in this study and sensitivity of bacterial cells under field conditions is unknown. Petri dish assays of bacterial sensitivity to copper, zinc, and streptomycin have been extensively used to detect bactericide resistance (6,7,17,24,39), and *in vitro* sensitivity appears to be a suitable proxy for sensitivity under field conditions (22). The efficacy of zinc sprays is currently under investigation in field trials.

Fatty acid and substrate utilization profiling revealed significant intraspecific diversity. Cluster analysis revealed limited grouping of strains by geographical region of origin based upon cellular fatty acid composition and, to a lesser extent, Biolog Microplate substrate utilization. However, the fatty acid and substrate utilization profiles were sufficiently variable among regions to allow for factor analysis and subsequent multiple logistic regression to generate a predictive model. The multiple logistic regression model developed in this study could correctly classify 69% of the strains included in this analysis into their geographical region of origin. Combining this model with rep-PCR-mediated DNA fingerprinting would provide a powerful tool for investigating dissemination of *X. axonopodis* pv. *allii* by seed transmission among onion production regions.

Identification of strains using the MIDI aerobe database and Biolog v. 4.1 database inconsistently identified strains. Fatty acid profiling identified the 49 strains of *X. axonopodis* pv. *allii* used in this study as 11 different *Xanthomonas* pathovars. Biolog identified the strains' closest match as 10 different pathovars. Users of these systems are encouraged to create a new profile for *X. axonopodis* pv. *allii* if fatty acids or Biolog substrate utilization are to be used for identification.

The conserved rep-PCR genomic fingerprints within geographic locations suggest that the population structure is largely clonal within production areas. The tight phylogenetic cluster formed within a given production region suggests *X. axonopodis* pv. *allii* was introduced into these production regions in one or a few events, perhaps on contaminated seed (3,33). The onion production regions considered in this study are sufficiently isolated that gene flow from other populations would be low and distinct clonal populations may have formed following genetic drift and selection of fit genotypes. The climates, cultivars, and production practices within the onion production regions considered in this study are different and may have selected for fitness and adapted genotypes unique for each region. However, the signature bands of *X. axonopodis* pv. *allii* pathogenic on onion are conserved among strains, and the recent evolution of *X.*

axonopodis pv. *allii* was revealed by the identical rDNA RFLP profiles and high sequence similarity of the non-conserved ITS region.

The relationship between *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *alfalfae*, *X. axonopodis* pv. *betlicola*, *X. axonopodis* pv. *citrumelo*, and *X. axonopodis* pv. *vesicatoria* and other DNA homology 9-2 strains remains unclear. Our study included a small subset of strains from each geographical region and few representative strains from other *Xanthomonas* species and pathovars. We are currently studying a larger collection of DNA homology group 9-2 strains to more fully describe the genetic and pathogenic relationships between these pathovars and *X. axonopodis* pv. *allii*.

The high level of genetic diversity within *X. axonopodis* pv. *allii* is in stark contrast to many other DNA fingerprinting studies with *Xanthomonas* and other plant pathogenic bacteria (5,20,32). Louws et al. (19) found nearly identical rep-PCR DNA fingerprints within the subspecies of *Clavibacter michiganensis*. Genetic similarity within subspecies was greater than 80%, but less than 40% between subspecies. The level of diversity within *X. axonopodis* pv. *allii* approaches that between *Clavibacter* subspecies. However, signature rep-PCR bands conserved within *X. axonopodis* pv. *allii*, identical rDNA RFLP profiles, and high ITS region sequence similarity do not suggest a polyphyletic population structure exists. Rademaker et al. (31) demonstrated rep-PCR fingerprinting explains 81% of the variability in DNA:DNA homology and can be used to reflect true genotypic and phylogenetic relationships. *X. axonopodis* pv. *allii* rep-PCR fingerprints among strains were at least 40% similar, corresponding approximately to at least 70% DNA:DNA homology (31). Therefore, despite high levels of genetic diversity within the population, *X. axonopodis* pv. *allii* appears to constitute a single species. Genetic drift of geographically isolated populations could account for the observed diversity among rep-PCR

DNA fingerprints. Knowledge of the population structure should aid in future epidemiological and ecological studies of *X. axonopodis* pv. *allii*.

Genetic diversity within *X. axonopodis* pv. *allii* may have important implications for resistance gene deployment and breeding. Host resistance to Xanthomonas leaf blight has only been reported in short day onion cultivars (27) and resistance appears to be associated with one or few genes. A race structure may exist within *X. axonopodis* pv. *allii* that could account for the variability in pathogenicity and virulence on pulse crops and other *Allium*. Studies are currently underway to further characterize genes involved in *X. axonopodis* pv. *allii* pathogenicity on onion and the existence of a population race structure.

LITERATURE CITED

1. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J. H., Zhange, Z., Miller, W., and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

2. Alvarez, A. M., Buddenhagen, I. W., Buddenhagen, E. S., and Domen, H. Y. 1978. Bacterial blight of onion, a new disease caused by *Xanthomonas* sp. Phytopathol. 68:1132-1136.

3. Audy P., Laroche, A., Saindon, G., Huang, H. C., and Gilbertson, R. L. 1994. Detection of the bean common blight bacteria *Xanthomonas campestris* pv. *phaseoli* and X. c. *phaseoli* var *fuscans*, using the polymerase chain-reaction. Phytopathol. 84:1185-1192.

4. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. 1998. Current Protocols in Molecular Biology. John Wiley & Sons, Inc., Hoboken, NJ. 672 pages.

5. Barak, J., and Gilbertson, R. L. 2003. Genetic diversity of *Xanthomonas campestris* pv. *vitians*, the casual agent of bacterial leaf spot of lettuce. Phytopathol. 93:596-603.

6. Bender, C. L., and Cooksey, D. A. 1986. Indigenous plasmids in *Pseudomonas syringae* pv. *tomato*: Conjugative transfer and role in copper resistance. J. Bacteriol. 165:534-541.

7. Bender, C. L., Malvick, D. K., Conway, K. E., George, S., and Pratt, P. 1990. Characterization of pXV10A, a copper resistance plasmid in *Xanthomonas campestris* pv. *vesicatoria*. Appl. and Environ. Microbiol. 56:170-175.

8. Bowen, P., Gibbs, H. A., and O'Garro, L. W. 1996. Garlic, chives, shallot, and leek are alternative hosts to *Xanthomonas campestris*, the pathogen of leaf blight of onion. Proc. Int. Congress of Plant Pathol. 7:696.

9. Cubero, J., and Graham, J. H. 2002. Genetic relationship among worldwide strains of *Xanthomonas* causing canker in citrus species and design of new primers for their identification by PCR. Appl. Environ. Microbiol. 86:1257-1264.

10. Dye, D. W., Bradbury, J. F., Goto, M., Hayward, A. C., Lelliott, R. A., and Schroth, M. N. 1980. International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. Review of Plant Pathol. 59:153-160.

11. Fenselau, S., Balbo, I., and Bonas, U. 1992. Determinants of pathogenicity in *Xanthomonas campestris* pv. *vesicatoria* are related to proteins involved in secretion in bacterial pathogens of animals. Mol. Plant Microbe Interact. 5:390-396.

12. Gent, D. H., Schwartz, H. F., Ishimaru, C. A., Louws, F. J., Cramer, R. A., and Lawrence, C. B. 2002. Polyphasic characterization of *Xanthomonas campestris* from onion. Pp. 48 in: Proc. 2002 National *Allium* Research Conference, Dec. 11 – 14, 2002, Pasco, WA.

13. Hauben, L., Vauterin, L., Swings, J., and Moore, E. R. B. 1997. Comparison of 16S ribosomal DNA sequences of all *Xanthomonas* species. Int. J. of Systematic Bacteriol. 47:328-335.

14. Isakeit, T., Miller, M. E., Barnes, L. W., Dickstein, E. R., and Jones, J. B. 2000. First report of leaf blight of onion caused by *Xanthomonas campestris* in the continental United States. Plant Dis. 84:201.

15. Jensen, M. A., Webster, J. A., and Straus, N. 1993. Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphisms. Appl. and Env. Microbiol. 59:945-952.

16. Kadota, I., Uehara, K., Shinohara, H., and Nishiyama, K. 2000. Bacterial blight of Welsh onion: A new disease caused by *Xanthomonas campestris* pv. *allii* pv. *nov.* J. Gen. Plant Pathol. 66:310-315.

17. Lee, Y., Hendson, M., Panopoulos, N. J., and Schroth, M. N. 1994. Molecular cloning, chromosomal mapping, and sequence analysis of copper resistance genes from *Xanthomonas campestris* pv. *juglandis*: Homology with small blue copper proteins and multicopper oxidase. J. Bacteriol. 176:173-188.

18. 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. and Env. Microbiol. 60:1068-1077.

19. Louws, F.J., Fulbright, D.W., Stephens, C.T., and de Bruijn, F. J. 1994. Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. Appl. and Env. Microbiol. 60:2286-2295.

20. Louws, F. J., Fulbright, D.W., Stephens, C. T., and de Bruijin, F. J. 1995. Differentiation of genomic structure by rep-PCR fingerprinting to rapidly classify *Xanthomonas campestris* pv. *vesicatoria*. Phytopathol. 85:528-536.

21. Louws, F. J., Bell, J., Medina-Mora, C. M., Smart, C. D., Opgenorth, D., Ishimaru, C. A., Hausbeck, M. K., de Bruijin, F. J., and Fulbright, D. W. 1998. rep-PCR-mediated genomic fingerprinting: A rapid and effective method to identify *Clavibacter michiganensis*. Phytopathol. 88:862-868.

22. Mahuku, G., Carlos, J, Henriquez, M. A., and Cuasquer, J. 2002. Diversity among *Xanthomonas axonopodis* pv. *phaseoli* strains from different geographical areas and pathogenic on common bean. Phytopathol. 92:S50.

23. Marco, G. M., and Stall, R. E. 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that in differ in sensitivity to copper. Plant Dis. 67:779-781.

24. Minsavage, G. V., Canteros, B. I., and Stall, R. E. 1990. Plasmid-mediated resistance to streptomycin in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathol. 80:719-723.

25. National Committee for Clinical Laboratory Standards. 2000. Methods for antimicrobial susceptibility tests for bacteria that grow aerobically - Fifth edition. Approved Standard M7-A5. NCCLS, Wayne, PA.

26. Nunez, J .J., Gilbertson, R. L., Meng, X., and Davis, R. M. 2002. First report of Xanthomonas leaf blight of onion in California. Plant Dis. 86:330.

27. O'Garro, L. W., and Paulraj, L. P. 1997. Onion leaf blight caused by *Xanthomonas campestris*: Alternative hosts and resistant onion genotypes. Plant Dis. 81:978-982.

28. Paulraj, L., and O'Garro, L. W. 1992. Aspects of the epidemiology of a leaf blight of onion in Barbados. Pp. 89-96 in: Proc. of the Tenth Annual Conference of the Barbados Society of Technologists in Agriculture. St. Micheal, Barbados, Nov 11-13, 1992.

29. Paulraj, L., and O'Garro, L. W. 1993. Leaf blight of onions in Barbados caused by *Xanthomonas campestris*. Plant Dis. 77:198-201.

30. Rademaker, J. L. W., Louws, F. J., and de Bruijn, F. J. 1999. Computer assisted pattern analysis of electrophoretic fingerprints and database construction. Pp. 1-33 In: Akkermans, A. D. L., van Elsas, J. D., and de Bruijn, F. J. (eds). Molecular Microbial Ecology Manual. Kluwer Academic Publishers, Dorrecht, Suppl. 4 Chapter 7.1.3.

31. Rademaker, J. L. W., Hoste, B., Louws, F. J., Kersters, K., Swings, J., Vauterin, L., Vauterin, P., and de Bruijn, F. J. 2000. Comparison of AFLP and rep-PCR genomic fingerprinting with DNA-DNA homology studies: *Xanthomonas* as a model system. Int. J. Syst. Bacteriol. 50:665-677.

32. Roberts, P. D., Hodge, N. C., Bouzar, H., Jones, J. B., Stall, R. E., Berger, R. D., and Chase, A. R. 1998. Relatedness of strains of *Xanthomonas fragariae* by restriction fragment length polymorphism, DNA-DNA reassociation, and fatty acid analyses. Appl. and Env. Microbiol. 64:3961-3965.

33. Roumagnac, P., Gagnevin, L., and Pruvost, O. 2000. Detection of *Xanthomonas* sp., the causal agent of onion bacterial blight, in onion seeds using a newly developed semi-selective isolation medium. Eur. J. Plant Path. 106:867-877.

34. Roumagnac, P., Gagnevin, L., Gardan, L., Sutra, L., Manceau, C., Dickstein, E. R., Jones, J. B., Rott, P., and Pruvost, O. 2004. Polyphasic characterization of xanthomonads isolated from onion, garlic and Welsh onion (*Allium* spp.) and their relatedness to different *Xanthomonas* species. Int. J. Syst. Evol. Microbio. 54:15-24.

35. Sanders, F. H., Langston, D. B., Brock, J. H., Gitaitis, R. D., Curry, D. E., and Torrance, R. L. 2003. First report of a leaf blight of onion caused by *Xanthomonas* spp. in Georgia. Plant Dis. 87:749

36. Schwartz, H. F., and Otto, K. J. 1998. Onion bacterial disease management in Colorado. Pp. 214-218 in: Proc. of the 1998 National Onion (and other *Allium*) Research Conference, Dec. 10 – 12, 1998, Sacramento, CA.

37. Schwartz, H. F., and Otto, K. 2000. First report of a leaf blight of onion by *Xanthomonas campestris* in Colorado. Plant Dis. 84:922.

38. Serfontein, J. J. 2001. Xanthomonas blight of onion in South Africa. Plant Dis. 85:442.

39. Sundin, G. W., Jones, A. L., and Fulbright, D. W. 1989. Copper resistance in *Pseudomonas syringae* from cherry orchards and its associated transfer *in vitro* and *in planta* with a plasmid. Phytopathol. 79:861-865.

40. Thomas, W. D. Jr., and Weinhold, A. R. 1953. *Xanthomonas striaformans*, a new bacterial pathogen of onion. J. of the Colo.-Wyo. Academy of Sci. 4(5):22.

41. Vauterin, L., Hoste, B., Kersters, K., and Swings, K. 1995. Reclassification of *Xanthomonas*. Int. J. of Syst. Bacteriol. 45:472-489.

42. Vauterin, L, Yang, P., and Swings, J. 1996. Utilization of fatty acid methyl esters for the differentiation of new *Xanthomonas* species. Int. J. of Syst. Bacteriol. 46:298-304.

43. Verniere, C., Pruvost, O., Civerolo, E. L., Gambin, O., Jacquemoud-Collet, J. P., and Luisetti, J. 1993. Evaluation of the Biolog substrate utilization system to identify and assess metabolic variation among strains of *Xanthomonas campestris* pv. *citri*. Appl. and Env. Microbiol. 59:243-249.

44. Weller, D. M., and Saettler, A. W. 1978. Rifampin-resistant *Xanthomonas phaseoli* var. *fuscans* and *Xanthomonas phaseoli*: Tools for field study of bean blight bacteria. Phytopathol. 68:778-781.

Table II. 1. *Xanthomonas* and *Stenotrophomonas* strains used in this study.

Strain	Other strain designations	Origin			Source[a]	ITS[b]	RFLP[b]	*hrp B6*[b]
		Host	Location	Year				
		Allium cepa	Hawaii	1977		...	...	...
A118-2a	...	*Allium cepa*	Hawaii	1977	A. Alvarez	...	...	...
A206-2a	...	*Allium cepa*	Hawaii	1977	A. Alvarez	...	...	+
A206-2b	...	*Allium cepa*	Hawaii	1977	A. Alvarez	+	...	+
A206-5	...	*Allium cepa*	Hawaii	1977	A. Alvarez	...	...	...
A225-2	...	*Allium cepa*	Hawaii	1977	A. Alvarez	...	...	...
A227-2	...	*Allium cepa*	Hawaii	1977	A. Alvarez	...	...	...
A229-1	...	*Allium cepa*	Hawaii	1977	A. Alvarez	+	+	+
A255-4	...	*Allium cepa*	Hawaii	1977	A. Alvarez	...	...	...
A256-3	...	*Allium cepa*	Hawaii	1977	A. Alvarez	...	...	...
A274-3	...	*Allium cepa*	Hawaii	1977	A. Alvarez	...	...	...
A274-7a	...	*Allium cepa*	Hawaii	1977	A. Alvarez	...	...	+
A551-3	...	*Allium cepa*	Hawaii	1980	A. Alvarez	...	...	...
A554-3	...	*Allium cepa*	Hawaii	1980	A. Alvarez	...	...	...
A579-7	...	*Allium cepa*	Hawaii	1980	A. Alvarez	...	...	...
A1889	...	*Allium cepa*	Hawaii	1987	A. Alvarez	...	...	...
A1890	...	*Allium cepa*	Hawaii	1987	A. Alvarez	...	...	...
JV 594	CFBP 6362	*Allium cepa*	Brazil	1987	O. Pruvost	+	...	+
JV 595	CFBP 6363	*Allium cepa*	Brazil	1987	O. Pruvost	+	...	+
ATCC 504	O130	*Allium cepa*	Colorado	1996	H. Schwartz	+	...	+
ATCC 505	O153	*Allium cepa*	Colorado	1996	H. Schwartz	...	...	+
ATCC 506	O154	*Allium cepa*	Colorado	1996	H. Schwartz	+	+	+
ATCC 507	O155	*Allium cepa*	Colorado	1996	H. Schwartz	+	...	+
ATCC 508	O177	*Allium cepa*	Colorado	1998	H. Schwartz	+	...	+
B458	...	*Phaseolus vulgaris*	Colorado	2000	H. Schwartz	+	+	+
F2:22	...	*Allium cepa*	Barbados	1996	L. O'Garro	+	...	+
JX 721	CFBP 6387	*Allium.cepa*	Venezuela	1997	O. Pruvost	+	...	...
JX 727	CFBP 6388	*Allium cepa*	Venezuela	2001	O. Pruvost	+	...	+
TX-1a	...	*Allium cepa*	Texas	1998	T. Isakeit	...	...	...
TX-2b	...	*Allium cepa*	Texas	1998	T. Isakeit	+	+	...
TX-2c	...	*Allium cepa*	Texas	1998	T. Isakeit	...	...	...
TX-3	...	*Allium cepa*	Texas	1998	T. Isakeit	+	...	+
TX-10	...	*Allium cepa*	Texas	1998	T. Isakeit	+	...	+
MAFF 311173	...	*Allium fistulosum*	Japan	1998	MAFF	+	...	+
MAFF 311174	...	*Allium fistulosum*	Japan	1998	MAFF	+	...	+
MAFF 311175	...	*Allium fistulosum*	Japan	1998	MAFF	...	...	+
MAFF 311176	...	*Allium fistulosum*	Japan	1998	MAFF	...	...	+
MAFF 311177	...	*Allium fistulosum*	Japan	1998	MAFF	...	+	...
MAFF 311178	...	*Allium fistulosum*	Japan	1998	MAFF	+	...	...
MAFF 311179	...	*Allium fistulosum*	Japan	1998	MAFF	...	...	...
ATCC BAA 575	BD 142	*Allium cepa*	South Africa	1999	ARC-PPRI	+	...	+

ATCC BAA 576	BD 143	*Allium cepa*	South Africa	1999	ARC-PPRI	+	...	+
ATCC BAA 577	BD 211	*Allium cepa*	South Africa	1999	ARC-PPRI	...	...	...
Xcu 200-2	...	*Allium cepa*	Georgia	2000	R. Gitaitis	...	...	...
Xcu 01-1	...	*Allium cepa*	Georgia	2001	R. Gitaitis	+	...	+
Xcu 01-2	...	*Allium cepa*	Georgia	2001	R. Gitaitis	+	...	+
Calandri-1	...	*Allium cepa*	California	2002	R. Gilbertson	+	...	...
Calandri-3	...	*Allium cepa*	California	2002	R. Gilbertson	...	...	...
Calon-1	...	*Allium cepa*	California	2002	R. Gilbertson	+	+	...
Calon-5	...	*Allium cepa*	California	2002	R. Gilbertson	+	...	+
ATCC 10547	...	*Dacus carota*	USA	...	ATCC	...	...	...
ATCC 11633	...	*Capsicum annuum*	USA	...	ATCC	...	...	...
ATCC 11672	...	*Xanthium strumarium*	India	1950	ATCC	...	...	...
ATCC 11765	...	*Medicago sativa*	India	...	ATCC	...	...	...
ATCC 13461	...	*Holcus sp.*	Texas	1978	ATCC	...	...	...
ATCC 13637	...	*Homo sapiens*	...	1960	ATCC	...	...	...
ATCC 19047	...	*Piper betle*	India	1964	ATCC	...	...	...
ATCC 19312	...	*Axonopu scoparius*	Columbia	...	ATCC	...	...	...
ATCC 19313	...	*Corylus maxima*	Oregon	...	ATCC	...	...	...
ATCC 29088	...	*Lolium multiflorium*	Switzerland	1975	ATCC	...	...	...
ATCC 29091	...	*Dactylis glomerata*	Switzerland	1975	ATCC	...	...	...
ATCC 33913	...	*Brassica oleracea*	UK	...	ATCC	...	...	...
ATCC 35937	...	*Lycopersicon lycopersicum*	New Zealand	...	ATCC	...	...	...
ATCC 49079	...	*Raphunus sativus*	USA	...	ATCC	...	...	...
ATCC 49083	...	*Juglans regia*	New Zealand	...	ATCC	...	...	...
ATCC 49119	...	*Phaseolus vulgaris*	USA	1989	ATCC	...	...	...
ATCC 49120	...	*Citrus sp.*	Florida	1989	ATCC	...	...	...

[a] Source: H. Schwartz, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO; R. Gilbertson, Department of Plant Pathology, University of California, Davis, CA; T. Isakeit, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX; MAFF=Ministry of Agriculture, Forestry, and Fisheries of Japan, Okinawa; ATCC=American Type Culture Collection, Manassas, VA; L. O'Garro, Department of Biology, University of the West Indies, Bridgetown, Barbados; A. Alvarez, Department of Plant Pathology, University of Hawaii, Honolulu, HI; R. Gitaitis, Department of Plant Pathology, University of Georgia, Tifton, GA; ARC-PPRI= Agricultural Research Council-Plant Protection Research Institute, Pretoria, South Africa; O. Pruvost, Centre de Coopération Internationale en Recherche Agronomique pour le Dévelopement, Reunion Island, France.

[b] + Indicates strain was used for this analysis; ... Indicates strain was not used for this analysis

Table II. 2. Cellular fatty acid composition of five representative onion strains of *Xanthomonas* from different geographical regions and belonging to five rep-PCR genotype groups.

		% Fatty Acid Composition														
					11:0			12:0				13:0				15:0
	rep-PCR		11:0	10:0	ISO	11:0	13:0	ISO	12:0	14:0		ISO	13:0	15:1	15:0	ANTE
Strain[y]	Genotype	10:0	iso	3OH	3OH	3OH	ISO	3OH	3OH	ISO	14:0	3OH	2OH	ISO F	ISO	ISO
TX-10	1	1.02	3.57	0.32	1.68	…	…	…	3.26	0.35	1.88	3.39	…	…	26.63	6.82
O177	2	0.92	3.68	0.38	1.50	…	…	…	2.27	…	1.80	2.50	…	…	29.37	7.48
Calon-5	3	0.79	3.63	0.33	1.66	0.16	0.31	0.21	2.71	0.32	1.57	3.61	0.28	0.20	28.22	8.61
MAFF 311173	4	0.78	4.13	0.34	1.80	…	…	…	2.91	0.34	1.42	3.96	0.29	…	29.10	8.16
JV 594	5	1.22	4.07	0.31	1.46	…	…	0.27	2.50	0.71	1.66	2.43	…	…	25.43	11.57

		% Fatty Acid Composition													
							ISO		17:0				17:0		
	rep-PCR	15:1		16:0			17:1	17:0	ANTE	17:1	18:1	18:1	ISO	Sum	
Strain	Genotype	ω 6c	15:0	ISO	16:1ω9c	16:0	ω9c	ISO	ISO	ω8c	ω9c	ω7c	3OH	F3[z]	Unknown
TX-10	1	…	1.52	1.86	2.16	7.19	4.86	8.36	0.52	1.02	0.77	0.91	…	20.34	1.60
O177	2	…	1.74	1.56	1.76	6.99	4.94	8.02	0.51	0.96	0.69	…	…	19.98	2.96
Calon-5	3	…	1.49	1.93	2.02	6.91	4.81	8.24	0.64	0.94	0.66	0.66	0.30	17.56	1.22
MAFF 311173	4	0.28	1.33	2.04	1.55	6.17	5.28	8.07	0.62	0.95	0.55	0.59	…	18.18	1.17
JV 594	5	0.47	1.66	3.69	1.90	5.47	4.54	6.06	0.69	1.09	0.65	0.67	…	18.97	2.50

[y] Fatty acid composition data for five of the 49 onion *Xanthomonas* strains analyzed are presented that represented the diversity of fatty acid profiles observed among strains pathogenic to onion.

[z] Sum F3=summed feature 3 representing 16:1ω7c and 15 ISO 2OH fatty acids. Fatty acids 10:0 2OH, 12:0, 17:1 ω6c, and 17:1 10methyl were occasionally detected in some strains, but comprised less than 1% of the total fatty acid profile. The representative strains presented here lacked these four fatty acids and are not presented.

Table II. 3. Maximum likelihood estimates of fatty acid methyl ester and substrate utilization profile multiple logistic regression used to predict geographic region of origin of onion strains of *Xanthomonas*.

Maximum Likelihood Estimates

Parameter[w]	DF[x]	Estimate[y]	Standard Error	Wald χ^2	Pr > χ^{2z}
Intercept Barbados	1	-4.662	1.08	18.48	<0.0001
Intercept Brazil	1	-3.529	0.68	26.56	<.00001
Intercept California	1	-2.495	0.49	26.44	<0.0001
Intercept Colorado	1	-1.670	0.39	18.56	<0.0001
Intercept Georgia	1	-1.260	0.35	12.68	0.0004
Intercept Hawaii	1	0.916	0.33	7.81	0.0052
Intercept Japan	1	1.704	0.38	19.71	<0.0001
Intercept South Africa	1	2.166	0.43	24.97	<0.0001
Intercept Texas	1	3.641	0.72	25.77	<0.0001
Factor 1	1	0.782	0.27	8.38	0.0038
Factor 9	1	0.865	0.27	9.91	0.0016

[w] Each equation is given by $Z= \beta_0 + 0.782(\text{Factor 1}) + 0.865(\text{Factor 2})$, where β_0 is the cumulative logit intercept for each region; where Intercept Barbados=-4.662 and Intercept Brazil (Barbados + Brazil)=-3.529....and Intercept Texas=(Barbados + Brazil.... +Texas) =3.641. The probability of group membership of a strain in a region (Zi) is described by $p(R_i) = e^{Zi}/(1 + e^{Zi})$, where e is the natural logarithm and the $p(R_{Venezuela})=1-p(R_{Texas})$.

[x] Degrees of freedom

[y] Intercept estimate= β_0

[z] Probability of obtaining a greater χ^2 statistic than that observed if the null hypothesis is true.

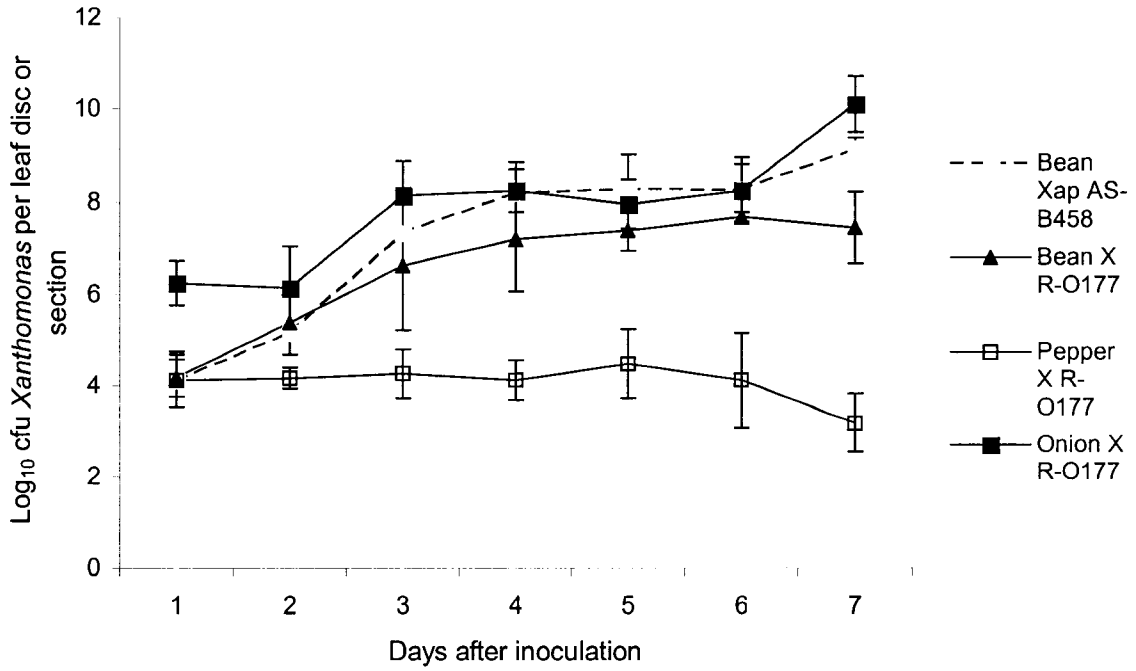


Figure II. 1. *In planta* population dynamics of onion *Xanthomonas* strain R-O177 and *X. axonopodis* pv. *phaseoli* strain AS-B458 in dry bean, onion, and pepper. Populations of *Xanthomonas* strain R-O177 in onion were enumerated every 2 days for 14 days, but are scaled to 7 days for comparison. *X. axonopodis* pv. *phaseoli* strain AS-B458 decreased to less than 10^3 CFU per leaf disk or section 7 days after inoculation in pepper and onion, respectively (data not shown). Data are mean of five replications repeated twice (n=10) ± standard error or mean. X=*Xanthomonas*; Xap=*Xanthomonas axonopodis* pv. *phaseoli*

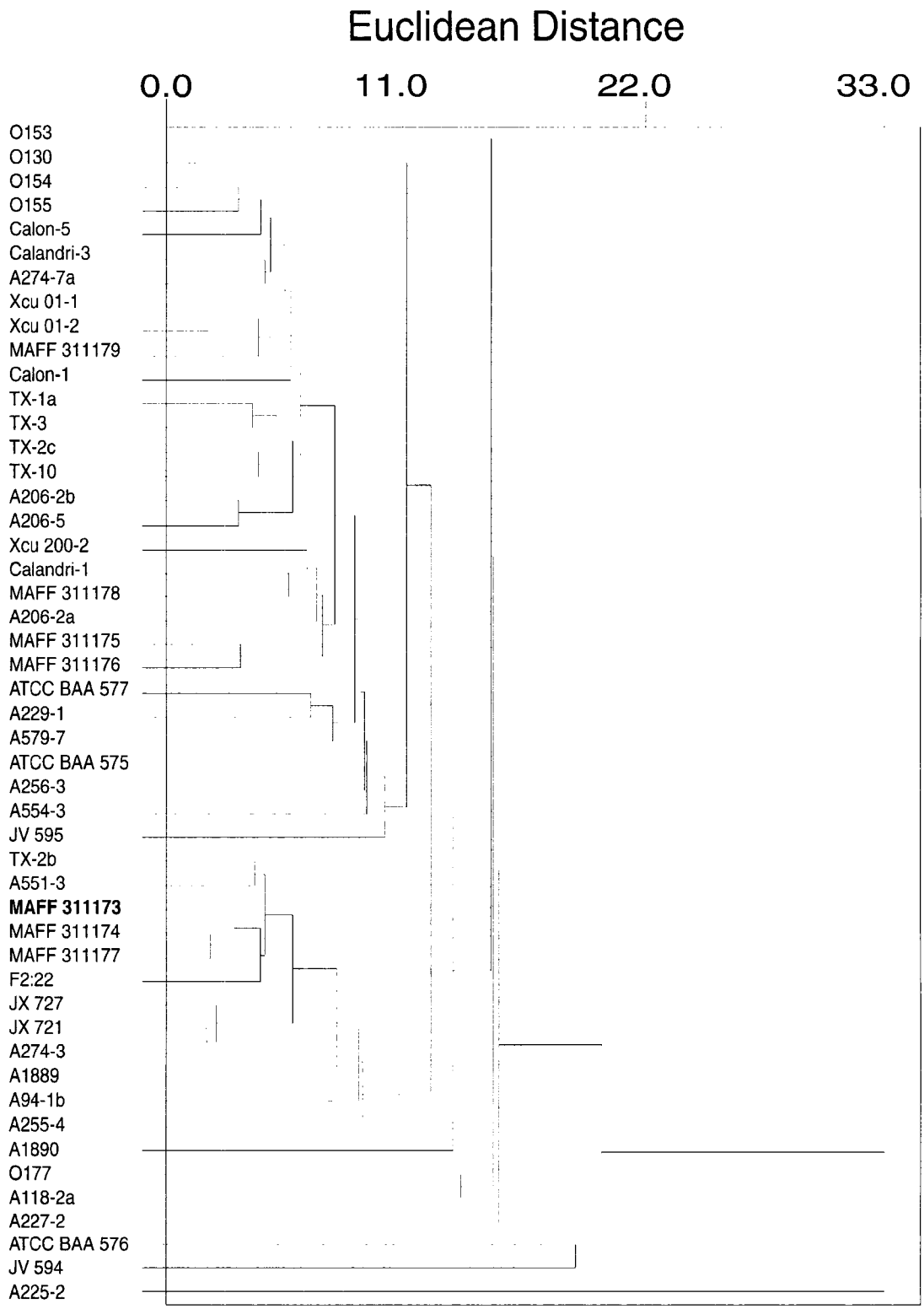


Figure II. 2. Relationships among 49 onion *Xanthomonas* strains isolated from various geographical regions based upon Biolog GN Microplate (Biolog Inc., Hayward, CA) substrate utilization patterns. The type strain of *Xanthomonas campestris* pv. *allii* MAFF 311173 is noted in bold.

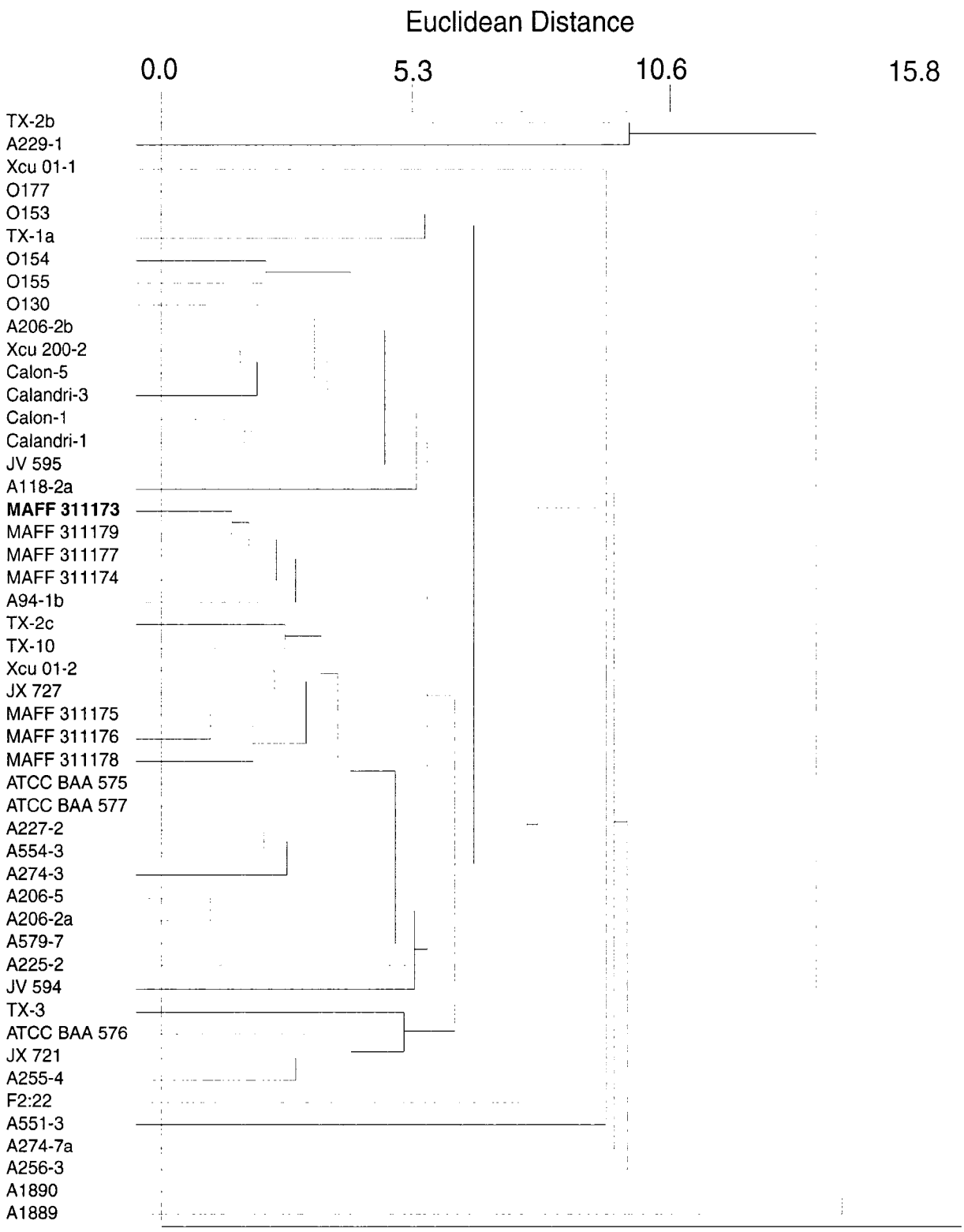
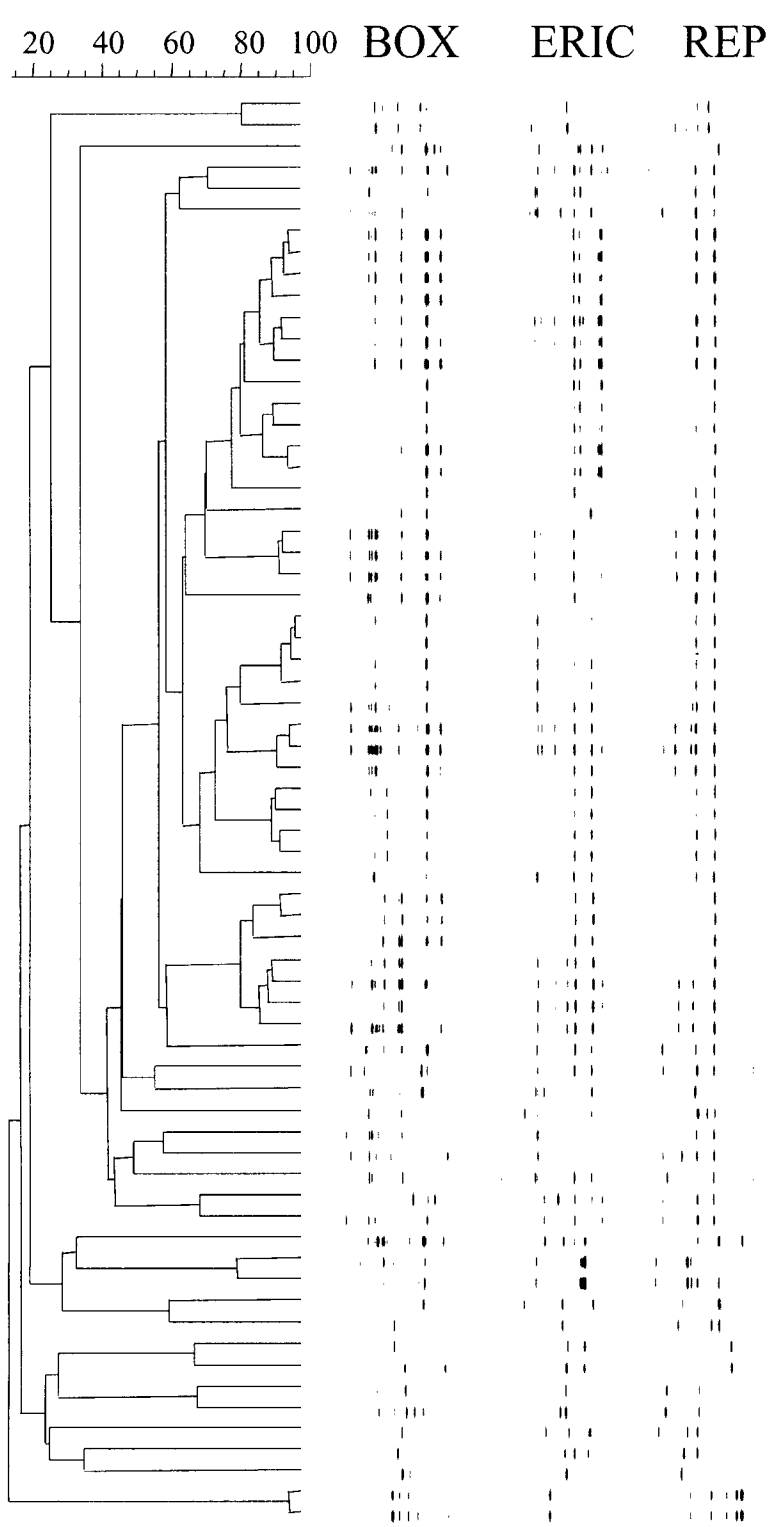


Figure II. 3. Relationships among 49 onion *Xanthomonas* strains isolated from various geographical regions based upon fatty acid composition. The type strain of *Xanthomonas campestris* pv. *allii* MAFF 311173 is noted in bold.



Pathovar	Strain	DNA homology group	
X ax phaseoli	49119	9	
X ax phaseoli	B458	9	
X ax axonopodis	19312	9	
X ax	TX-1a	-	1
X ax	TX-10	-	1
X ax	TX-3	-	1
X ax	A225-2	-	2
X ax	A255-4	-	2
X ax	A256-3	-	2
X ax	A206-5	-	2
X ax	A274-3	-	2
X ax	A551-3	-	2
X ax	A274-7a	-	2
X ax	A206-2a	-	2
X ax	A94-1b	-	2
X ax	A118-2a	-	2
X ax	A554-3	-	2
X ax	A579-7	-	2
X ax	O177	-	2
X ax citrumelo	49120	9	2
X ax	A206-2b	-	2
X ax	A229-1	-	2
X ax	A227-2	-	2
X ax betlicola	19047	9	
X ax	Calandril-1	-	3
X ax	Calandril-3	-	3
X ax	Calon-5	-	3
X ax	Calon-1	-	3
X ax	JX 721	-	3
X ax	Xcu01-2	-	3
X ax	Xcu01-1	-	3
X ax	Xcu200-2	-	3
X ax	O153	-	3
X ax	O154	-	3
X ax	O130	-	3
X ax	O155	-	3
X ax	JX 727	-	3
X ax	**MAFF 311173**	-	4
X ax	MAFF 311174	-	4
X ax	MAFF 311179	-	4
X ax	MAFF 311175	-	4
X ax	MAFF 311176	-	4
X ax	MAFF 311177	-	4
X ax	MAFF 311178	-	4
X ax alfalfae	11765	9	
X ax	F2:22	-	5
X ax	TX-2c	-	5
X ax vesicatoria	11633	9	5
X ax	JV 594	-	5
X ax	JV 595	-	5
X ax	BAA 577	-	5
X ax	BAA 575	-	5
X ax	BAA 576	-	5
X vasicola holcicola	13461	10	
X	TX-2b	-	
X campestris carotae	10547	4	
X arboricola juglandis	49083	4	
X arboricola corylina	19313	4	
X campestris campestris	33913	15	
X campestris raphani	49079	15	
X tranlucens graminis	29088	16	
X translucens graminis	29091	16	
X campestris vesicatoria	35937	14	
S maltophila	13637	21	
X campestris badrii	11672	8	
X	A1890	-	
X	A1889	-	

Figure II. 4. Similarity among rep-PCR-mediated DNA fingerprints of onion *Xanthomonas* strains from various geographical areas and between other species and pathovars of *Xanthomonas*. DNA fingerprints were generated using primers corresponding to prokaryotic enterobacterial repetitive intergenic consensus (ERIC) sequences, repetitive extragenic palindromic (REP) sequences, and the BOXA subunit of the BOX element (BOX); and analyzed using the product-moment correlation coefficient (*r*). DNA homology groups correspond to those reported by Rademaker et al. (31). The type strain of *Xanthomonas campestris* pv. *allii* MAFF 311173 is noted in bold. Genotype groups 1 to 5 are noted with brackets. X=*Xanthomonas*, X ax=*Xanthomonas axonopodis*, S maltophilia=*Stenotrophomonas maltophilia*, - = not known

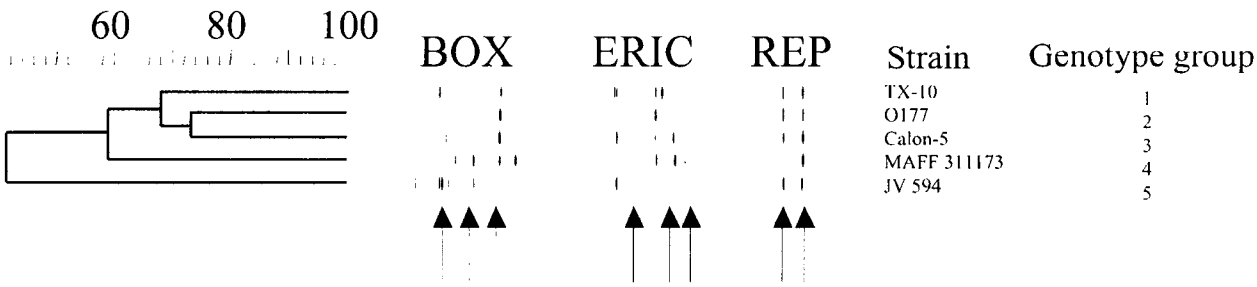


Figure II. 5. Diversity of rep-PCR DNA fingerprints among onion *Xanthomonas* strains isolated from various geographical regions and representing different genotype groups. Arrows indicate DNA bands that are conserved across all strains pathogenic on onion. The scale indicates the product-moment correlation coefficient (*r*). Strain MAFF 311173 is the type strain of *X. campestris* pv. *allii*. MAFF=Ministry of Agriculture, Forestry, and Fisheries GENE BANK accession.

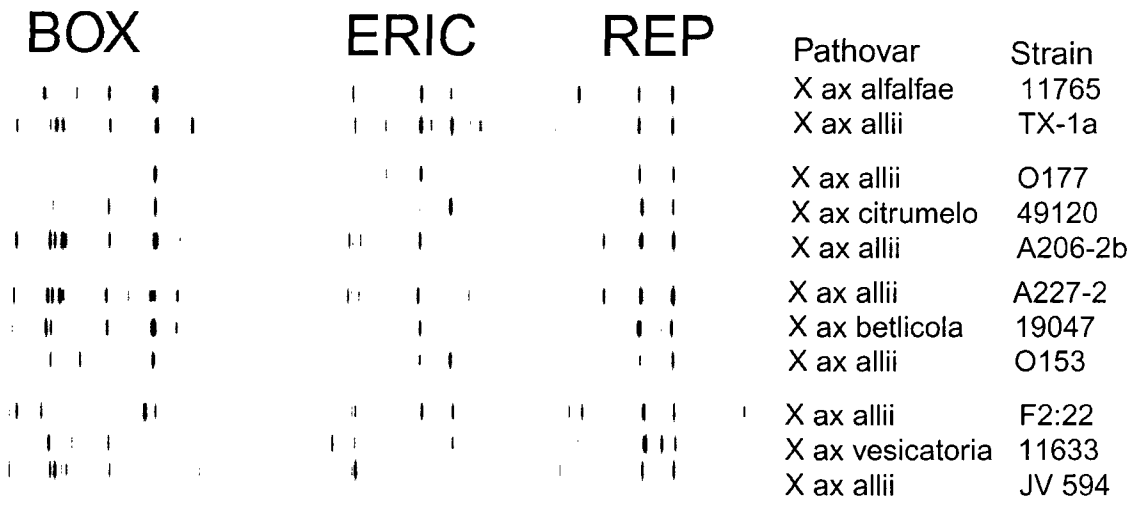


Figure II. 6. rep-PCR DNA fingerprint profile matches of onion *Xanthomonas* strains and other *Xanthomonas* pathovars within DNA homology group 9-2. X ax=*Xanthomonas axonopodis*

CHAPTER III

EPIPHYTIC SURVIVAL OF XANTHOMONAS AXONOPODIS PV. ALLII AND X. AXONOPODIS PV. PHASEOLI ON LEGUMINOUS HOSTS AND ONION

INTRODUCTION

Xanthomonas leaf blight of onion (*Allium cepa*), caused by *Xanthomonas axonopodis* pv. *allii*, is a yield limiting disease in Colorado (32,33) and several tropical, subtropical, and semi-arid onion producing regions of the world (1,12,14,21,25,26,28,34). Disease symptoms are varied, but include leaves with lenticular water-soaked lesions that elongate into chlorotic streaks, necrosis, tip dieback, and stunting of plants that reduces bulb size. A bulb rot has never been reported, but yield losses of 19% to 100% have been reported (32,34).

The host range of *X. axonopodis* pv. *allii* appears limited to onion and a few *Allium* species (1,6,10,14,27), although some strains are reportedly pathogenic to leguminous hosts such as snap bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*), soybean (*Glycine max*), winged bean (*Psophocarpus tetragonolobus*), moth bean (*Vigna aconitifolia*), and field pea (*Pisum sativum*) (22). Gent et al. (10) reported that *X. axonopodis* pv. *allii* did not induce common bacterial blight symptoms on dry bean, but the pathogen multiplied *in planta* to greater than 10^7 CFU per 20 mm^2 leaf disk under experimental conditions.

Onion is frequently rotated with leguminous hosts such as dry bean and soybean in the Colorado because few pests in this region are known to attack both onion and these crops, and production

practices tend to be compatible (30). Although *X. axonopodis* pv. *allii* is capable of *in planta* multiplication in dry bean under high humidity and temperature growth chamber conditions, it is unknown if the bacterium is capable of pathogenic or epiphytic survival on leguminous hosts under field conditions in Colorado. Similarly, *X. axonopodis* pv. *phaseoli* may be capable of epiphytic survival on onion, but no studies have evaluated this under field conditions. Close rotation of onion and leguminous hosts (such as dry bean) may allow *X. axonopodis* pv. *allii* and/or *X. axonopodis* pv. *phaseoli* to survive asymptomatically in onion and legume cropping systems, negating the effects of crop rotation. Therefore, this study was initiated to determine if *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* are capable of epiphytical survival on both leguminous hosts and onion.

MATERIALS AND METHODS

Bacterial strains and culture. A rifampicin mutant of *X. axonopodis* pv. *allii* strain O177 (ATCC 508) was generated as previously described (36), and is referred to as R-O177. Strain R-O177 is resistant to greater than 200 μg/ml rifampicin, but selection routinely was performed on nutrient agar amended with 50 μg/ml of rifampicin. Other strains of *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* were routinely cultured on nutrient agar or broth lacking antibiotic during incubation at 29°C. Bacterial strains were preserved in 15% nutrient glycerol broth at -80°C for long-term storage.

Pathogenicity and host range. All presumptive *X. axonopodis* isolates recovered from dry bean and onion were tested for pathogenicity to onion (cv. Vantage) and dry bean (cv. Sacramento light red kidney) in growth chamber assays. The youngest, fully extended leaves of 8-week old onion plants were pin-pricked three times at 2.5 cm intervals with a 22 gauge needle bearing a bacterial matrix of a given isolate removed from a 72-hour old nutrient agar culture

plate. Each pin-pricked leaf area was inoculated with a bacterial matrix approximately equal in size to the needle tip. Plants serving as negative controls were pin-pricked with a sterile needle. Pathogenicity to dry bean (cv. Sacramento light red kidney) was evaluated by spray inoculation. A colony of the isolate to be tested was transferred to 3 ml of nutrient broth in a 15 ml culture tube, and was incubated at 26°C with vigorous shaking (250 oscillations/min) for 24 h. The bacterial cells were collected by centrifugation before adjusting to approximately 10^7 CFU/ml in sterile magnesium phosphate buffer (0.01 M magnesium sulfate and 0.01M potassium phosphate pH 7.2). Three- to four-week old dry bean plants were sprayed (Crown SpraTool, Aerovoe Industries, Inc., Gardnerville, NV) to runoff with the bacterial suspension. At least three plants were inoculated with each isolate. Control plants were inoculated with sterile buffer.

The plants were placed in a growth chamber and incubated for 14 days with a 28°C/24°C day/night temperature regime, light intensity of 350 $\mu Ms^{-1}m^{-2}$, 100% relative humidity, and daily misting with tap water to runoff. Plants were observed daily for symptom development.

Growth chamber epiphytic population assays. Epiphytic development of strain R-O177 was monitored on several leguminous hosts and onion in growth chamber assays. Plants of soybean (cv. S40C1), lentil (*Lens culinaris* cv. Brewer), chickpea (*Cicer arietinum* cv. Sanford), dry bean (cv. Sacramento Light Red Kidney), alfalfa (*Medicago sativa*, cv. Haygrazer) or onion (cv. Cometa) were grown under greenhouse conditions (approximately 24°C/20°C day/night temperature regime and 14-hour photoperiod, with approximately 2-h of supplemental incandescent lighting) until they were three- to four- (soybean, lentil, chickpea, dry bean, and alfalfa) or six- to eight (onion) weeks old. Three plants of each species were seeded individually in MetroMix 200 potting soil (Grace Sierra Horticultural Products Company, Milpitas, CA) in 1 l pots.

Twenty pots of each plant species were inoculated by spraying to runoff with a 10^5 CFU/ml bacterial suspension using a Crown Spratool. Inoculum of strain R-O177 was cultured by inoculating 3 ml of nutrient broth in 15 ml culture tubes, and incubating at 26°C with vigorous shaking (250 oscillations/min) for 24 h. The bacterial cells were collected by centrifugation before adjusting to approximately 10^5 CFU/ml in sterile magnesium phosphate buffer. After inoculation, plants were allowed to air dry and then sampled immediately by removing all aboveground plant material from four pots of each plant species and placing it individually into plastic bags. Another set of four pots of each plant species was destructively sampled each day for four days. An experimental unit consisted of one pot of a given plant species that contained three plants destructively sampled each day. Plants were placed in a growth chamber and incubated at 28°C/24°C day/night temperature regime, light intensity of 350 $\mu Ms^{-1}m^{-2}$, 100% relative humidity, and daily misting with tap water to runoff.

Harvested plant samples were weighed and placed into sterile 250 ml flasks containing 100 ml of magnesium sulfate-potassium phosphate buffer, and shaken at 250 oscillations/min for 60 min at room temperature (approximately 22°C). Aliquots (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium phosphate buffer before plating in duplicate onto nutrient agar amended with 50 µg/ml rifampicin and cycloheximide. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 26°C, and a subset of rifampicin-resistant colonies were confirmed as *X. axonopodis* pv. *allii* by standard physiological and biochemical tests (29), including Gram stain reaction, pigmentation on yeast dextrose carbonate medium, fluorescence on King's medium B, indole test, growth on 0.1% tetrazolium chloride, oxidase test, starch hydrolysis, oxidative utilization of glucose, catalase test, production of H_2S from cysteine, presence of arginine dihydrolase, and casein hydrolysis test. The experiment was repeated twice over time.

Inoculated field epiphytic population studies. Field studies were established at the Agricultural Research, Development, and Education Center near Fort Collins, CO to determine if *X. axonopodis* pv. *allii* is capable of surviving epiphytically on leguminous hosts under field conditions. Soybean (cv. S40C1), lentil (cv. Brewer), chickpea (cv. Sanford), dry bean (cv. Sacramento light red kidney), and onion (cv. Vantage) were planted into plots from April to early June (depending on the plant species) in 2003 and 2004. Plants were established from seed planted approximately 0.1 m apart in beds on 0.76 m centers. Each bed contained two rows of each crop spaced 0.15 m apart. The field was furrow irrigated once to twice weekly and did not receive any fertilizer. A plot consisted of four contiguous 0.76 m-wide beds 7.6 m in length of each plant species. Each plot was replicated four times in a randomized complete block design.

The center two rows of each plot were inoculated to runoff with 10^6 CFU/ml strain R-O177 amended with 0.1% Silwet L-77 (Loveland Industries, Greeley, CO) using a CO_2-pressurized backpack sprayer on 25 July, 2003 (206 day of year) and 23 July, 2004 (205 day of year). Inoculum was prepared by harvesting cells of strain R-O177 from 72-h old rifampicin-amended nutrient agar plates grown at 29°C. The culture was adjusted spectrophotometically to 10^8 CFU/ml (OD_{600}=0.12) and then diluted 100-fold in sterile magnesium phosphate buffer.

Approximately 20 to 40 grams of asymptomatic leaf material were arbitrarily collected from each plot every 4 to 8 days after inoculation, placed into resealable plastic bags, and promptly transported to the laboratory on ice for epiphyte enumeration. Five to 10 grams of this bulked leaf sample was weighed and used in epiphyte enumeration assays as described previously, except leaf rinsates were plated in duplicate using a spiral-plating system (Autoplate 4000, Spiral Biotech, Inc., Norwood, MA). Representative colonies were confirmed as *X. axonopodis* pv. *allii* by physiological and biochemical tests, and pathogenicity on onion as described previously.

Recovery of epiphytic *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* from commercially-produced onion and dry bean. Epiphytic xanthomonad populations were recovered and enumerated from commercial dry bean and onion fields in northern Colorado to determine if *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* can survive asymptomatically on dry bean and onion, respectively. Field locations and production practices are summarized in Table 1. Fourteen dry bean and onion fields were monitored in 2003, which consisted of three onion fields planted to a non-host (winter wheat) of *X. axonopodis* pv. *allii* or *X. axonopodis* pv. *phaseoli* in 2002, five dry bean fields planted to dry bean in 2002, four onion fields planted to dry bean in 2002, and two dry bean fields planted to wheat in 2002. Epiphytic xanthomonad populations were monitored in twenty fields in 2004, which included six onion fields planted to a non-host in 2003 (field corn, sugar beet, or winter wheat), nine onion fields planted to dry bean in 2003, and five dry bean fields planted to a non-host (winter wheat or field corn) in 2003.

Approximately 20 to 40 grams of leaf material was collected from each field every 18 to 26 days by walking in a W pattern and arbitrarily collecting the youngest fully-extended leaves from 10 to 20 asymptomatic plants. The leaves were placed into resealable plastic bags, and promptly transported to the laboratory on ice for epiphyte enumeration. Four bulked samples (each 5 to 10 grams) from each field were used in epiphyte enumeration assays as described previously, except leaf rinsates were plated in duplicate using a spiral-plating system (Autoplate 4000, Spiral Biotech, Inc., Norwood, MA) onto modified MXP medium (9) containing 50 µg/ml kasugamycin, 50 µg/ml cephalexin, and 50 µg/ml cycloheximide to reduce growth of other bacteria and fungi. Plates were incubated in the dark at 29°C for 96 h before counting characteristic xanthomonad colonies surrounded by a zone of starch hydrolysis. Representative xanthomonad colonies were picked from plates and confirmed as *X. axonopodis* pv. *allii* or *X. axonopodis* pv. *phaseoli* by physiological and biochemical tests, Biolog substrate utilization profiles, and pathogenicity to onion or dry bean.

Statistical analysis. Growth chamber assays were organized as a completely randomized design with four replications. All bacterial population data were log transformed to achieve independently and normally distributed experimental errors with a common variance. Statistical analysis was performed using the PROC MIXED model in SAS v. 9.1 (SAS Institute, Cary, NC). Inoculated field studies were arranged as a randomized complete block design with four replications. Data were analyzed using PROC MIXED, where block and year were considered random and crop treatments fixed effects. Epiphytic xanthomonad populations monitored over time in commercial onion and dry bean fields were analyzed by repeated measures analysis with unequal spacing over time using PROC MIXED. The experiment was arranged as a completely randomized design, where an entire field following the same crop rotation pattern was considered an experimental unit.

RESULTS

Growth chamber epiphytic population assays. Epiphtyic populations of *X. axonopodis* pv. *allii* strain R-O177 increased on all hosts evaluated in warm, humid conditions favorable to the pathogen (Figure III. 1). Host and sample date affected the culturable epiphytic populations (*P*-value<0.0001). Epiphytic populations (CFU/g fresh weight) four days after inoculation were 1.67 x 10^4, 3.03 x 10^5, 4.32 x 10^5, 5.5 x 10^5, 7.90 x 10^5, and 2.17 x 10^6 for chickpea, lentil, soybean, dry bean, alfalfa, and onion, respectively. Small water-soaked lesions were apparent on onion 4 days after inoculation, but water-soaked lesions and other typical bacterial disease symptoms were absent on all other hosts. Epiphytic populations increased 3.05 logarithmic units/g fresh weight of plant tissue on onion over the time course of the experiment, which was at least 1.0 logarithmic unit greater than the leguminous plants evaluated; suggesting onion is a more suitable epiphytic host of *X. axonopodis* pv. *allii* than the leguminous plants studied in this experiment.

Epiphytic populations increased only 0.79 and 0.83 logarithmic units/g fresh weight tissue on soybean and chickpea, respectively, despite favorable temperatures and high relative humidity.

Inoculated field epiphytic population studies. Inoculated field experiments were established to determine if *X. axonopodis* pv. *allii* is capable of epiphytic survival under uncontrolled and variable weather conditions. Crop species and sampling date were significant in the mixed model (*P*-value=0.029 and <0.0001, respectively) where replication and year were considered random factors. Although plots were uniformly inoculated with 10^6 CFU/ml of *X. axonopodis* pv. *allii* strain R-O177, culturable epiphytic populations 33 days after inoculation varied among crop species. Epiphytic populations decreased at least 0.8 logarithmic units/g fresh weight on leaves of leguminous hosts and increased 2.72 logarithmic units/g fresh weight on onion leaves (Figure III.2). *X. axonopodis* pv. *allii* populations on onion increased on six of seven sampling dates, and 3.21 x 10^7 CFU/g fresh weight of leaves were recovered on the last sampling date. No epiphytic bacteria were recovered from leaf rinsates of chickpea or soybean, and less than 10 CFU/g fresh weight were recovered from dry bean leaves on the last sampling date. Small epiphytic populations were recovered from lentil on six of seven sampling dates throughout the season, and 8.29 x 10^2 CFU/g fresh weight of leaves were recovered on the last sampling date.

In 2004, epiphytic populations of *X. axonopodis* pv. *allii* again decreased on all crops except onion (Figure III.3). Fifty-three days after inoculation, epiphytic populations varied from undetectable (chickpea and soybean) to greater than 2.10 x 10^5 CFU/g fresh weight of leaves (onion). Final season bacterial populations on dry bean and lentil did not differ from those on the first sampling date, suggesting dry bean and lentil allowed the bacterium to persist but not multiply. *X. axonopodis* pv. *allii* populations on onion were greater on all sampling dates than those on the first sampling date, but varied from 2.10 x 10^5 to 3.66 x 10^7 CFU/g fresh weight of leaves among sampling dates.

Recovery of epiphytic *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* from commercially-produced onion and dry bean. Fourteen commercial dry bean and onion fields under different cropping systems were monitored for naturally-occurring epiphytic xanthomonad populations in 2003. Epiphytic *X. axonopodis* pv. *phaseoli* was recovered on at least one sampling date from five of five (100%) dry bean fields preceded by dry bean, two of two (100%) dry bean fields preceded by winter wheat, and four of four onion (100%) fields preceded by dry bean in 2002. *X. axonopodis* pv. *phaseoli* was not recovered from the three onion fields preceded by winter wheat in 2002. *X. axonopodis* pv. *allii* was not recovered from any field sampled.

Among fields sampled in 2003, *X. axonopodis* pv. *phaseoli* populations ranged from 0 to 1.23 x 10^5 CFU/ g leaf tissue on dry bean preceded by dry bean, 0 to 3.16 x 10^2 CFU/g leaf tissue on dry bean preceded by winter wheat, and 0 to 2.1 x 10^1/g leaf tissue on onion preceded by dry bean the previous season. Sampling date was not significant in the mixed model (Table III. 2). Epiphytic *X. axonopodis* pv. *phaseoli* populations were greatest in dry bean fields preceded by dry bean or winter wheat in 2002, but *X. axonopodis* pv. *phaseoli* populations did not differ among dry bean preceded by winter wheat and onion preceded by dry bean (Figure III. 4) Least square means estimates, significance, and confidence limits for culturable epiphytic xanthomonad populations in relation to crop rotation are presented in Table III. 3.

In 2004, 20 commercial dry bean and onion fields following different crop rotations were monitored for naturally-occurring xanthomonad populations. Epiphytic *X. axonopodis* pv. *phaseoli* was recovered on at least one sampling date from seven of nine (78%) onion fields planted to dry bean the previous season, and culturable xanthomonad populations ranged from 0 to 9.51 x 10^3 CFU/g of leaves among fields and sampling dates. *X. axonopodis* pv. *phaseoli* was not recovered from onion fields preceded by a nonhost (field corn, sugar beet, or winter wheat) or

dry bean fields preceded by a nonhost (field corn or winter wheat) the previous season (Figure III. 5). *X. axonopodis* pv. *allii* was not recovered during 2004.

Epiphytic xanthomonad populations on dry bean and onion varied among years. Epiphytic xanthomonad populations on onion preceded by dry bean were not different significantly from those on onion preceded by winter wheat in the first year of this study, but were greater in 2004 (Table III.3). In 2003, epiphytic populations on dry bean preceded by winter wheat were greater than 0 (α level=0.1), but were not different from 0 values in 2004.

DISCUSSION

Common bacterial blight of dry bean and Xanthomonas leaf blight of onion are endemic in some Colorado dry bean and onion production regions. Onion and dry bean are often grown in rotation with one another in the central High Plains production regions because few pests are known to attack both crops, and production practices for each crop are generally compatible (30). Epiphytic survival of *X. axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *allii* on onion and dry bean, respectively, may necessitate a change in current disease management tactics. Strains of *X. axonopodis* pv. *allii* currently present in the U.S. do not incite common bacterial blight on leguminous crops (1,10,27), and *X. axonopodis* pv. *phaseoli* does not attack onion (10), but asymptomatic survival of these pathogens in the phyllosphere of onion and other rotational crops could complicate the effectiveness of disease management strategies.

In this study, we demonstrated *X. axonopodis* pv. *allii* is capable of epiphytic multiplication and survival under growth chamber and field conditions on several leguminous crops commonly grown in rotation with onion. *X. axonopodis* pv. *allii* achieved populations that were at least 10-fold greater on onion than on leguminous hosts in growth chamber studies, suggesting the

bacterium is more adapted to the onion phyllosphere than those of leguminous nonhosts. Epiphytic populations varied among these nonhosts, but still increased on all plants evaluated.

Survival of *X. axonopodis* pv. *allii* on several leguminous hosts under high humidity growth chamber conditions is not surprising. Under greenhouse conditions, *X. axonopodis* pv. *phaseoli* is capable of colonizing many diverse plants, including corn, beet, *Chenopodium album* (common lambsquarter), *Amaranthus retroflexus* (redroot pigweed), and *Echinochloa crus-galli* (barnyard grass; but the populations on these hosts are 10 to 10,000-fold smaller than on dry bean and large epiphytic populations do not persist for long periods of time (8). Under humid conditions, enteric clinical pathogens such as *Salmonella enterica* and *Escherichia coli* are capable of epiphytic survival on diverse plants, but they are not adapted to stressful conditions and their populations decrease under dry conditions (7,22). When wet conditions return, however, *S. enterica* can multiply and reestablish large epiphytic populations on leaves (7). A similar occurrence may be possible with *X. axonopodis* pv. *allii* on leguminous hosts, where small populations of the bacterium can persist during unfavorable conditions but recover when the environmental and/or host condition(s) change.

Inoculated field plots revealed *X. axonopodis* pv. *allii* can persist on several leguminous hosts, in the absence of disease symptoms, for extended periods of time under variable and stressful conditions. In both 2003 and 2004, measurable *X. axonopodis* pv. *allii* populations were recovered from dry bean and lentil at least 35 days after inoculation, ranging from 10^1 to greater than 10^6 CFU/g. Chickpea and soybean appear to be poor epiphytic hosts of *X. axonopodis* pv. *allii* under the environmental conditions (high temperatures and low moisture) present during these studies. The pathogen was not detectable on chickpea 10 or 37 days after inoculation in 2003 and 2004, respectively. On soybean, detectable populations were absent 15 and 45 days after inoculation in 2003 and 2004, respectively. The epiphyte recovery method used in these

studies could not detect less than 199 culturable *X. axonopodis* pv. *allii* CFU/gram of tissue in an experimental unit, and populations less than this level were considered 0. It is possible small, undetected populations of the pathogen were present on chickpea and soybean on sampling dates in which no epiphytes were recovered. Whether these small populations were present or the pathogen was extinct on these plant species, chickpea and soybean do not appear to be important epiphytic hosts of *X. axonopodis* pv. *allii* in semi-arid production regions.

Larger epiphytic populations of *X. axonopodis* pv. *allii* were recovered from dry bean and lentil in 2004 as compared to 2003, and may be related to weather conditions. Weather conditions monitored by a local Colorado Agricultural Meteorological Network weather station less than 1 km from the field site recorded measurable precipitation on 2 and 6 days during July and August 2003, respectively, totaling 0.3 cm in July and 2.7 cm in August. During the same periods in 2004, measurable rainfall was recorded on 6 and 11 days in July and August, respectively, totaling 1.8 cm and 3.5 cm. Mean daily high and low temperatures each month were nearly 5°C and 3°C higher in 2003 as compared to 2004, respectively. Temperature and moisture are known to influence the epiphytic behavior of many phytopathogenic and nonpathogenic bacteria (13,15,22), and may adversely affect *X. axonopodis* pv. *allii* colonization and persistence on nonhosts.

In contrast, *X. axonopodis* pv. *allii* epiphytic populations on symptomless onion leaves in 2003 were lower at the end of the 2004 season as compared to the 2003 season. Samples were collected longer into the growing season in 2004 as compared to 2003 and may suggest a seasonal population decline, but epiphytic populations were trending downward over the final four sampling dates in 2004. The basis for these differences are unknown, but different environmental conditions may influence *X. axonopodis* pv. *allii* survival and multiplication on the leaves of host and nonhost plants. Relatively large (generally greater than 10^6 CFU/g) epiphytic populations of

X. axonopodis pv. *allii* were recovered from onion across sampling dates, and suggests the pathogen is relatively more adapted to survive warm, dry conditions on onion as compared to leguminous plants.

In commercial dry bean and onion fields, *X. axonopodis* pv. *phaseoli* was recovered consistently from onion following dry bean in rotation, but rarely from onion following a nonhost such as field corn, sugar beet, or winter wheat. Although Cafati and Saettler (8) reported *X. axonopodis* pv. *phaseoli* can colonize field corn and sugar beet leaves when artificially inoculated under greenhouse conditions, the epiphytic populations decreased significantly after 21 days and these hosts are unlikely to support large epiphytic populations of *X. axonopodis* pv. *phaseoli* under field conditions. Under semi-arid conditions in Colorado, *X. axonopodis* pv. *phaseoli* was recovered from onion following a nonhost from only one field on one sampling date during these studies.

Recovery of *X. axonopodis* pv. *phaseoli* from symptomless onion in close rotation with dry bean but not other crops suggests the pathogen was present in the dry bean crop, over wintered in the field, and subsequently colonized onion in the following season. Epiphytic *X. axonopodis* pv. *phaseoli* also persisted on onion throughout most of the 2003 and 2004 seasons. Several epiphytic hosts of phytopathogenic xanthomonads have been identified, but generally host-specific xanthomonads do not persist epiphytically on nonhost for extended periods of time (2,4,37). That *X. axonopodis* pv. *phaseoli* was recovered from onion throughout much of the growing season may be epidemiologically significant. Cropping of dry bean and onion in sequential seasons may provide a bridge for small populations of *X. axonopodis* pv. *phaseoli* to survive in the absence of its primary host, and thus persist in the field. However, not all onion fields surveyed supported epiphytic *X. axonopodis* pv. *phaseoli*, and epiphytic populations from those fields that did support *X. axonopodis* pv. *phaseoli* were relatively small (less than 10^2 and 10^4 in 2003 and 2004, respectively). If minimum tillage practices were followed in these fields,

or if incorporation of onion crop debris was incomplete, *X. axonopodis* pv. *phaseoli* could potentially overwinter in association with infested onion crop debris (11). Deep incorporation of onion crop debris would likely prevent *X. axonopodis* pv. *phaseoli* from overwintering.

X. axonopodis pv. *allii* was not recovered from onion or dry bean in commercial fields monitored in this study, but its persistence on inoculated dry bean and lentil under experimental field conditions suggest the pathogen may be able to survive between onion crops on these hosts. In Barbados, onion rotation with leguminous crops is not recommended because prevalent strains of *X. axonopodis* pv. *allii* in Barbados are reportedly pathogenic to such crops (23). *X. axonopodis* pv. *allii* did not cause any visible disease symptoms on the epiphytic hosts (except onion) evaluated under growth chamber or field conditions in this study; and *X. axonopodis* pv. *allii* strains from other geographical regions of the world are not pathogenic to leguminous plants (1,10,27). Although *X. axonopodis* pv. *allii* is not an aggressive pathogen of dry bean or lentil in the Central High Plains production region, the potential and significance of its epiphytic survival on common rotation crops with onion requires more investigation.

The potential for *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* to colonize the phyllosphere of plants that serve as host to other pathovars of *X. axonopodis* may have implications for horizontal gene transfer and evolution of new genotypes of each pathogen. The phyllosphere is a harsh environment for microbes because of quickly-changing temperature, humidity, free moisture, and ultraviolet radiation, and bacteria survive most successfully in this environment in large cell aggregates (17,19,20). The location and aggregation of *X. axonopodis* cells on dry bean and onion is unknown, but these phyllosphere environments are likely to be conducive to conjugative transfer of plasmid and chromosomal DNA among bacteria (17). Since *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* can both survive epiphytically on onion and dry bean, the rate of horizontal gene transfer among the pathovars may increase.

Epiphytic survival of multiple pathovars of *X. axonopodis* on the same host may increase the risk of bactericide or antibiotic resistance developing in both pathogens. Copper and streptomycin resistance is not widespread in *X. axonopodis* pv. *allii* (10,24) or *X. axonopodis* pv. *phaseoli*, but is common in many phytopathogenic bacteria (5,18). Copper tolerance genes may also occur on plasmids carrying avirulence genes (35). In Colorado, copper bactericides are routinely applied to both dry bean and onion for common bacterial blight and Xanthomonas leaf blight management, respectively (31,32). *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* may be exposed to copper residues on both hosts, exerting a strong selection pressure for resistance in both pathogens. Epiphytic survival of both pathogens on dry bean and onion could lead to rapid development of copper resistance in either pathovar, and subsequent transfer to the other by horizontal gene transfer.

In this study we have established, for the first time, that *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* are capable of epiphytic survival on both onion and dry bean. The implications of epiphytic survival of these pathogens on crops commonly grown in close rotation remain unclear, but cropping systems that avoid close rotations of onion and dry beans and encourage rapid breakdown of crop residues should reduce potential inoculum sources of both pathogens in the Central High Plains.

LITERATURE CITED

1. Alvarez, A. M., Buddenhagen, I. W., Buddenhagen, E. S., and Domen, H. Y. 1978. Bacterial blight of onion, a new disease caused by *Xanthomonas* sp. Phytopathol. 68:1132-1136.

2. Angeles-Ramos, R., Vidaver, A. K.., and Flynn, P. 1991. Characterization of epiphytic *Xanthomonas campestris* pv. *phaseoli* and pectolytic xanthomonads recovered from symptomless weeds in the Dominican Republic. Phytopathol. 81:677-681.

3. Audy P., Laroche, A., Saindon, G., Huang, H. C., and Gilbertson, R. L. 1994. Detection of the bean common blight bacteria *Xanthomonas campestris* pv. *phaseoli* and *X. c. phaseoli* var *fuscans*, using the polymerase chain-reaction. Phytopathol. 84:1185-1192.

4. Barak, J. D., Koike, S. T., and Gilbertson, R. L. 2001. Role of crop debris and weeds in the epidemiology of bacterial leaf spot of lettuce in California. Plant Dis. 85:169-178.

5. Bender, C. L., and Cooksey, D. A. 1986. Indigenous plasmids in *Pseudomonas syringae* pv. *tomato*: Conjugative transfer and role in copper resistance. J. Bacteriol. 165:534-541.

6. Bowen, P., Gibbs, H. A., and O'Garro, L. W. 1996. Garlic, chives, shallot, and leek are alternative hosts to *Xanthomonas campestris*, the pathogen of leaf blight of onion. Proc. Int. Congress of Plant Pathol. 7:696.

7. Brandl, M. T., and Mandrell, R. E. 2002. Fitness of *Salmonella enterica* serovar Thompson in the cilantro phyllosphere. Appl. Environ. Microbiol. 68:3614-3621.

8. Cafati, C. R., and Saettler, A. W. 1980. Role of nonhost species as alternate inoculum sources of *Xanthomonas phaseoli*. Plant Dis. 64:194-196.

9. Claflin, L. E., Vidaver, A. K., and Sasser, M. 1987. MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathol. 77:730-734.

10. Gent, D. H., Schwartz, H. F., Ishimaru, C. A., Louws, F. J., Cramer, R. A., and Lawrence, C. B. 2004. Polyphasic characterization of *Xanthomonas* strains from onion. Phytopathol. 94:184-195.

11. Gilbertson, R. L., Rand, R. E., and Hagedorn, D. J. 1990. Survival of *Xanthomonas campestris* pv. *phaseoli* and pectolytic strains of *X. campestris* in bean debris. Plant Dis. 74:322-327.

12. Isakeit, T., Miller, M. E., Barnes, L. W., Dickstein, E. R., and Jones, J. B. 2000. First report of leaf blight of onion caused by *Xanthomonas campestris* in the continental United States. Plant Dis. 84:201.

13. Johnson, K. B., Stockwell, V. O., Sawyer, T. L., and Sugar, D. 2000. Assessment of environmental factors influencing growth and spread of *Pantoea agglomerans* on and among blossoms of pear and apple. Phytopathol. 90:1285-1294.

14. Kadota, I., Uehara, K., Shinohara, H., and Nishiyama, K. 2000. Bacterial blight of Welsh onion: A new disease caused by *Xanthomonas campestris* pv. *allii* pv. *nov.* J. Gen. Plant Pathol. 66:310-315.

15. Kinkel, L. L., Wilson, M., and Lindow, S. E. 2000. Plant species and plant incubation conditions influence variability in epiphytic bacterial population size. Microbial Ecol. 39:1-11.

16. 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. and Env. Microbiol. 60:1068-1077.

17. Lindow, S. E., and Brandl, M. T. 2003. Microbiology of the phyllosphere. Applied and Environ. Biol. 69:1875-1883.

18. Minsavage, G. V., Canteros, B. I., and Stall, R. E. 1990. Plasmid-mediated resistance to streptomycin in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathol. 80:719-723.

19. Monier, J. M., and Lindow, S. E. 2004. Frequency, size, and location of bacterial aggregates on bean leaf surfaces. Applied and Environ. Microbiol. 70:346-355.

20. Monier, J. M., and Lindow, S. E. 2003. Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. Proc. Nat. Acad. Sci. of the United States of America. 100:15977-15982.

21. Nunez, J .J., Gilbertson, R. L., Meng, X., and Davis, R. M. 2002. First report of Xanthomonas leaf blight of onion in California. Plant Dis. 86:330.

22. O'Brien, R. D., and Lindow, S. E. 1989. Effect of plant species and environmental conditions on epiphytic population sizes of *Pseudomonas syringae* and other bacteria. Phytopathol. 79:619-627.

23. O'Garro, L. W., and Paulraj, L. P. 1997. Onion leaf blight caused by *Xanthomonas campestris*: Alternative hosts and resistant onion genotypes. Plant Dis. 81:978-982.

24. Paulraj, L., and O'Garro, L. W. 1992. Aspects of the epidemiology of a leaf blight of onion in Barbados. Pp. 89-96 in: Proc. of the Tenth Annual Conf. of the Barbados Society of Technologists in Agriculture. St. Micheal, Barbados, Nov 11-13, 1992.

25. Paulraj, L., and O'Garro, L. W. 1993. Leaf blight of onion in Barbados caused by *Xanthomonas campestris*. Plant Dis. 77:198-201.

26. Roumagnac, P., Gagnevin, L., and Pruvost, O. 2000. Detection of *Xanthomonas* sp., the causal agent of onion bacterial blight, in onion seeds using a newly developed semi-selective isolation medium. Eur. J. Plant Path. 106:867-877.

27. Roumagnac, P., Gagnevin, L., Gardan, L., Sutra, L., Manceau, C., Dickstein, E. R., Jones, J. B., Rott, P., and Pruvost, O. 2004. Polyphasic characterization of xanthomonads isolated from onion, garlic and Welsh onion (*Allium* spp.) and their relatedness to different *Xanthomonas* species. Int. J. Syst. Evol. Microbio. 54: 15-24.

28. Sanders, F. H., Langston, D. B., Brock, J. H., Gitaitis, R. D., Curry, D. E., and Torrance, R. L. 2003. First report of a leaf blight of onion caused by *Xanthomonas* spp. in Georgia. Plant Dis. 87:749.

29. Schaad, N.W., Jones, J.B., and Chun, W. eds 2001. Laboratory Guide for the Identification of Plant Pathogenic Bacteria. 3rd ed. The American Phytopathological Society Press, St. Paul, MN. 398 pp.

30. Schwartz, H.F., and Bartolo, M.E. eds. 1995. Colorado Onion Production and Integrated Pest Management. Bull. 547A Colorado State University, Fort Collins, CO. 39 pp..

31. Schwartz, H.F., Brick, M.A., Nuland, D.S., and Franc, G.D. (Editors). 1996. Dry Bean Production and Pest Management. Reg. Bull. No. 562A, Fort Collins, CO. 106 pp.

32. Schwartz, H. F., and Otto, K. J. 1998. Onion bacterial disease management in Colorado. Pp. 214-218 in: Proc. of the 1998 National Onion (and other *Allium*) Research Conference, Dec. 10 – 12, 1998, Sacramento, CA.

33. Schwartz, H. F., and Otto, K. 2000. First report of a leaf blight of onion by *Xanthomonas campestris* in Colorado. Plant Dis. 84:922.

34. Serfontein, J. J. 2001. Xanthomonas blight of onion in South Africa. Plant Dis. 85:442.

35. Stall, R. E., Loschke, D. C., and Jones, J. B. 1986. Linkage of copper resistance and avirulence loci on a self-transmissible plasmid in *Xanthmonas campestris* pv. *vesicatoria*. Phytopathol. 76:240-243.

36. Weller, D. M., and Saettler, A. W. 1978. Rifampin-resistant *Xanthomonas phaseoli* var. *fuscans* and *Xanthomonas phaseoli*: Tools for field study of bean blight bacteria. Phytopathol. 68:778-781.

37. Zhao, Y., Damicone, J. P., and Bender, C. L. 2002. Detection, survival, and sources of inoculum for bacterial diseases of leafy crucifers in Oklahoma. Plant Dis. 86:883-888.

Table III.1. Dry bean and onion fields monitored for epiphytic xanthomonad populations in 2003 and 2004. Wheat=winter wheat, Corn=field corn, and Bean=dry bean.

Crop				
2002	**2003**	**2004[z]**	**Location (county)**	**Irrigation method**
2003 fields monitored				
Wheat	Onion	--	Weld	Furrow
Wheat	Onion	--	Weld	Furrow
Wheat	Onion	--	Weld	Furrow
Bean	Onion	--	Weld	Drip
Bean	Onion	--	Morgan	Sprinkler
Bean	Onion	--	Morgan	Sprinkler
Bean	Onion	--	Morgan	Sprinkler
Bean	Bean	--	Weld	Furrow
Bean	Bean	--	Weld	Furrow
Bean	Bean	--	Weld	Drip
Bean	Bean	--	Weld	Drip
Bean	Bean	--	Weld	Drip
Wheat	Bean	--	Weld	Furrow
Wheat	Bean	--	Weld	Furrow
2004 fields monitored				
--	Corn	Onion	Weld	Furrow
Bean	Potato	Onion	Morgan	Sprinkler
--	Sugar-beet	Onion	Weld	Furrow
Potato	Wheat	Onion	Larimer	Furrow
Bean	Wheat	Onion	Weld	Drip
Bean	Bean	Onion	Weld	Drip
--	Bean	Onion	Weld	Drip
Bean	Bean	Onion	Weld	Furrow
--	Bean	Onion	Weld	Furrow
--	Bean	Onion	Weld	Furrow
--	Bean	Onion	Larimer	Furrow
Wheat	Bean	Onion	Morgan	Sprinkler
Wheat	Bean	Onion	Morgan	Sprinkler
Wheat	Bean	Onion	Morgan	Sprinkler
Wheat	Bean	Onion	Morgan	Sprinkler
--	Corn	Bean	Weld	Furrow
--	Wheat	Bean	Weld	Furrow
--	Wheat	Bean	Weld	Furrow
--	Wheat	Bean	Weld	Furrow
--	Wheat	Bean	Weld	Drip

[z] -- Indicates crop was unknown

Table III. 2. Type 3 test of mixed model fixed effects with spatial exponential repeated measure analysis of 2003 and 2004 epiphytic xanthomonad populations in relation to crop rotation and sampling time.

Effect	Numerator DF	Denominator DF	F Value	P-Value
2003				
Rotation	3	10	4.73	0.0265
Sampling time	4	40	1.22	0.3195
Rotation*Samping time	12	40	0.79	0.6592
2004				
Rotation	2	17	5.87	0.0115
Sampling time	3	51	1.07	0.3691
Rotation*Samping time	6	51	0.55	0.7709

Table III. 3. Least square mean estimates, significance, and confidence limits for culturable epiphytic xanthomonad populations in relation to crop rotation. Values were calculated as logarithmic colony forming units/g fresh weight of leaves.

Rotation[y]	DF	Least square means estimate	*t* value	Prob *t*>0[z]	Least square means confidence limits	
					Lower	Upper
2003						
Nonhost-onion	10	0.00	0.00	1.0000	-1.09	1.09
Dry bean-onion	10	0.29	0.69	0.5045	-0.65	1.24
Dry bean-dry bean	10	2.02	5.32	0.0003	1.17	2.87
Wheat-dry bean	10	1.14	1.89	0.0877	-0.20	2.47
2004						
Nonhost-onion	17	0.00	1.00	0.3327	-0.22	0.61
Dry bean-onion	17	0.83	5.20	<0.0001	0.50	1.17
Nonhost-dry bean	17	0.20	1.00	1.0000	-0.45	0.45

[y] Epiphytic xanthomonad populations monitored over time in commercial onion and dry bean fields were analyzed by repeated measures analysis with unequal spacing over time using SAS v 9.1 PROC MIXED. Sampling date was not significant in the mixed model. The experiment was arranged as a completely randomized design, where an entire field following the same crop rotation pattern was considered an experimental unit. Nonhost refers to rotational crops that are not hosts of *X. axonopodis* pv. *allii* or *X. axonopodis* pv. *phaseoli*, and included field corn, sugar beet, potato, and winter wheat.

[z] Probability mean population of epiphytic xanthomonads is greater than 0 based on a *t* test.

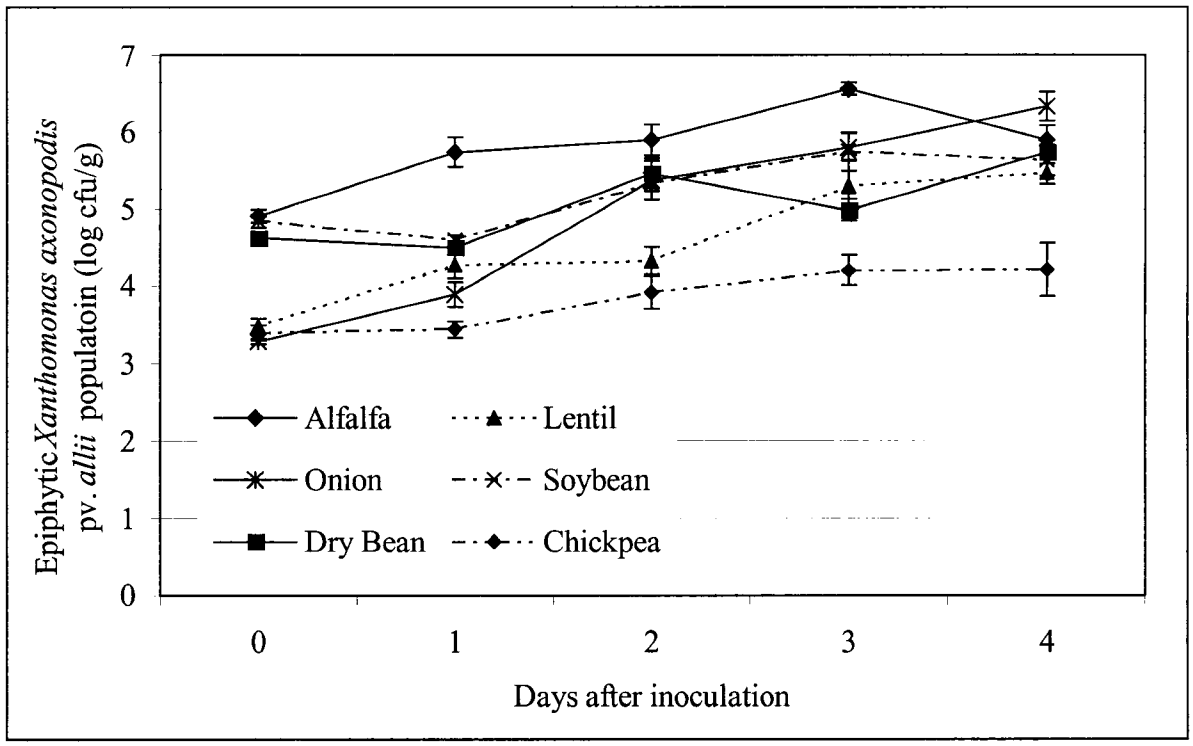


Figure III.1. Epiphytic population dynamics of *Xanthomonas axonpodis* pv. *allii* strain R-O177 on leguminous hosts and onion under high humidity and temperature growth chamber conditions. Data are mean of four replications repeated over two experiments (n=8) ± standard error of the mean.

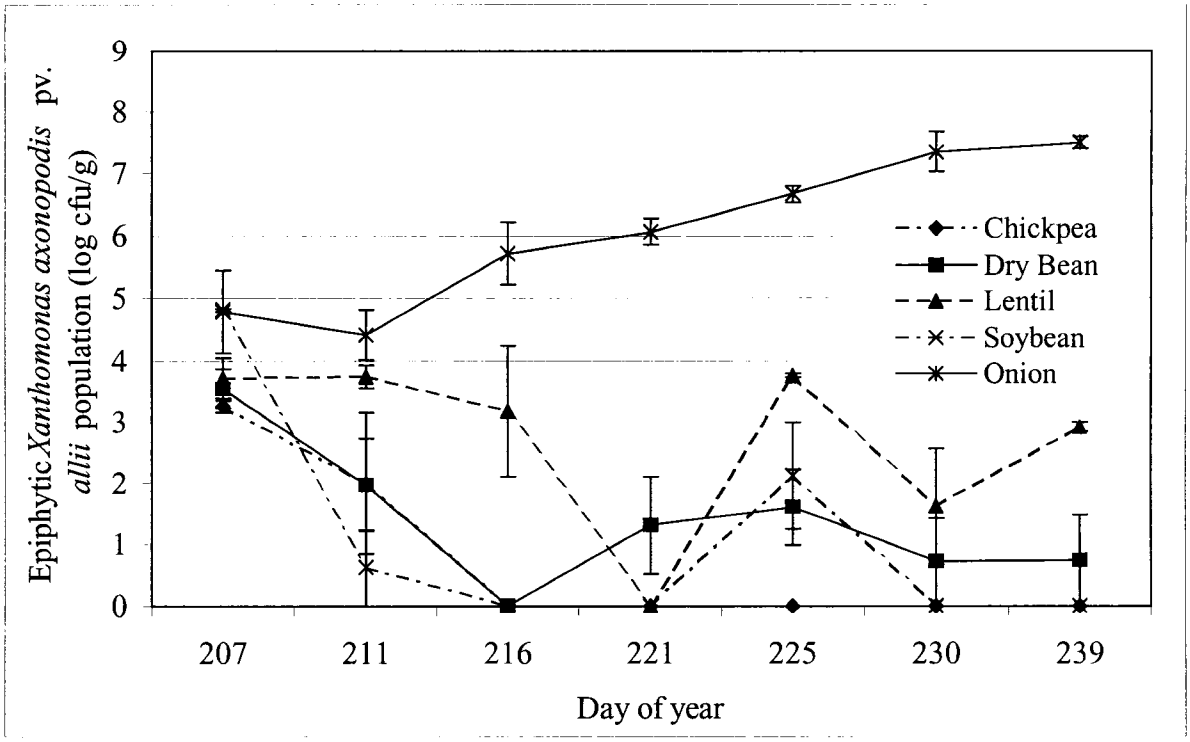


Figure III. 2. Epiphytic population dynamics of *Xanthomonas axonpodis* pv. *allii* strain R-O177 on leguminous hosts and onion in inoculated experimental plots near Fort Collins, CO, 2003. Plots were inoculated with a 10^6 cfu/ml suspension of *X. axonpodis* pv. *allii* strain R-O177 on 25 July, 2003 (206 day of year). Data are mean of four replications (n=4) ± standard error of the mean.

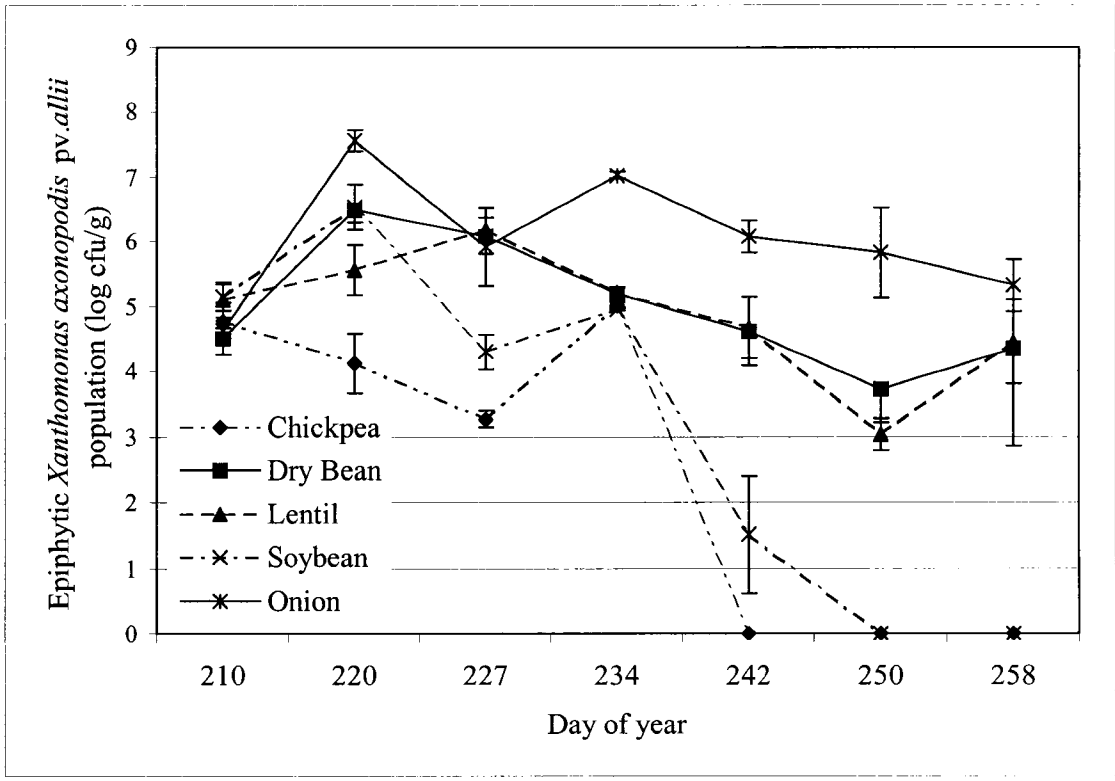


Figure III. 3. Epiphytic population dynamics of *Xanthomonas axonpodis* pv. *allii* strain R-O177 on leguminous hosts and onion in inoculated experimental plots near Fort Collins, CO, 2004. Plots were inoculated with a 10^6 cfu/ml suspension of *X. axonpodis* pv. *allii* strain R-O177 on 23 July, 2004 (205 day of year). Data are mean of four replications (n=4) ± standard error of the mean.

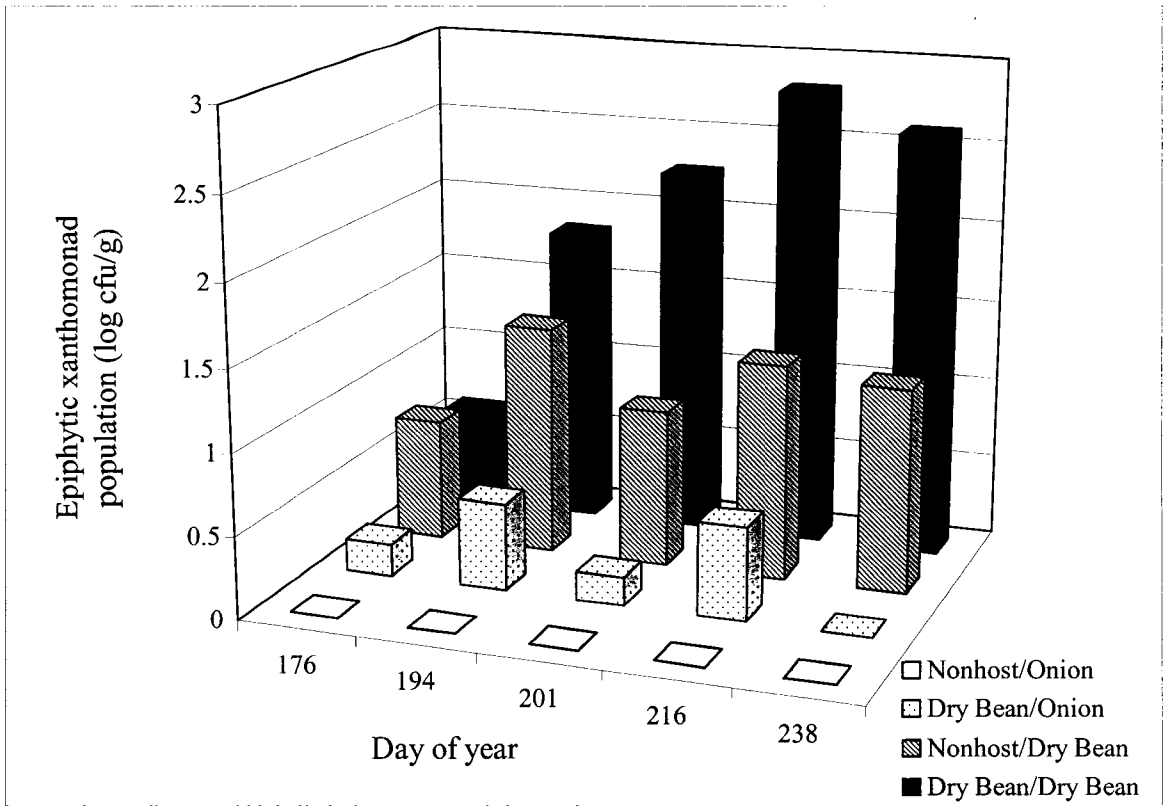


Figure III. 4. Epiphytic xanthomonad populations in 2003 on dry bean and onion in relation to cropping system. Epiphytic populations were estimated from bulked samples collected on 25 June, 13 July, 4 August, and 26 August. The number of fields assayed for epiphytic populations varied among crop rotations; n=3 for nonhost-onion rotations, n=4 for dry bean-onion rotations, n=2 for nonhost-dry bean rotations, and n=5 for dry bean-dry bean rotations.

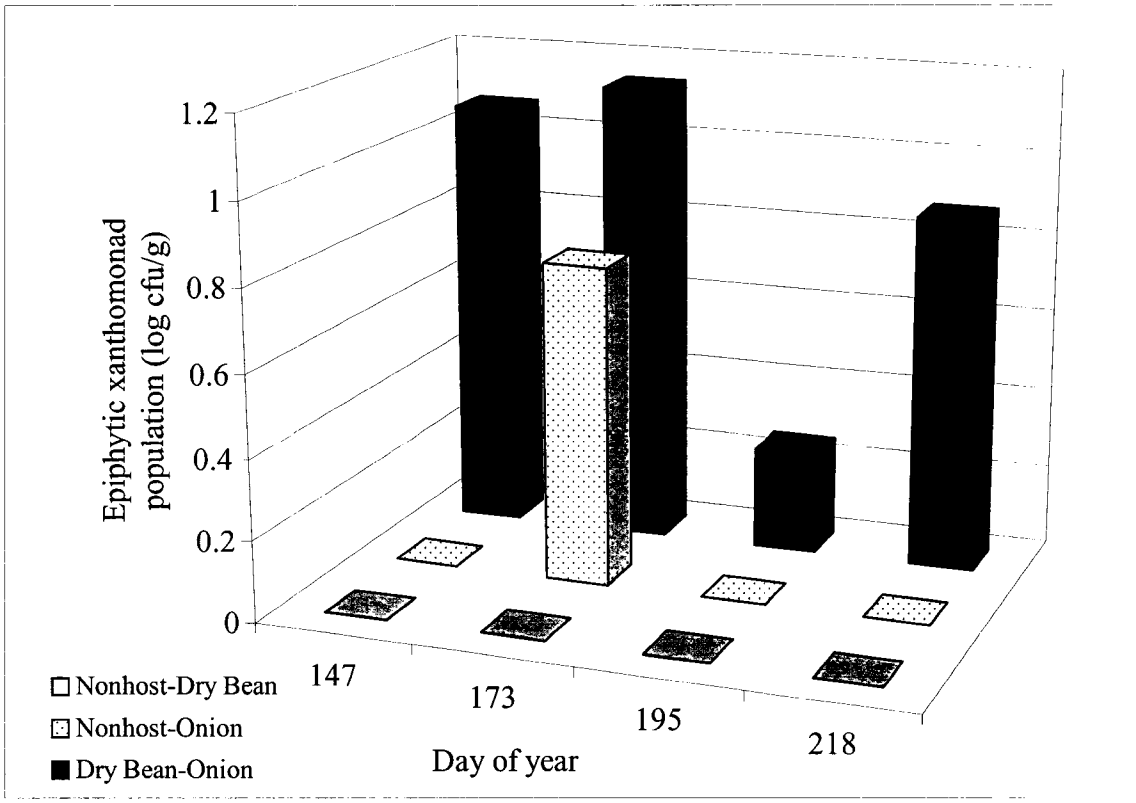


Figure III. 5. Epiphytic xanthomonad populations in 2004 on dry bean and onion in relation to cropping system. Epiphytic populations were estimated from bulked samples collected on 26 May, 21 June, 13 July, and 5 August. Nonhost refers to rotation crops that are not hosts of *Xanthomonas axonopodis* pv. *allii* or *X. axonopodis* pv. *phaseoli*, and included field corn, sugar beet, and winter wheat. The number of fields assayed for epiphytic populations varied among crop rotations; n=5 for nonhost-dry bean rotations, n=6 for nonhost-onion rotations, and n=9 for dry bean-onion rotations.

CHAPTER IV

INOCULUM SOURCES OF XANTHOMONAS AXONOPODIS PV. ALLII IN COLORADO

INTRODUCTION

Xanthomonas leaf blight of onion (*Allium cepa*), caused by *Xanthomonas axonopodis* pv. *allii*, is a yield limiting disease in Colorado (24). Disease symptoms are varied but include leaves with lenticular water-soaked lesions that elongate into chlorotic streaks, necrosis, tip dieback, and stunting of plants that reduces bulb size. This reduction in leaf area results in undersized bulbs at harvest that can reduce yields significantly (17,24,25). A bulb rot is not known to occur.

Few disease management strategies have been developed for Xanthomonas leaf blight of onion because basic knowledge of *X. axonopodis* pv. *allii* survival and dissemination is lacking. The planting of pathogen-free seed is essential because seed can be contaminated by *X. axonopodis* pv. *allii* (20), and this contamination appears important epidemiologically in tropical production regions (18). Crop rotation has been suggested in Barbados since *X. axonopodis* pv. *allii* strains common in this region reportedly attack leguminous host such as snap bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*), and soybean (*Glycine max*) (15). Disease symptoms have not been observed in Colorado on any leguminous host, but *X. axonopodis* pv. *allii* colonized and persisted epiphytically on dry bean (*P. vulgaris*) and lentil (*Lens culinaris*) (Gent and Schwartz, unpublished data). The bacterium can also attack several *Allium* species such as *A. fistulosum*, *A.*

sativum, *A. porrum*, and *A. cepa* var. *ascalonicum* (5,8,10,19). Two Xanthomonas leaf blight resistant cultivars have been identified (15), but are not adapted in Colorado and are no longer available commercially.

In the absence of effective cultural practices, host resistance, or biological controls, growers in Colorado rely upon copper-based bactericides to manage Xanthomonas leaf blight (24). Copper bactericides alone or amended with maneb suppress the disease, but spray applications must be applied preventatively and regularly (every five to 10 days) to be effective (23). Ten or more applications are made each season, but spray timing may be improved by disease forecasting (26). Copper resistance was not observed in a worldwide collection of 49 *X. axonopodis* pv. *allii* strains (8), but was reported in strains from Barbados (16), and is common among other phytopathogenic bacteria (4,13,27). Reliance upon copper bactericides alone for Xanthomonas leaf blight management is not sustainable.

Sustainable management of Xanthomonas leaf blight will likely require a multi-tactic approach that reduces or avoids primary inoculum sources, but these inoculum sources are unknown. Therefore, we sought to identify and quantify *X. axonopodis* pv. *allii* primary inoculum sources in Colorado onion production regions.

MATERIALS AND METHODS

Bacterial strains and culture. A rifampicin mutant of *X. axonopodis* pv. *allii* strain O177 (ATCC 508) was generated as previously described (28), and is referred to as R-O177. Strain R-O177 is resistant to greater than 200 μg/ml rifampicin, but selection routinely was performed on nutrient agar amended with 50 or 100 μg/ml of rifampicin and 50 μg/ml cycloheximide. Other xanthomonads isolated from weeds, crops, or water were routinely cultured on nutrient agar at

29°C. Bacterial strains were preserved in 15% nutrient glycerol broth at -80°C for long-term storage.

Pathogenicity and host range. All presumptive xanthomonads recovered were tested for pathogenicity on onion (cv. Vantage) and dry bean (cv. Sacramento light red kidney) in growth chamber assays. The youngest, fully-extended leaves of 8 week-old onion plants were pin-pricked three times at 2.5 cm intervals with a 22 gauge needle bearing a bacterial matrix of a given isolate removed from 72-hour old nutrient agar culture plates. Each pin-pricked leaf area was inoculated with a bacterial matrix approximately equal in size to the needle tip. Plants serving as negative controls were pin-pricked with a sterile needle. Pathogenicity on dry bean was evaluated by spray inoculation. A single colony of the isolate to be tested was picked from a 72-hr old nutrient agar plate and transferred into 3 ml of nutrient broth in 15 ml culture tubes, and was incubated at 26°C with vigorous shaking (250 oscillations/min) for 24 h. The bacterial cells were collected by centrifugation before adjusting to approximately 10^7 CFU/ml in sterile magnesium sulfate-potassium phosphate buffer (0.01 M magnesium sulfate and 0.01M potassium phosphate pH 7.2). Three- to four-week old dry bean plants were sprayed (Crown SpraTool, Aerovoe Industries, Inc., Gardnerville, NV) to runoff with the bacterial suspension. Control plants were inoculated with sterile buffer. The plants were placed in a growth chamber and incubated for 7 days with a 28°C/24°C day/night temperature regime, light intensity of 350 $\mu Ms^{-1}m^{-2}$, 100% relative humidity, and daily misting with tap water to runoff. At least three plants were inoculated with each isolate. Plants were observed daily for symptom development. Isolates were considered nonpathogenic to a given host if disease symptoms failed to develop within 14 days.

Growth chamber epiphytic population assays. Epiphytic development of strain R-O177 was monitored on weeds commonly found in Colorado onion fields in growth chamber assays. Plants

of field bindweed (*Convolvulus arvensis*), common lambsquarter (*Chenopodium album*), hairy nightshade (*Solanum sarrachoides*), yellow nutsedge (*Cyperus esculentus*), and redroot pigweed (*Amaranthus retroflexus*) were grown under greenhouse conditions (approximately 24°C/20°C day/night temperature regime and 14-hour photoperiod, with approximately 2-h of supplemental incandescent lighting) until they were three- to four- (weeds) or six- to eight-(onion) weeks old. Three seeds of each species were planted individually into MetroMix 200 potting soil (Grace Sierra Horticultural Products Company, Milpitas, CA) in 1 l pots. Weed seed was collected from biotypes of each species found at and near the Colorado State University Agricultural Research, Development, and Education Center (ARDEC) near Fort Collins, CO; and were assumed to be collected from apparently non-contaminated mature plants.

Twenty pots of each plant species were inoculated by spraying to runoff with a 10^5 CFU/ml bacterial suspension using a Crown Spratool. Inoculum of strain R-O177 was cultured by inoculating 3 ml of nutrient broth in 15 ml culture tubes, and incubating at 26°C with vigorous shaking (250 oscillations/min) for 24 h. The bacterial cells were collected by centrifugation before adjusting to approximately 10^5 CFU/ml in sterile magnesium sulfate-potassium phosphate buffer. After inoculation, plants were allowed to air dry and then sampled immediately by removing all aboveground plant material from four pots of each plant species and placing each individually into a plastic bag. Another set of four pots of each plant species were destructively sampled each day for four days. An experimental unit consisted of one pot of a given plant species that contained three plants destructively sampled each day. Plants were maintained at 28°C/24°C day/night temperature regime, light intensity of 350 $\mu Ms^{-1}m^{-2}$, 100% relative humidity, and daily misting with tap water to runoff after inoculation.

Harvested plant samples were weighed and placed into sterile 250 ml flasks containing 100 ml of magnesium sulfate-potassium phosphate buffer, and shaken at 250 oscillations/min for 60 min at

room temperature (approximately 22°C). Aliquots (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium sulfate-potassium phosphate buffer before plating in duplicate onto nutrient agar amended with 50 µg/ml rifampicin and cycloheximide. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 26°C, and a subset of rifampicin resistant colonies were confirmed as *X. axonopodis* pv. *allii* by standard physiological and biochemical tests (21), including Gram reaction, pigmentation on yeast dextrose carbonate medium, fluorescence on King's medium B, indole test, growth on 0.1% tetrazolium chloride, oxidase test, starch hydrolysis, oxidative and fermentative utilization of glucose, production of catalase, production of H_2S from peptone, production of arginine dihydrolase, and casein hydrolysis test. The experiment was repeated twice over time.

Recovery of *X. axonopodis* pv. *allii* from weed, crop, and volunteer onion plants. Weed, crop, and volunteer onion plant surveys were conducted in 2003 and 2004 in three major onion-growing regions of the state; namely the Arkansas Valley, north central Colorado, and northeastern Colorado. Commonly-occurring weed, crop, and volunteer onion (where found) plants were collected from within and adjacent to fields that were currently planted to or had been planted to onion the previous year. Five sites were surveyed for *X. axonopodis* pv. *allii* in 2003, and 6 sites were surveyed in 2004. Xanthomonas leaf blight was confirmed at Sites 1 and 5 in 2002, and sites 1,4,and 5 in 2003. The disease was not observed in onion crops at the other sites during 2002 or 2003.

At each location, four bulked samples (each 5 to 10 grams) from each field were used for epiphyte recovery assays. Plant samples were weighed and placed into sterile 250 ml flasks containing 100 ml of magnesium sulfate-potassium phosphate buffer, and shaken at 250 oscillations/min for 60 min at room temperature (approximately 22°C). Aliquots (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium sulfate-potassium phosphate buffer before

plating in duplicate onto modified MXP medium (7) containing 50 mg/l kasugamycin, 30 mg/l cephalexin, and 50 mg/l cycloheximide or plated in duplicate onto modified MXP medium using a spiral-plating system (Autoplate 4000, Spiral Biotech, Inc., Norwood, MA). Culture plates were incubated in the dark at 29°C for 72 to 96 h before observing plates for characteristic xanthomonad colonies surrounded by a zone of starch hydrolysis. Representative xanthomonad colonies were picked from plates and confirmed as *X. axonopodis* by physiological and biochemical tests and Biolog (Biolog, Inc., Hayward, CA) substrate utilization profiles. Identification to the pathovar level (*X. axonopodis* pv. *allii* or *phaseoli*) was determined by pathogenicity to onion or dry bean.

Recovery of *X. axonopodis* pv. *allii* from irrigation water. Irrigation water entering and leaving onion fields was assayed for the presence of *X. axonopodis* pv. *allii* in 2003 and 2004. Field plots were established at ARDEC near Fort Collins, CO and the Arkansas Valley Research Center near Rocky Ford, CO to study the epiphytic survival of *X. axonopodis* pv. *allii* on leguminous hosts, determine the influence of nitrogen fertilization on Xanthomonas leaf blight severity, and screen copper and other bactericides efficacy for Xanthomonas leaf blight management. Plots in these studies were inoculated with 10^6 to 10^8 CFU/ml of *X. axonopodis* pv. *allii* strain R-O177 to initiate disease epidemics. Data from these studies is not presented here, but irrigation water entering and leaving these fields was collected and assayed for *X. axonopodis* pv. *allii* in 2003 (1 field near Fort Collins) and 2004 (2 fields near Fort Collins designated fields 1 and 2, and 1 field near Rocky Ford). Cultivars Vantage and X-201 were planted each year at Fort Collins and Rocky Ford, respectively.

X. axonopodis pv. *allii* populations were measured from water collected during each irrigation in sterile 50-ml centrifuge tubes from both the top and bottom ends of each field. Water sampling began when Xanthomonas leaf blight symptoms were first detected, and continued until the last

irrigation before harvest. Water entering the top of fields was collected randomly from 3 to 10 locations as it left gated, polyvinvyl chloride delivery pipe (Fort Collins) or siphon tubes (Rocky Ford) before it contacted any plant or area of the field where plants were growing. Tail water was collected randomly from 3 to 10 locations at the bottom of each field from individual furrows before it mixed with water from other rows. Random rows sampled at the top of the field were not necessarily the same random rows sampled from the bottom of the field. Water used for irrigation in fields sampled near Fort Collins was derived from ground water delivered to the field in buried plastic pipe, while water used for irrigation in the field sampled near Rocky Ford originated from the Arkansas River and was delivered to the field through the Rocky Ford Canal system. An individual water sample collected on a given sampling date from a location was considered an experimental unit. Each field was approximately 150 m in length, and was irrigated once to twice weekly except when rainfall >2.5 cm occurred since the previous irrigation.

Water samples were placed at 4°C until assays were conducted. All samples were processed within 24 h from collection, but samples were generally processed within 1 h from the time of collection. Aliquots (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium sulfate-potassium phosphate buffer or spiral-plated directly (in duplicate) onto nutrient agar amended with 100 µg/ml rifampicin and 50 µg/ml cycloheximide. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 29°C days, and a subset of rifampicin resistant colonies were confirmed as *X. axonopodis* pv. *allii* by pathogenicity to onion and by physiological and biochemical tests.

Survival of *X. axonopodis* pv. *allii* in crop debris. *X. axonopodis* pv. *allii* survival in onion crop debris was determined in furrow-irrigated plots near Fort Collins and Rocky Ford from September 2003 to May (Rocky Ford) or June 2004 (Fort Collins). Leaves of onion cv. Vantage with characteristic Xanthomonas leaf blight symptoms were arbitrarily collected (irrespective of

leaf age) in September 2003 from plants grown in experimental plots near Fort Collins. Leaves were only collected from plants that did not receive any bactericide treatment, and were dried at room temperature (approximately 24°C) for 72 h. Leaves were examined carefully, and any leaf with atypical Xanthomonas leaf blight symptomology was discarded. The remaining diseased leaves were cut into 12.7 cm lengths, which contained large lenticular-shaped, water-soaked lesions. Leaves with lesions covering at least 50% of the flat side of the leaf were collected and weighed, on average 0.23 g each. Four of these leaves were placed into a nylon stocking and placed into mesh onion sacks (four nylon stockings per bag) to aid in recovery of the nylon-mesh stockings if they decomposed over time. The mesh onion sacks were placed on the soil surface of a 75 cm wide bed, and anchored with wooden stakes or buried 25 cm deep to simulate over wintering without or with deep tillage, respectively. An experimental unit was considered an individual nylon stocking containing four diseased onion leaves, sampled on a given sampling date. The field near Fort Collins was left fallow and not irrigated until onion was again planted in April 2004. The field was then irrigated once to twice weekly until the study concluded. At Rocky Ford, the field was planted to winter wheat (*Triticum aestivum*) and was irrigated five times throughout the duration of the study.

A buried and non-buried mesh onion sack each was recovered monthly beginning 1 October 2003. A nylon stocking was removed from the mesh onion sack, and placed into a sterile mortar before the addition of approximately 150 ml of liquid nitrogen. After evaporation, the nylon stocking and associated diseased leaves were cut into small pieces with sterile scissors and ground with a sterile pestle. After the mortars thawed at room temperature, 20 ml of sterile magnesium sulfate-potassium phosphate buffer was added and used to thoroughly rinse the nylon stocking and associated leaf material. Aliquots (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium sulfate-potassium phosphate buffer before plating in duplicate onto nutrient agar amended with 100 µg/ml rifampicin and 50 µg/ml cycloheximide. Characteristic *X.*

axonopodis pv. *allii* colonies were enumerated after 72 h of incubation at 29°C, and a subset of rifampicin-resistant colonies were confirmed as *X. axonopodis* pv. *allii* by pathogenicity to onion and physiological and biochemical tests.

Statistical analyses. Growth chamber assays were organized as a completely randomized design with four replications. All bacterial population data were log transformed to achieve independently and normally distributed experimental errors with a common variance. Statistical analysis was performed using PROC MIXED in SAS v. 9.1 (SAS Institute, Cary, NC). Populations of *X. axonopodis* pv. *allii* recovered in water collected from the top and bottom of fields were analyzed by *t* tests on each sampling date from each location. Populations of *X. axonopodis* pv. *allii* recovered in crop buried or non-buried crop debris was analyzed by one-sided *t*- tests assuming unequal variances on each sampling date from each location.

RESULTS

Growth chamber epiphytic population assays. *X. axonopodis* pv. *allii* population dynamics varied among weed species evaluated in growth chamber assays. Culturable populations of the bacterium four days after inoculation increased 1.44, 1.77, and 1.84 logarithmic units/g fresh weight on *Convolvulus arvensis*, *Cyperus esculentus*, and *Solanum sarrachoides*, respectively. During the same period, epiphytic populations decreased by 1.2 and 2.7 logarithmic units/g fresh weight on *A. retroflexus* and *C. album*, respectively. Angular, water-soaked lesions and other symptoms characteristic of bacterial pathogen infection were not visible on any plants throughout the experiment.

Recovery of *X. axonopodis* pv. *allii* from weed, crop, and volunteer onion plants. *X. axonopodis* pv. *allii* and nonpathogenic xanthomonads were recovered from several weed and crop plants sampled in 2003 at sites where an epidemic of Xanthomonas leaf blight occurred the previous year. At site 1 in Larimer County, epiphytic xanthomonads were recovered from volunteer onion, *Amaranthus retroflexus*, *Echinochloa crus-galli*, *Polygonum convolvulus*, *Cirsium arvense*, *Convolvulus arvensis*, and *Malva neglecta*. These isolates were yellow-pigment, non-fluorescent, Gram-negative, obligate aerobes, positive for the presence of catalase, produced H_2S from cysteine, hydrolyzed casein and starch, were negative for the production of arginine dihydrolase, indole, and oxidase, and did not grow on 0.1% tetrazolium chloride medium. These isolates were identified to the genus *Xanthomonas* based upon substrate utilization profiles on Biolog GN microplates (data not presented), but most were nonpathogenic to onion and dry bean. Isolates pathogenic to onion, and therefore classified as *X. axonopodis* pv. *allii*, were recovered only from *M. neglecta*.

At site 5 in Otero County, an epidemic of Xanthomonas leaf blight occurred in 2002. In 2003, epiphytic *X. axonopodis* pv. *allii* was recovered from symptomless *Medicago sativa*, *A. retroflexus*, *Anoda cristata*, *K. scoparia*, and *Solanum rostratum*. Isolates recovered from these hosts were confirmed as *X. axonopodis* pv. *allii* by physiological and biochemical tests as described above, Biolog substrate utilization profiles, and pathogenicity to onion but not dry bean. Characteristic Xanthomonas leaf blight symptoms of lenticular water-soaked lesions most prominent on the flattened sides of leaves, necrotic streaks, and tip dieback were observed on volunteer onion plants at site 5. Isolation of the casual organism from diseased plants yielded typical *X. axonopodis* pv. *allii* colonies, which were confirmed as the Xanthomonas leaf blight bacterium as described above. Epiphytic *Xanthomonas* species were not recovered from weeds sampled at sites 2, 3, or 4 in 2003. Xanthomonas leaf blight did not occur at these sites in 2002.

In 2004, weeds and crops at 6 locations were monitored for epiphytic xanthomonads, and epiphytic xanthomonads were recovered from all sites except site 2. At site 1, an epidemic of Xanthomonas leaf blight occurred in 2003, and epiphytic xanthomonads were recovered from volunteer onion, *A. retroflexus*, *Helianthus annus*, and *Lens culinaris*. *X. axonopodis* pv. *allii* was recoverd from volunteer onion, but only *X. axonopodis* pv. *phaseoli* was recovered from *A. retroflexus*. Xanthomonads nonpathogenic to onion or dry bean were recovered from *Helianthus annus* and *Lens culinaris*. An epidemic of Xanthomonas leaf blight did not occur at site 3 in 2003, and *X. axonopodis* pv. *allii* was not recovered. *X. axonopodis* pv. *phaseoli* was recovered from *A. retroflexus* and *S. halepense* at site 3. A Xanthomonas leaf blight epidemic occurred at site 4 in 2003, and *X. axonopodis* pv. *allii* was recovered from volunteer onion, but not the weeds sampled. However, both *X. axonopodis* pv. *phaseoli* and nonpathogenic xanthomonads were recovered from *Convolvulus arvensis*. An epidemic of Xanthomonas leaf blight occurred at site 5 in 2003, and *X. axonopodis* pv. *allii* was recovered from all plants sampled, namely volunteer onion, *A. retroflexus*, *Cenchrus longispinus*, *Chenopodium album*, *H. annuus*, *K. scoparia*, and *S. sarrachoides*. A Xanthomonas leaf blight epidemic did not occur at site 6 in 2003, and *X. axonopodis* pv. *allii* was not recovered from any plant sampled. Epiphytic xanthomonads were recovered from all plants sampled though, namely *C. arvensis*, *C. eculentus*, *H. annuus*, *K. scoparia*, *M. sativa*, *S. rostratum*, and *T. officinale*, but these xanthomonads were not pathogenic to dry bean or onion.

Recovery of *X. axonopodis* pv. *allii* from irrigation water. *X. axonopodis* pv. *allii* was recovered consistently from irrigation tail water leaving onion fields where Xanthomonas leaf blight symptoms were visible (Figures IV. 2 to IV. 4). At Fort Collins in 2003, 9.90 x 10^2 to 4.11 x 10^3 CFU/ml were recovered from irrigation tail water, but *X. axonopodis* pv. *allii* was not recovered from water entering the field. From field 1 at Fort Collins in 2004, small populations

of *X. axonopodis* pv. *allii* (8.93 to 7.53 x 10^1 CFU/ml) were recovered from irrigation tail water in the week following the first appearance of Xanthomonas leaf blight symptoms, but as many as 3.02 x 10^4 CFU/ml were recovered later in the season. *X. axonopodis* pv. *allii* was not recovered on any sampling date from water entering the field. In field 2 at Fort Collins in 2004, very small populations of *X. axonopodis* pv. *allii* (7.03 CFU/ml) were again recovered from irrigation tail water within 7 days of Xanthomonas leaf blight symptom appearance in the field, and these populations increased on all but the last sampling date throughout the season. The bacterium was not recovered from water entering field 2 on any sampling date.

At Rocky Ford in 2004, only two irrigation events were sampled after Xanthomonas leaf blight symptoms were apparent in the field because timely and consistent rainfall supplied much of the water requirement for the crop. On 1 and 14 August, no xanthomonads were recovered from irrigation water entering the field, but 1.55 x 10^1 and 8.71 x 10^2 CFU/ml were recovered from irrigation water at the bottom of the field, respectively.

Survival of *X. axonopodis* pv. *allii* in crop debris. Culturable populations of *X. axonopodis* pv. *allii* were not greater in onion leaves on the soil surface as compared to buried leaves at Fort Collins or Rocky Ford (P-value 0.50 and 0.52) on the first sampling date, but were greater on all other sampling dates across both locations (Table IV. 2 and Figures IV. 5 and IV. 6). At Fort Collins, culturable *X. axonopodis* pv. *allii* populations decreased more than 100-fold in leaves on the soil surface, as compared to the original population, over the 9-month duration of the study. In the same time period, culturable *X. axonopodis* pv. *allii* populations decreased more than 1,000,000,000-fold in leaves buried 25-cm deep. Similarly at Rocky Ford, populations in leaves left on the surface decreased greater than 10,000-fold over the 8 months they were monitored, but decreased greater than 100,000,000 in buried leaves. On the last sampling date, 8.32 x 10^7 and 1.32 x 10^6 CFU/leaf were cultured from leaves left on the soil surface at Fort Collins and Rocky

Ford, respectively. Culturable populations in buried leaves on the same dates were 9.12 and 5.37 $x10^2$ CFU/leaf from Fort Collins and Rocky Ford, respectively.

DISCUSSION

Xanthomonas leaf blight was first observed in Colorado in 1996, and annual occurrences of the disease since its appearance suggest it has become endemic in southern Colorado. Management of Xanthomonas leaf blight has largely been limited to copper bactericide applications because basic elements of the disease epidemiology are unknown, and inoculum sources of the pathogen have not been identified. In this study, we have identified and quantified several primary inoculum sources of *X. axonopodis* pv. *allii*. Reservoirs of the pathogen were identified in or on other crop, and weed, and volunteer onion plants, contaminated irrigation water, and infested crop debris. The design of onion production systems must consider multiple *X. axonopodis* pv. *allii* inoculum sources to reduce recurring losses from Xanthomonas leaf blight.

Several weeds common in onion production systems in Colorado were found to be sources of epiphytic *X. axonopodis* pv. *allii*, as well as *X. axonopodis* pv. *phaseoli* and other xanthomonads. *X. axonopodis* pv. *allii* was only recovered from sites where Xanthomonas leaf blight occurred the previous year, and its recovery from many weeds was not consistent across sites or time. In 2003, *X. axonopodis* pv. *allii* was recovered only from *M. neglecta* at site 1, but was recovered from five weed species and alfalfa at site 5; an epidemic of Xanthomonas leaf blight occurred at both locations in 2002. Similarly in 2004, the bacterium was not recovered from any weed or other crop plants at sites 1,2,3 or 4, but was recovered from six weed species at site 5. In growth chamber studies under conditions favorable to the pathogen, epiphytic populations of *X. axonopodis* pv. *allii* increased on three of five weed species evaluated, but decreased nearly 1000-fold on *C. album*. Plant species and environmental conditions are known to influence epiphytic

bacterial populations (14), but the conditions that allow *X. axonopodis* pv. *allii* to successfully colonize weeds are unclear. When the results of growth chamber and field studies are taken together, they suggest that not all weed species support epiphytic growth of *X. axonopodis* pv. *allii*. We recovered *X. axonopodis* pv. *phaseoli* and nonpathogenic xanthomonads from several weed and crop plants, and sanitation of weeds may eliminate potential reservoirs of both *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli*.

Volunteer onion, however, consistently was a source of *X. axonopodis* pv. *allii*. Xanthomonas leaf blight symptoms or epiphytic *X. axonopodis* pv. *allii* were observed on or recovered from volunteer onion at four or five sites where Xanthomonas leaf blight occurred the previous year. At site 5 in 2003 and site 5 in 2004, Xanthomonas leaf blight symptoms were observed on volunteer onion before the disease appeared in nearby onion fields, suggesting volunteer onion is an early-season source of the pathogen.

X. axonopodis pv. *allii* does not appear to persist for long periods of time on weed hosts. We did not recover *X. axonopodis* pv. *allii* from weeds or other crops if Xanthomonas leaf blight did not occur the previous season. This finding is consistent with studies of *X. axonopodis* pv. *phaseoli* (1,6) and *X. campestris* pv. *vitians* (3). Angeles-Ramos et al. (1) found *X. axonopodis* pv. *phaseoli* was most readily recovered from weeds within dry bean fields where common bacterial blight symptoms were present, but few weeds outside of such fields harbored epiphytic *X. axonopodis* pv. *phaseoli*. These epiphytic populations were short-lived. Within 7 days from harvest, epiphytic *X. axonopodis* pv. *phaseoli* cells were not detected on weeds within the field. Similarly, Barak et al. (3) reported *X. campestris* pv. *vitians* was not recovered unless lettuce (*Lactuca sativa*) with bacterial leaf spot symptoms were nearby, and the bacterium did not survive on weeds during a two-month fallow period between lettuce crops. In the current study, *X. axonopodis* pv. *allii* was recovered from only a few weeds in or near fields where

Xanthomonas leaf blight was present less than 10 months prior. Therefore, epiphytic survival of *X. axonopodis* pv. *allii* on weeds may be important in some fields, but does not appear to be the primary means for bacterial persistence in the absence of onion.

Irrigation water appears to be an efficient medium for the dispersal of *X. axonopodis* pv. *allii*. Large populations of the bacterium, sometimes in excess of 10^4 CFU/ml, were recovered from irrigation water leaving fields where Xanthomonas leaf blight symptoms were observed. However, irrigation water originating from ground water was not found to harbor *X. axonopodis* pv. *allii* when plated onto rifampicin-amended nutrient agar or modified MXP medium (data not presented). Onion crops are irrigated primarily by furrow irrigation in Colorado (Schwartz and Bartolo, 1995), and the tail water leaving these fields is returned to canal systems and reused in other down-stream fields. Xanthomonads are known to be readily disseminated by irrigation water (11) and avoiding reuse of irrigation water and relying upon ground water for irrigation where possible should reduce this source of *X. axonopodis* pv. *allii*.

Crop debris can be an epidemiologically important source of xanthomonads (3,9,22), and we found significant populations of *X. axonopodis* pv. *allii* overwintering in diseased onion leaves buried 25-cm deep or left on the soil surface. Culturable populations of the bacterium were decreased eight to nine orders of magnitude in buried leaves over the nine month time course of this study, but decreased only two to four orders of magnitude in leaves left on the soil surface. Greater than 10^6 CFU/leaf were recovered from onion leaves on the soil surface after eight months, but only 10^4 CFU/leaf were recovered from buried leaves when sampled in March, the month in which most onions are planted in Colorado. Deep incorporation of diseased crop debris would not eliminate overwintering populations of the bacterium before an onion crop would be planted the following spring. In June, small populations (less than 10 CFU/leaf) of the pathogen were recovered from buried leaves, and these bacteria would likely not survive until the following

season. Therefore, prompt and thorough incorporation of infested crop debris after harvest and crop rotation of at least two years between onion are necessary to reduce and avoid overwintering *X. axonopodis* pv. *allii* in Colorado.

Integrated management of Xanthomonas leaf blight in Colorado must consider multiple inoculum sources of *X. axonopodis* pv. *allii*, including contaminated seed (18,20), weeds, leguminous crops, infested crop debris, irrigation water, and volunteer onion. Onion production systems that practice strict sanitation of weed and volunteer onion plants, follow a two-year or longer rotation to nonhosts such as small grains, avoid reuse of irrigation tail water, use only pathogen-free seed, and promote rapid breakdown of crop debris by deep tillage should reduce *X. axonopodis* pv. *allii* survival and minimize reliance upon copper bactericides for disease management.

LITERATURE CITED

1. Angeles-Ramos, R., Vidaver, A. K., and Flynn, P. 1991. Characterization of epiphytic *Xanthomonas campestris* pv. *phaseoli* and pectolytic xanthomonads recovered from symptomless weeds in the Dominican Republic. Phytopathol. 81:677-681.

2. Audy P., Laroche, A., Saindon, G., Huang, H. C., and Gilbertson, R. L. 1994. Detection of the bean common blight bacteria *Xanthomonas campestris* pv. *phaseoli* and *X.* c. *phaseoli* var *fuscans*, using the polymerase chain-reaction. Phytopathol. 84:1185-1192.

3. Barak, J. D., Koike, S. T., and Gilbertson, R. L. 2001. Role of crop debris and weeds in the epidemiology of bacterial leaf spot of lettuce in California. Plant Dis. 85:169-178.

4. Bender, C. L., and Cooksey, D. A. 1986. Indigenous plasmids in *Pseudomonas syringae* pv. *tomato*: Conjugative transfer and role in copper resistance. J. Bacteriol. 165:534-541.

5. Bowen, P., Gibbs, H. A., and O'Garro, L. W. 1996. Garlic, chives, shallot, and leek are alternative hosts to *Xanthomonas campestris*, the pathogen of leaf blight of onion. Proc. Int. Congress of Plant Pathol. 7:696.

6. Cafati, C. R., and Saettler, A. W. 1980. Role of nonhost species as alternate inoculum sources of *Xanthomonas phaseoli*. Plant Dis. 64:194-196.

7. Claflin, L. E., Vidaver, A. K., and Sasser, M. 1987. MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathol. 77:730-734.

8. Gent, D. H., Schwartz, H. F., Ishimaru, C. A., Louws, F. J., Cramer, R. A., and Lawrence, C. B. 2002. Polyphasic characterization of *Xanthomonas* strains from onion. Phytopathol. 94:184-195.

9. Gilbertson, R. L., Rand, R. E., and Hagedorn, D. J. 1990. Survival of *Xanthomonas campestris* pv. *phaseoli* and pectolytic strains of *X. campestris* in bean debris. Plant Dis. 74:322-327.

10. Kadota, I., Uehara, K., Shinohara, H., and Nishiyama, K. 2000. Bacterial blight of Welsh onion: A new disease caused by *Xanthomonas campestris* pv. *allii* pv. *nov.* J. Gen. Plant Pathol. 66:310-315.

11. King, C. J., and Brinkerhoff, L. A. 1949. The dissemination of *Xanthomonas malvacearum* by irrigation water. Phytopathol. 39:88-90.

12. 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. and Env. Microbiol. 60:1068-1077.

13. Minsavage, G. V., Canteros, B. I., and Stall, R. E. 1990. Plasmid-mediated resistance to streptomycin in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathol. 80:719-723.

14. O'Brien, R. D., and Lindow, S. E. 1989. Effect of plant species and environmental conditions on epiphytic population sizes of *Pseudomonas syringae* and other bacteria. Phytopathol. 79:619-627.

15. O'Garro, L. W., and Paulraj, L. P. 1997. Onion leaf blight caused by *Xanthomonas campestris*: Alternative hosts and resistant onion genotypes. Plant Dis. 81:978-982.

16. Paulraj, L., and O'Garro, L. W. 1992. Aspects of the epidemiology of a leaf blight of onion in Barbados. Pp. 89-96 in: Proc. of the Tenth Annual Conf. of the Barbados Society of Technologists in Agriculture. St. Micheal, Barbados, Nov 11-13, 1992.

17. Paulraj, L., and O'Garro, L. W. 1993. Leaf blight of onion in Barbados caused by *Xanthomonas campestris*. Plant Dis. 77:198-201.

18. Roumagnac, P., Pruvost, O., Chiroleu, F., and Hughes, G. 2004. Spatial and temporal analyses of bacterial blight of onion caused by *Xanthomonas axonopodis* pv. *allii*. Phytopathol. 94:138-146.

19. Roumagnac, P., Gagnevin, L., Gardan, L., Sutra, L., Manceau, C., Dickstein, E. R., Jones, J. B., Rott, P., and Pruvost, O. 2004. Polyphasic characterization of xanthomonads isolated from onion, garlic and Welsh onion (*Allium* spp.) and their relatedness to different *Xanthomonas* species. Int. J. Syst. Evol. Microbio. 54: 15-24.

20. Roumagnac, P., Gagnevin, L., and Pruvost, O. 2000. Detection of *Xanthomonas* sp., the causal agent of onion bacterial blight, in onion seeds using a newly developed semi-selective isolation medium. Eur. J. Plant Path. 106:867-877.

21. Schaad, N.W., Jones, J.B., and Chun, W. eds 2001. Laboratory Guide for the Identification of Plant Pathogenic Bacteria. 3rd ed. The American Phytopathological Society Press, St. Paul, MN. 398 pp.

22. Schaad, N. W., and White, W. C. 1974. Survival of *Xanthomonas campestris* in soil. Phytopathol. 64:1518-1520.

23. Schwartz, H.F., and Bartolo, M.E. 1995. Colorado Onion Production and Integrated Pest Management. Bulletin 547A. Colo. State Univ., Ft. Collins, 39 pp.

24. Schwartz, H. F., and Otto, K. J. 1998. Onion bacterial disease management in Colorado. Pp. 214-218 in: Proc. of the 1998 National Onion (and other *Allium*) Research Conference, Dec. 10 – 12, 1998, Sacramento, CA.

25. Schwartz, H. F., and Otto, K. 2000. First report of a leaf blight of onion by *Xanthomonas campestris* in Colorado. Plant Dis. 84:922.

26. Schwartz, H. F., Otto, K. L., and Gent, D. H. 2003. Relation of temperature and rainfall to development of Xanthomonas and Pantoea leaf blights of onion in Colorado. Plant Dis. 87:11-14.

27. Stall, R. E., Loschke, D. C., and Jones, J. B. 1986. Linkage of copper resistance and avirulence loci on a self-transmissible plasmid in *Xanthmonas campestris* pv. *vesicatoria*. Phytopathol. 76:240-243.

28. Weller, D. M., and Saettler, A. W. 1978. Rifampin-resistant *Xanthomonas phaseoli* var. *fuscans* and *Xanthomonas phaseoli*: Tools for field study of bean blight bacteria. Phytopathol. 68:778-781.

Table IV. 1. Location, field history, and results of isolation of *Xanthomonas axonopodis* from sites in Colorado during 2003 to 2004.

	Crop[y]						
Site	**2002**	**2003**	**2004**	**Date**	**County**	**Plant species**	**Epiphtyic *Xanthomonas sp*[z]**
2003							
1	Onion	Onion	--	23-Jun	Larimer	*Allium cepa*	+
1	Onion	Onion	--	23-Jun	Larimer	*Amaranthus retroflexus*	+
1	Onion	Onion	--	23-Jun	Larimer	*Chenopodium album*	-
1	Onion	Onion	--	23-Jun	Larimer	*Echinochloa crus-galli*	+
1	Onion	Onion	--	23-Jun	Larimer	*Polygonum convolvulus*	+
1	Onion	Onion	--	25-Jul	Larimer	*Amaranthus retroflexus*	+
1	Onion	Onion	--	25-Jul	Larimer	*Cirsium arvense*	+
1	Onion	Onion	--	25-Jul	Larimer	*Convolvulus arvensis*	+
1	Onion	Onion	--	25-Jul	Larimer	*Echinochloa crus-galli*	+
1	Onion	Onion	--	25-Jul	Larimer	*Malva neglecta*	+
2	Dry bean	Onion	--	26-Jun	Morgan	*Amaranthus retroflexus*	-
2	Dry bean	Onion	--	26-Jun	Morgan	*Chenopodium album*	-
2	Dry bean	Onion	--	26-Jun	Morgan	*Helianthus annuus*	-
2	Dry bean	Onion	--	26-Jun	Morgan	*Helianthus annuus*	-
3	Dry bean	Onion	--	26-Jun	Morgan	*Kochia scoparia*	-
3	Dry bean	Onion	--	26-Jun	Morgan	*Kochia scoparia*	-
3	Dry bean	Onion	--	26-Jun	Morgan	*Solanum sarrachoides*	-
4	Dry bean	Onion	--	30-Jun	Prowers	*Chenopodium album*	-
5	Onion	Onion	--	30-Jun	Otero	*Allium cepa*	+
5	Onion	Onion	--	30-Jun	Otero	*Chenopodium album*	-
5	Onion	Onion	--	8-Jul	Otero	*Allium cepa*	+
5	Onion	Onion	--	8-Jul	Otero	*Amaranthus retroflexus*	+
5	Onion	Onion	--	8-Jul	Otero	*Anoda cristata*	+
5	Onion	Onion	--	8-Jul	Otero	*Chenopodium album*	-
5	Onion	Onion	--	8-Jul	Otero	*Kochia scoparia*	-
5	Onion	Onion	--	8-Jul	Otero	*Medicago sativa*	+
5	Onion	Onion	--	8-Jul	Otero	*Solanum rostratum*	+

2004

1	Onion	Onion	Onion	27-May	Larimer	*Allium cepa*	+
1	Onion	Onion	Onion	27-May	Larimer	*Amaranthus retroflexus*	-
1	Onion	Onion	Onion	27-May	Larimer	*Helianthus annuus*	-
1	Onion	Onion	Onion	27-May	Larimer	*Kochia scoparia*	-
1	Onion	Onion	Onion	27-May	Larimer	*Lens culinaris*	+
1	Onion	Onion	Onion	27-May	Larimer	*Solanum sarrachoides*	-
1	Onion	Onion	Onion	9-Jun	Larimer	*Amaranthus retroflexus*	+
1	Onion	Onion	Onion	9-Jun	Larimer	*Helianthus annuus*	+
1	Onion	Onion	Onion	9-Jun	Larimer	*Kochia scoparia*	-
1	Onion	Onion	Onion	9-Jun	Larimer	*Lens culinaris*	-
1	Onion	Onion	Onion	9-Jun	Larimer	*Polygonum convolvulus*	-
1	Onion	Onion	Onion	9-Jun	Larimer	*Solanum sarrachoides*	-
2	Dry bean	Onion	Potato	7-Jun	Morgan	*Allium cepa*	-
2	Dry bean	Onion	Potato	7-Jun	Morgan	*Kochia scoparia*	-
2	Dry bean	Onion	Potato	7-Jun	Morgan	*Solanum sarrachoides*	-
3	--	Dry bean	Onion	13-Jul	Morgan	*Amaranthus retroflexus*	+
3	--	Dry bean	Onion	13-Jul	Morgan	*Kochia scoparia*	-
3	--	Dry bean	Onion	13-Jul	Morgan	*Sorghum halepense*	+
4	Onion	Onion	Soybean	8-Jun	Otero	*Allium cepa*	+
4	Onion	Onion	Soybean	8-Jun	Otero	*Glycine max*	-
4	Onion	Onion	Soybean	8-Jun	Otero	*Kochia scoparia*	-
4	Onion	Onion	Field corn	8-Jun	Otero	*Convolvulus arvensis*	+
5	Field corn	Onion	Field corn	7-Jun	Yuma	*Allium cepa*	+
5	Field corn	Onion	Field corn	7-Jun	Yuma	*Amaranthus retroflexus*	+
5	Field corn	Onion	Field corn	7-Jun	Yuma	*Cenchrus longispinus*	+
5	Field corn	Onion	Field corn	7-Jun	Yuma	*Chenopodium album*	+
5	Field corn	Onion	Field corn	7-Jun	Yuma	*Helianthus annuus*	

5	Field corn	Onion	Field corn	7-Jun	Yuma	*Kochia scoparia*	+
5	Field corn	Onion	Field corn	7-Jun	Yuma	*Solanum sarrachoides*	+
6	Alfalfa	Alfalfa	Onion	7-Jun	Prowers	*Cyperus esculentus*	+
6	Alfalfa	Alfalfa	Onion	7-Jun	Prowers	*Helianthus annuus*	+
6	Alfalfa	Alfalfa	Onion	7-Jun	Prowers	*Kochia scoparia*	+
6	Alfalfa	Alfalfa	Onion	7-Jun	Prowers	*Medicago sativa*	+
6	Alfalfa	Alfalfa	Onion	7-Jun	Prowers	*Solanum rostratum*	+
6	Alfalfa	Alfalfa	Onion	7-Jun	Prowers	*Taraxacum officinale*	+

[y] Commonly occurring weed, crop, and volunteer onion (where found) plants were collected from within and adjacent to fields that were currently planted to or had been planted to onion the previous year. Four bulked samples (each 5 to 10 grams) of each weed from each field were used in epiphyte recovery assays. Plant samples were weighed and placed into sterile 250 ml flasks containing 100 ml of magnesium sulfate-potassium phosphate buffer, and shaken at 250 oscillations/min for 60 min at room temperature (approximately 22°C). Aliquots (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium sulfate-potassium phosphate buffer before plating in duplicate onto modified MXP medium (7) containing 50 mg/l kasugamycin, 30 mg/l cephalexin, and 50 mg/l cycloheximide or plated in duplicate onto modified MXP medium using a spiral-plating system. Culture plates were incubated in the dark at 29°C for 96 h before counting characteristic xanthomonad colonies surrounded by a zone of starch hydrolysis.

[z] Presumptive xanthomonads were confirmed as *X. axonopodis* by physiological and biochemical tests and Biolog (Biolog, Inc., Hayward, CA) substrate utilization profiles. Identification to the pathovar level (*X. axonopodis* pv. *allii* or *phaseoli*) was determined by pathogenicity to onion and dry bean. Nonpathogenic xanthomonads were recovered from several weed hosts, including *A. retroflexus*, *C. arvense*, *C. arvensis*, *C. eculentus*, *E. crus-galli*, *H. annuus*, *K. scoparia*, *L. culinaris*, *M. sativa*, *S. rostratum*, and *T. officinale*.

Table IV. 2. Survival of *Xanthomonas axonopodis* pv. *allii* strain R-O177 in leaves of onion cultivar Vantage buried 25 cm deep or left on the soil surface near Fort Collins, CO.

	Sampling date[y]								
	Oct-03	**Nov-03**	**Dec-03**	**Jan-04**	**Feb-04**	**Mar-04**	**Apr-04**	**May-04**	**Jun-04**
	Colony forming units (logarithmic CFU/leaf)								
Fort Collins									
Buried	10.03	9.60	10.84	9.08	8.46	9.85	8.97	7.99	7.92
Surface	10.12	6.89	4.95	5.33	3.95	4.06	3.77	3.21	0.96
t-test[z]	0.5000	0.0018	0.0007	0.0004	0.0002	<0.0001	<0.0001	<0.0001	0.0005
Rocky Ford									
Surface	10.94	9.03	8.82	9.01	8.14	8.13	8.88	6.12	--
Buried	11.02	7.13	4.92	3.81	5.10	6.52	2.46	2.73	--
t-test	0.5213	0.0008	<0.0001	<0.0001	0.0006	0.0372	0.0001	0.0052	--

[y] Leaves of onion cv. Vantage with characteristic Xanthomonas leaf blight symptoms were arbitrarily collected (irrespective of leaf age) in September 2003 in experimental plots near Fort Collins from plants that did not receive any bactericide treatment, and were dried at room temperature for 72 h. Leaves were cut into 12.7 cm lengths, each at least 50% covered by water-soaked lesions. Four of these leaves were transferred to a nylon stocking and placed into mesh onion sacks (four nylon stockings per bag) to aid in recovery of the nylon-mesh stockings if they decomposed over time. The mesh onion sacks were placed on the soil surface and anchored with wooden stakes or buried 25 cm deep to simulate over wintering without or with deep tillage, respectively. An experimental unit was considered an individual nylon stocking containing four diseased onion leaves, sampled on a given date. Samples were collected monthly beginning 1 October, 2003and *Xanthomonas axonopodis* pv. *allii* strain R-O177 populations were estimated by grinding the nylon stocking and associated diseased leaves in liquid nitrogen, suspending the ground leaf material in 20 ml of sterile magnesium phosphate buffer, and plating 100µl aliquots of serial dilutions onto nutrient agar amended with 100 µg/ml rifampicin and 50 µg/ml cycloheximide. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 29°C.

[z] Probability observed differences in culturable *X. axonopodis* pv. *allii* colony forming units among samples is due to chance. One-sided student *t*-tests assumed unequal variances.

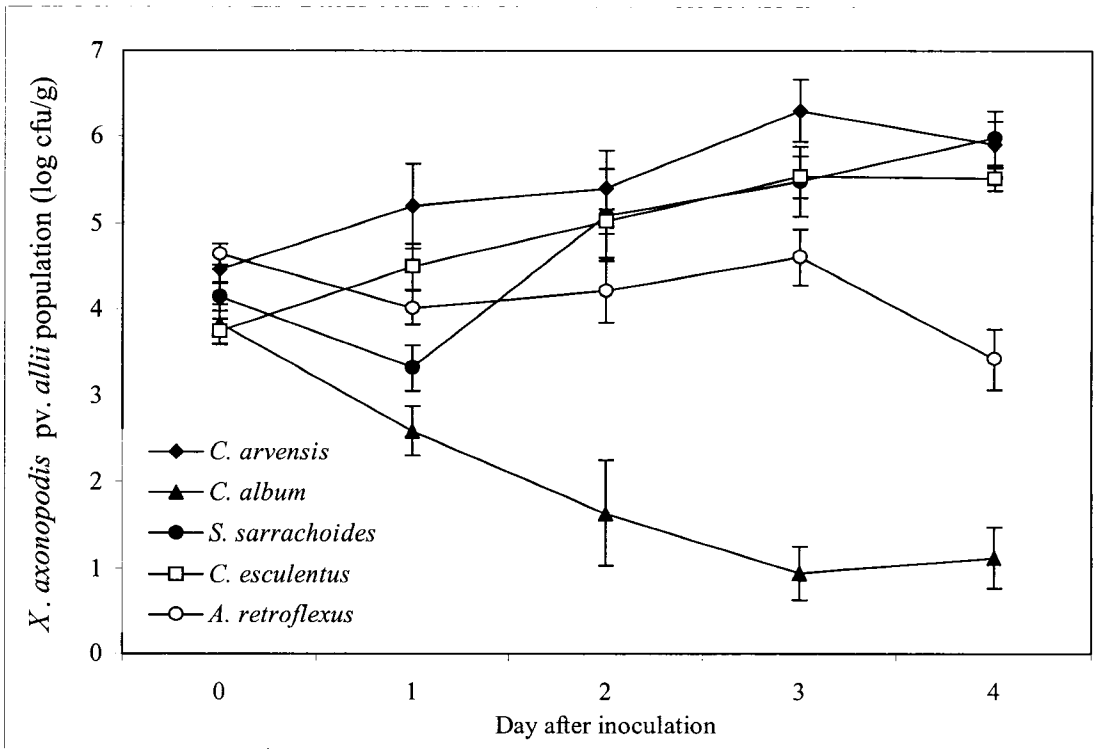


Figure IV. 1. Epiphytic populations of *Xanthomonas axonopodis* pv. *allii* strain R-O177 on weeds under high temperature and humidity conditions in a growth chamber. Three- to four-week old plants of field bindweed (*Convolvulus arvensis*), common lambsquarter (*Chenopodium album*), hairy nightshade (*Solanum sarrachoides*), yellow nutsedge (*Cyperus esculentus*), and redroot pigweed (*Amaranthus retroflexus*) were spray inoculated to runoff with a 10^5 CFU/ml bacterial suspension and maintained at 28°C/24°C day/night temperature regime, light intensity of 350 $\mu Ms^{-1}m^{-2}$, 100% relative humidity, and daily misting with tap water to runoff. Epiphytic *X. axonopodis* pv. *allii* strain R-O177 populations were quantified by leaf-rinsing and subsequent serial dilution onto rifampicin-amended nutrient agar. Data are mean of four replications repeated twice (n=8)±standard error.

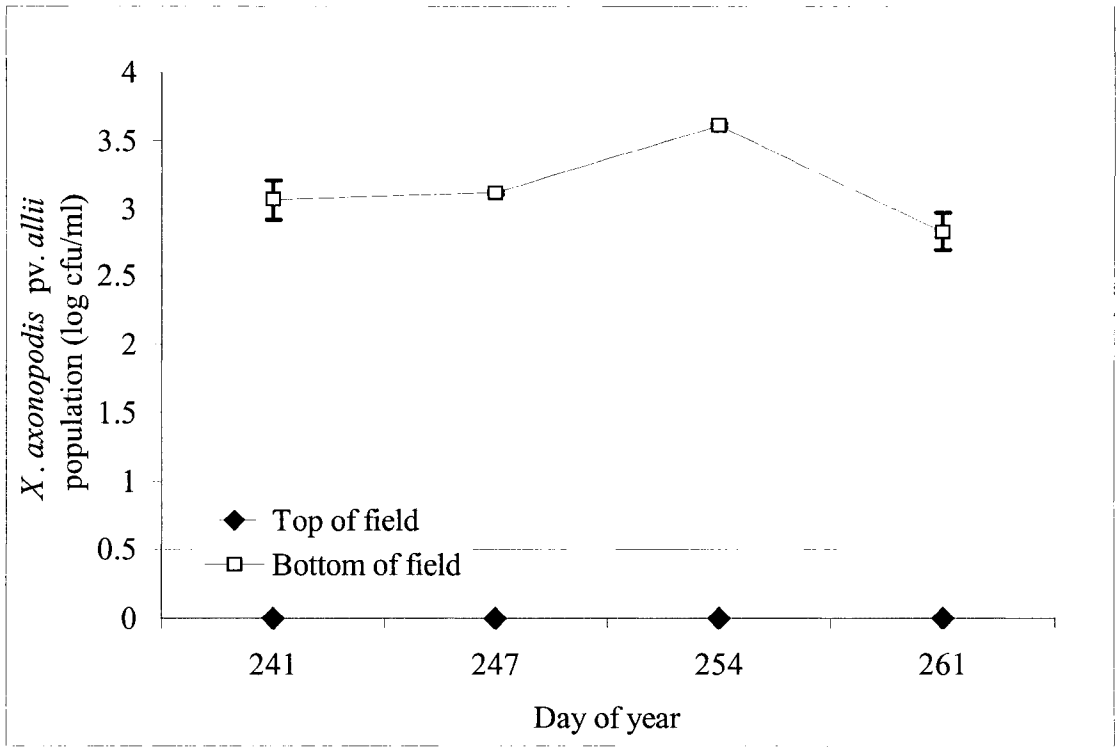


Figure IV. 2. Recovery of *Xanthomonas axonopodis* pv. *allii* strain R-O177 in irrigation water entering and leaving an onion field near Fort Collins, CO in 2003 during an epidemic of Xanthomonas leaf blight. *X. axonopodis* pv. *allii* populations in water were measured from water collected during each irrigation in sterile 50-ml centrifuge tubes from both the top and bottom ends. Water sampling began when Xanthomonas leaf blight symptoms were first detected, and continued until the last irrigation before harvest. Water entering the top of the field was collected arbitrarily from 3 to 10 locations as it left gated polyvinvyl chloride delivery pipe before it contacted any plant or area of the field where plants were growing. Tail water was collected arbitrarily from 3 to 10 locations at the bottom of the field from individual furrows before it mixed with water from other rows. Random rows sampled at the top of the field were not necessarily the same random rows sampled from the bottom of the field. Aliquots of water (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium sulfate-potassium phosphate buffer or spiral-plated directly (in duplicate) onto nutrient agar amended with 100 µg/ml rifampicin and 50 µg/ml cycloheximide. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 29°C.

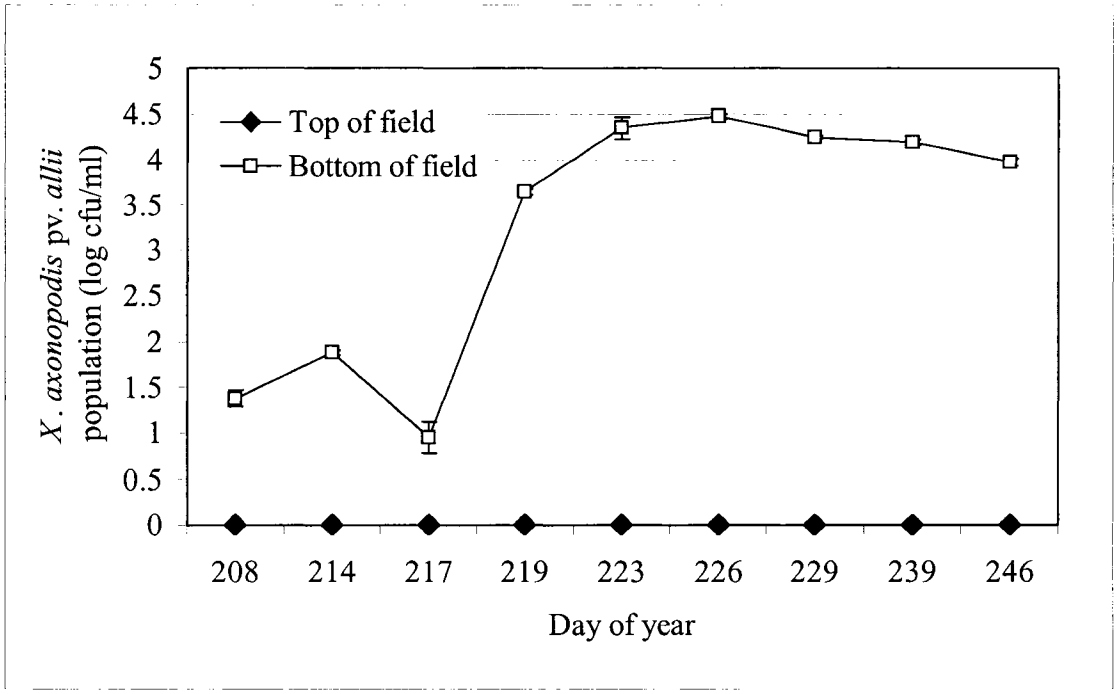


Figure IV. 3. Recovery of *Xanthomonas axonopodis* pv. *allii* strain R-O177 in irrigation water entering and leaving an onion field near Fort Collins, CO in 2004 during an epidemic of Xanthomonas leaf blight. *X. axonopodis* pv. *allii* populations in water were measured from water collected during each irrigation in sterile 50-ml centrifuge tubes from both the top and bottom ends. Water sampling began when Xanthomonas leaf blight symptoms were first detected, and continued until the last irrigation before harvest. Water entering the top of the field was collected arbitrarily from 3 to 10 locations as it left gated polyvinvyl chloride delivery pipe before it contacted any plant or area of the field where plants were growing. Tail water was collected arbitrarily from 3 to 10 locations at the bottom of the field from individual furrows before it mixed with water from other rows. Random rows sampled at the top of the field were not necessarily the same random rows sampled from the bottom of the field. Aliquots of water (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium sulfate-potassium phosphate buffer or spiral-plated directly (in duplicate) onto nutrient agar amended with 100 µg/ml rifampicin and 50 µg/ml cycloheximide. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 29°C.

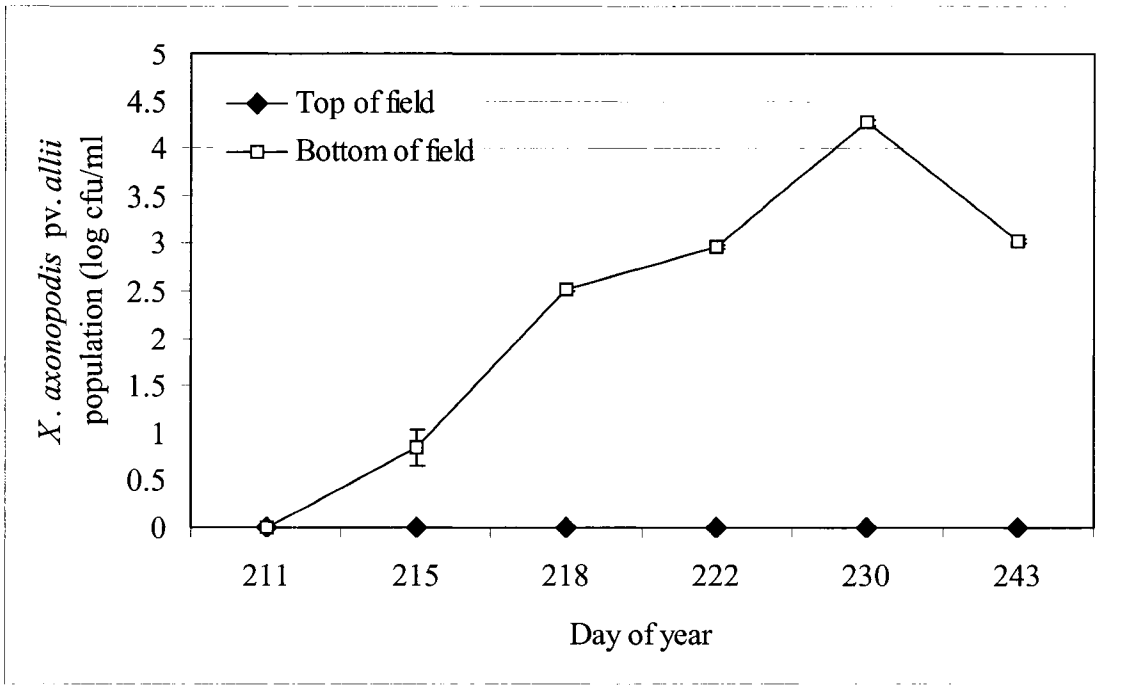


Figure IV. 4. Recovery of *Xanthomonas axonopodis* pv. *allii* strain R-O177 in irrigation water entering and leaving an onion field near Fort Collins, CO in 2004 during an epidemic of Xanthomonas leaf blight. *X. axonopodis* pv. *allii* populations in water were measured from water collected during each irrigation in sterile 50-ml centrifuge tubes from both the top and bottom ends. Water sampling began when Xanthomonas leaf blight symptoms were first detected, and continued until the last irrigation before harvest. Water entering the top of the field was collected arbitrarily from 3 to 10 locations as it left gated polyvinvyl chloride delivery pipe before it contacted any plant or area of the field where plants were growing. Tail water was collected arbitrarily from 3 to 10 locations at the bottom of the field from individual furrows before it mixed with water from other rows. Random rows sampled at the top of the field were not necessarily the same random rows sampled from the bottom of the field. Aliquots of water (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium sulfate-potassium phosphate buffer or spiral-plated directly (in duplicate) onto nutrient agar amended with 100 µg/ml rifampicin and 50 µg/ml cycloheximide. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 29°C.

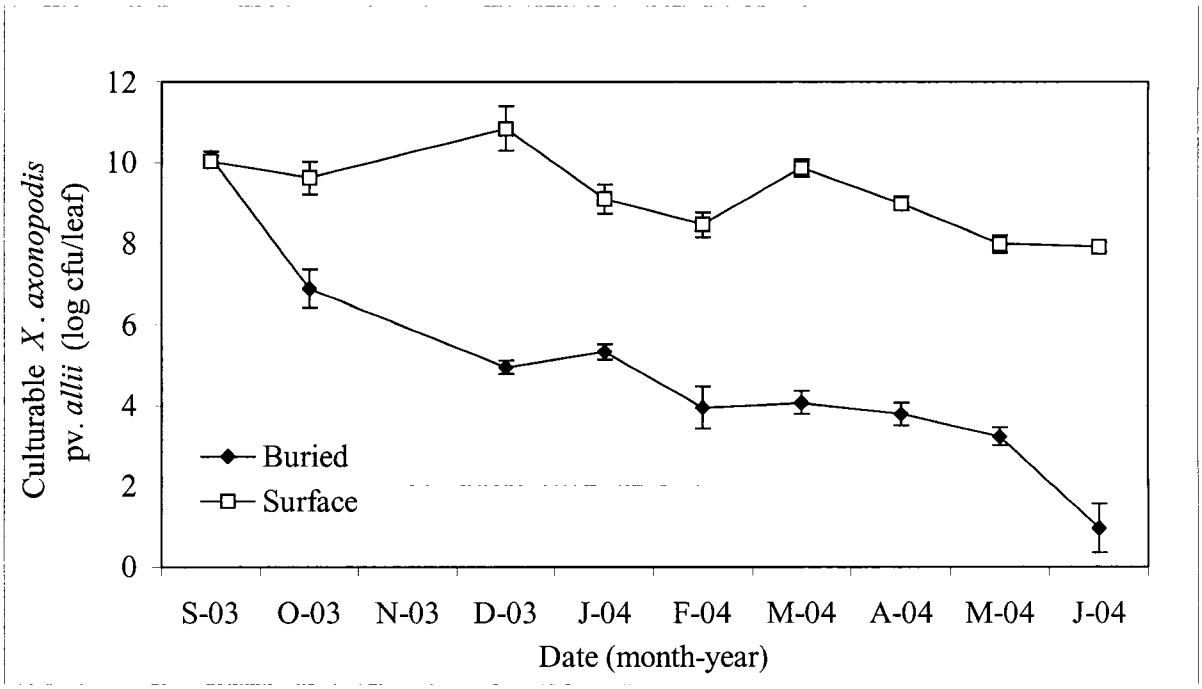


Figure IV. 5. Survival of *Xanthomonas axonopodis* pv. *allii* strain R-O177 in crop debris buried 25 cm deep or left on the soil surface near Fort Collins, CO. Leaves of onion cv. Vantage with characteristic Xanthomonas leaf blight symptoms were arbitrarily collected (irrespective of leaf age) in September 2003 experimental plots near Fort Collins from plants that did not receive any bactericide treatment, and were dried at room temperature for 72 h. Leaves were cut into 12.7 cm lengths, each at least 50% covered by water-soaked lesions. Four of these leaves were transferred to nylon stocking and placed into mesh onion sacks (four nylon stockings per bag) to aid in recovery of the nylon-mesh stockings if they decomposed over time. The mesh onion sacks were placed on the soil surface and anchored with wooden stakes or buried 25 cm deep to simulate over wintering without or with deep tillage, respectively. An experimental unit was considered an individual nylon stocking containing four diseased onion leaves, sampled on a given date. Samples were collected monthly and *Xanthomonas axonopodis* pv. *allii* strain R-O177 populations were estimated by grinding the nylon stocking and associated diseased leaves in liquid nitrogen, suspending the ground leaf material in 20 ml of sterile magnesium phosphate buffer, and plating 100µl aliquots of serial dilutions onto nutrient agar amended with 100 µg/ml rifampicin and 50 µg/ml cycloheximide. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 29°C.

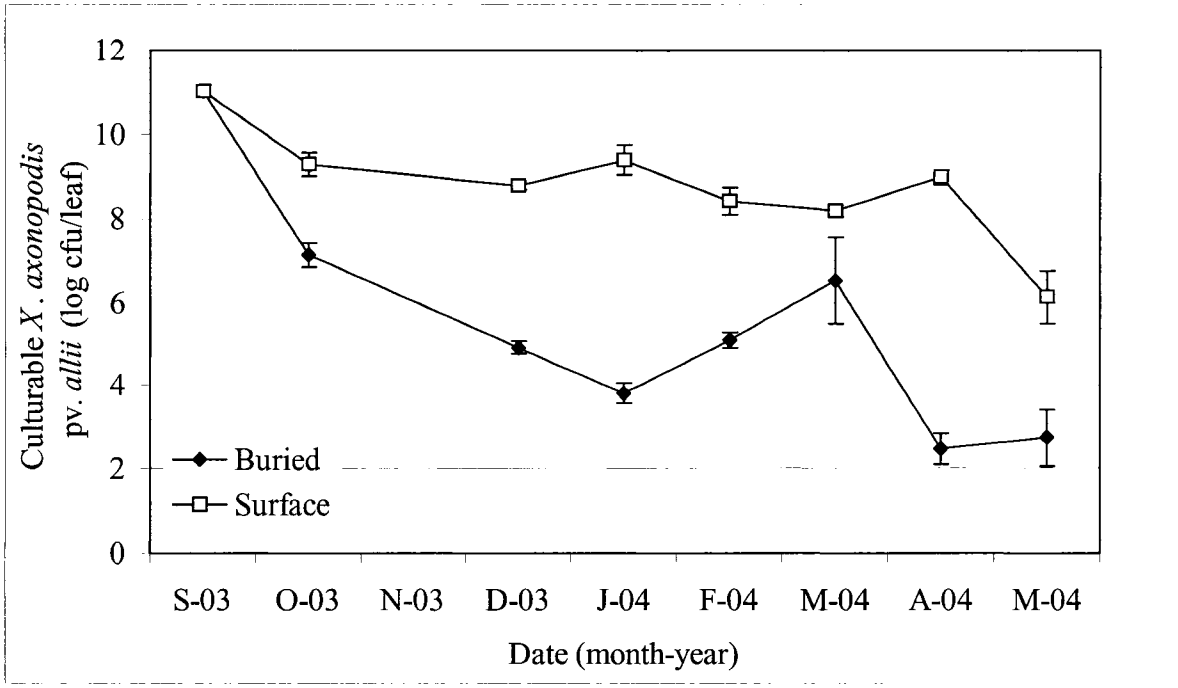


Figure IV. 6. Survival of *Xanthomonas axonopodis* pv. *allii* strain R-O177 in crop debris buried 25 cm deep or left on the soil surface near Fort Collins, CO. Leaves of onion cv. Vantage with characteristic Xanthomonas leaf blight symptoms were arbitrarily collected (irrespective of leaf age) in September 2003 experimental plots near Fort Collins from plants that did not receive any bactericide treatment, and were dried at room temperature for 72 h. Leaves were cut into 12.7 cm lengths, each at least 50% covered by water-soaked lesions. Four of these leaves were transferred to a nylon stocking and placed into mesh onion sacks (four nylon stockings per bag) to aid in recovery of the nylon-mesh stockings if they decomposed over time. The mesh onion sacks were placed on the soil surface and anchored with wooden stakes or buried 25 cm deep to simulate over wintering without or with deep tillage, respectively. An experimental unit was considered an individual nylon stocking containing four diseased onion leaves, sampled on a given date. Samples were collected monthly and *Xanthomonas axonopodis* pv. *allii* strain R-O177 populations were estimated by grinding the nylon stocking and associated diseased leaves in liquid nitrogen, suspending the leaf material in 20 ml of sterile magnesium phosphate buffer, and plating 100µl aliquots of serial dilutions onto nutrient agar amended with 100 µg/ml rifampicin and 50 µg/ml cycloheximide. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 29°C.

CHAPTER V

MINIMIZING CLASS B2 CARCINOGEN USE FOR XANTHOMONAS LEAF BLIGHT OF ONION MANAGEMENT

INTRODUCTION

Xanthomonas leaf blight of onion (*Allium cepa*), caused by the bacterium *Xanthomonas axonopodis* pv. *allii*, is a yield limiting disease of onion in Colorado (31,32). The disease symptoms can be varied, but generally appear as lenticular-shaped chlorotic spots that quickly develop into water-soaked lesions during rainy or humid weather. Chlorotic streaks, necrosis, and tip dieback develop as the disease progresses, resulting in stunting of plants and a reduction in or cessation of bulb development. Lesions are most prominent on the flattened sides of leaves. A bulb rot has not been reported, but yield losses can be significant in Colorado (31).

Few management strategies have been developed for Xanthomonas leaf blight. Crop rotation to nonhosts (5,11,19,26), use of resistant cultivars (19) and planting pathogen-free seed have been suggested (25,27). Adapted and commercially acceptable cultivars resistant to Xanthomonas leaf blight have not been identified in for local producers (Schwartz, unpublished), and the disease continues to cause losses in certain years in Colorado.

Copper bactericides amended with an ethylenebisdithiocarbamate (EBDC) fungicide can suppress Xanthomonas leaf blight and other bacterial diseases of onion in Colorado, but applications must be applied preventatively and regularly to be effective (31). Consequently, growers may make eight or more copper/EBDC applications per season to suppress disease. This approach to

disease management adds expense to onion production and may not be sustainable. Also, EBDC fungicides are class B2 carcinogens and may not be available for use on onion in the future. Copper resistance has not been observed among *Xanthomonas axonopodis* pv. *allii* strains prevalent in Colorado (8), but has been reported in Barbados (20). Bactericide resistance is widespread among phytopathogenic bacteria (4,17,33), and copper resistance is likely to appear in *Xanthomonas axonopodis* pv. *allii* with continued reliance upon this chemical for disease suppression. Copper bactericides alone provide little control of copper-tolerant bacteria (16,29). Therefore, new management strategies for Xanthomonas leaf blight are critical to delay or prevent the development of copper-tolerance, minimize EBDC fungicide use, and reduce production costs.

Copper bactericide and associated EBDC fungicide use may be reduced in Colorado by improving the timing and efficiency of applications. Currently, the timing of bactericide applications is based upon crop phenology (31). But the appearance of Xanthomonas leaf blight is variable across time and fields, and growers may make applications more often then needed to effectively manage the disease. Schwartz et al. (32) developed multiple regression models to predict the appearance and severity of Xanthomonas leaf blight in southern Colorado, and the use of these models to time bactericide applications may reduce unnecessary bactericide applications. Additionally, EBDC fungicides may not be necessary for Xanthomonas leaf blight management since copper-tolerant strains of *Xanthomonas axonopodis* pv. *allii* are not prevalent in Colorado (8).

Novel chemical and biological control agents appear promising for reducing traditional bactericide use on onion. Paulraj and O'Garro (21) evaluated *Pantoea agglomerans* in growth chamber assays, and found prophylactic applications of the bacterium to onion foliage greatly reduced Xanthomonas leaf blight severity. Additionally, systemic acquired resistance (SAR) can

be induced by acibenzolar-S-methyl (Actigard 50WG, Syngenta Crop Protection, Greensboro, NC), a structural analog of salicylic acid (9). Acibenzolar-S-methyl can be an effective alternative to many bactericides and fungicides for control of numerous plant diseases (1,2,6,9,18,22). Resistance to acibenzolar-S-methyl has not been reported in any pathogen, and its use can suppress bacterial spot and speck of tomato caused by copper-tolerant strains of *X. axonopodis* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*, respectively (16). However, acibenzolar-S-methyl and other inducers of systemic acquired resistance must be used carefully as phytotoxicity and yield depression can occur on some hosts or genotypes (6,24); and its use may aggrevate other pest problems (34,35). No studies have evaluated acibenzolar-S-methyl for Xanthomonas leaf blight suppression on onion.

Despite the potential for reduced copper and EBDC fungicide use to manage Xanthomonas leaf blight in Colorado, few field level studies have evaluated disease forecasting, EBDC fungicide rates, and novel chemical and biological controls. New management strategies for Xanthomonas leaf blight are necessary to improve disease control, avoid potential environmental impacts, and reduce onion production costs. Therefore, we launched this series of studies to reduce copper and EBDC fungicides used for Xanthomonas leaf blight management by improving the timing of bactericide applications, and identifying novel chemical treatments and biological control agents.

METHODS AND MATERIALS

Bacterial strains and culture. A rifampicin mutant of *X. axonopodis* pv. *allii* strain O177 (ATCC 508) was generated as previously described (36), and is referred to as R-O177. Strain R-O177 is resistant to greater than 200 µg/ml rifampicin, but selection routinely was performed on nutrient agar amended with 50 µg/ml of rifampicin. Other strains of *X. axonopodis* pv. *allii* were routinely cultured on nutrient agar or broth lacking antibiotic during incubation at 29°C. To

prepare inoculum of strain R-O177, loopfuls of bacteria were streaked onto rifampicin-amended nutrient before incubating culture plates at 29°C for 72 h in the dark. Cells were harvested from plates by flooding with deionized water and gently scraping the plates with a small, flame-sterilized spatula. The cell suspension was adjusted to 10^8 CFU/ml spectrophotometrically (OD_{600}=0.12) before adjustment to the desired concentration for an experiment. The bacterial strain was preserved in 15% nutrient glycerol broth at -80°C for long-term storage.

Epiphytic population assays. Experiments were conducted in a growth chamber to determine the effect of acibenzolar-S-methyl and copper hydroxide on epiphytic multiplication of *X. axonopodis* pv. *allii* strain R-O177. Plants of onion cultivars Cometa and Vantage were grown under greenhouse conditions (approximately 24°C/20°C day/night temperature regime and 14-hour photoperiod, with approximately 2-h of supplemental incandescent lighting) until they were six- to eight-weeks old. Three plants of each species were seeded individually into 1 l pots in MetroMix 200 potting soil (Grace Sierra Horticultural Products Company, Milpitas, CA). Plants treated with acibenzolar-S-methyl (Actigard 50WG) were sprayed with 39 g a.i./ha amended with 0.25 v/v Latron AG-98 (Dow AgroSciences, Indianapolis, IN) in 90 l of water/ ha in a spray chamber pressurized to 275 kPa with compressed CO_2. Plants receiving the copper hydroxide treatment were similarly treated with copper hydroxide-mancozeb (ManKocide, Griffin, L. L. C., Valdosta, GA) at 1.03 kg a.i./ha (copper hydroxide)+ 0.34 kg a.i./ha (mancozeb) amended with 0.25% v/v Latron AG-98 1 h prior to inoculation. Untreated plants were sprayed with water amended with 0.25% v/v Latron AG-98.

Twenty pots of each cultivar were inoculated by spraying to runoff with a 10^5 CFU/ml bacterial suspension using a Crown SpraTool (Aerovoe Industries, Inc., Gardnerville, NV). Inoculum of strain R-O177 was cultured by inoculating 3 ml of nutrient broth in 15 ml culture tubes, and incubating at 26°C with vigorous shaking (250 oscillations/min) for 24 h. The bacterial cells

were collected by centrifugation before adjusting to approximately 10^5 CFU/ml in sterile magnesium phosphate buffer (0.01 M magnesium sulfate and 0.01M potassium phosphate, pH 7.2). After inoculation, plants were allowed to air dry and then sampled immediately by removing all aboveground plant material from four pots of each cultivar and placing it individually into plastic bags. Plants were placed in a growth chamber and incubated at 28°C/24°C day/night temperature regime, light intensity of 350 $\mu Ms^{-1}m^{-2}$, 100% relative humidity, and daily misting with tap water to runoff. Another set of four pots of each cultivar were destructively sampled each day for four days. An experimental unit consisted of one pot of a given cultivar that contained three plants destructively sampled each day.

Harvested plant samples were weighed and placed into sterile 250 ml flasks containing 100 ml of magnesium phosphate buffer, and shaken at 250 oscillations/min for 60 min at room temperature (approximately 22°C). Aliquots (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium phosphate buffer before plating in duplicate onto nutrient agar amended with 50 µg/ml rifampicin and cycloheximide. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 29°C, and a subset of rifampicin resistant colonies were confirmed as *X. axonopodis* pv. *allii* by standard physiological and biochemical tests (28); including Gram stain reaction, pigmentation on yeast dextrose carbonate medium, fluorescence on King's medium B, indole test, growth on 0.1% tetrazolium chloride, oxidase test, starch hydrolysis, oxidative utilization of glucose, catalase test, production of H_2S from cysteine, presence of arginine dihydrolase, and casein hydrolysis test. The experiment was repeated twice over time.

***In planta* population dynamics.** Multiplication of *X. axonopodis* pv. *allii* strain R-O177 in onion was quantified as previously described (8). Plants were treated with acibenzolar-S-methyl, copper hydroxide, or water as described above. The youngest, fully-extended leaves of 8-week-

old onion plants (cv. Cometa and Vantage) were pin-pricked seven times at 1 cm intervals with a 22 gauge needle bearing strain R-O177 removed from 72-hour old nutrient agar culture plates. Each pin-pricked leaf area was inoculated with a bacterial matrix approximately equal in size to the needle tip. Leaf sections (7 by 1 cm in length), each having an inoculated area, were removed daily, vortexed in 5 ml of sterile magnesium phosphate buffer amended with 0.25% v/v X-77 nonionic surfactant (Helena Chemical Co., Fresno, CA) to remove surface copper hydroxide-mancozeb residues, surface disinfested in 95% ethanol, rinsed in sterile magnesium phosphate buffer, and ground aseptically in 1 ml of sterile magnesium phosphate buffer with a mortar and pestle. The homogenate was serially diluted and plated onto nutrient agar amended with 50 μg/ml rifampicin and cycloheximide. Rifampicin-resistant colonies were enumerated after 72 hr of incubation at 29° C, and representative colonies were confirmed as strain R-O177 by physiological and biochemical tests.

Evaluation of acibenzolar-S-methyl and biological control agents. Field plots were established from 2002 to 2004 near Fort Collins, CO at the Colorado State University Agricultural Research, Development, and Education Center, near Rocky Ford, CO at the Arkansas Valley Research Center, and near Yuma, CO at the Irrigation Research Foundation to evaluate acibenzolar-S-methyl and biological control agents for suppression of Xanthomonas leaf blight. Plots at Fort Collins (2002 to 2004) and Yuma (2003 and 2004) were established with the yellow onion cultivar Vantage from seed planted approximately 0.10 m apart in beds on 0.76 m centers; each bed contained one (Yuma) or two (Fort Collins) rows spaced 0.15 m apart. At Rocky Ford, seeds of yellow onion cultivar X-201 was planted approximately 0.10 m apart in beds on 1.06 m centers; each bed contained two rows spaced 0.15 m apart. Fields at Fort Collins and Rocky were irrigated by furrow irrigation, but irrigation was provided by center-pivot in fields at Yuma. Fertilizer, herbicides, and insecticides were applied according to standard production practices (30). A plot consisted of one bed 1.06- (Rocky Ford) or 0.76 m (Fort Collins

and Yuma) wide by 4.6 m long bed separated by a single untreated row. Treatments were applied as described in the bactericide timing study described above.

Treatments were the same at all locations during a given year, but varied among years. Experiments were arranged in a randomized split-block design with four replications. Whole plot treatments consisted of a given chemical or biological control agent (untreated, copper hydroxide, acibenzolar-S-methyl, *Pantoea agglomerans*, *P. agglomerans*/*P. fluorescens*), and the subplot factor was the presence or absence of weekly copper hydroxide-mancozeb (ManKocide) applications. Plots were monitored weekly to determine the initial appearance and subsequent disease development. Average percent disease severity was estimated for each plot using a modified Horsfall-Barrett scale and then converted to a percentage. Weekly disease severity ratings were used to calculate the relative area under the disease progress curve (RAUDPC). At maturity, a 3 m subsection of each subplot was mechanically topped and harvested, graded, and weighed to estimate yields.

In 2002, plots were established near Rocky Ford and Fort Collins. Treatments consisted of 10 weekly applications of copper hydroxide (Kocide 2000) at 0.90 kg a.i./ha or acibenzolar-S-methyl at 39 g a.i./ha, with or without copper hydroxide-mancozeb (ManKocide) at 1.03 kg a.i./ha (copper hydroxide) + 0.34 kg a.i./ha (mancozeb). Ten weekly applications were made beginning 108 or 76 days after planting at Rocky Ford or Fort Collins, respectively. Untreated rows separating treated plots were inoculated with 10^8 CFU/ml water of *X. axonopodis* pv. *allii* strain O177 to initiate an epidemic of Xanthomomas leaf blight. These rows were inoculated 151 and 164 or 113, 118, and 127 days after planting at Rocky Ford and Fort Collins, respectively.

In 2003, experiments were conducted near Rocky Ford, Fort Collins, and Yuma. Treatments consisted of 9 or 10 applications of copper hydroxide (Kocide 2000) at 0.90 kg a.i./ha or

acibenzolar-S-methyl (Actigard 50WG) at 39 g a.i./ha with or without copper hydroxide/mancozeb (ManKocide) at 1.03 kg a.i./ha (copper hydroxide) + 0.34 kg a.i./ha (mancozeb). Copper hydroxide and copper hydroxide-mancozeb treatments were applied weekly beginning 105, 99, or 97 days after planting at Rocky Ford, Fort Collins, or Yuma, respectively. Ten applications were made at Rocky Ford and Yuma, but 9 applications were made at Fort Collins. Four weekly acibenzolar-S-methyl applications were made at each location in 2003, beginning 105, 99, or 97 days after planting at Rocky Ford, Fort Collins, or Yuma, respectively. Plots were inoculated with 10^8 CFU/ml water *X. axonopodis* pv. *allii* strain R-O177 124, 136, 143, 150, and 157, or 133, 140, and 144, or 126 and 133 days after planting at Rocky Ford, Fort Collins, or Yuma, respectively.

In 2004, the experiments were repeated at the three locations. Treatments and rates were as described in 2003, but BlightBan C9-1 (lyophilized cells of *Pantoea agglomerans* strain C9-1, NuFarm, Laverton, Australia) and BlightBan C9-1/A506 (equal mixture of lyophilized cells of *Pantoea agglomerans* strain C9-1 and *P. fluorescens* strain A506) were included. BlightBan C9-1 was applied at the rate of 10^{10} CFU/ml *P. agglomerans* strain C9-1, and BlightBan C9-1/A506 was applied at the rate of 5 x 10^9 CFU/ml each of *P. agglomerans* strain C9-1 and *P. fluorescens* strain A506. Weekly applications of treatments began 101, 101, 98 days after planting at Rocky Ford, Fort Collins, and Yuma. A total of 11, 9, and 12 copper hydroxide applications were made at Rocky Ford, Fort Collins, and Yuma, respectively. Four weekly applications were made of acibenzolar-S-methyl and two weekly applications were made of the biological control agents beginning on the same days as the copper hydroxide treatment.

Identification of the critical period for bactericide applications. Experiments were established at the Arkansas Valley Research Center near Rocky Ford, CO from 2002 to 2004 to determine the critical period for Xanthomonas leaf blight control with copper bactericides. A plot

consisted of a 7.5 m length of one bed containing two lines of the onion cultivar X-201. Plots were arranged in a randomized split-block design with three replications, where the timing of the first copper hydroxide application was the whole plot factor and EBDC fungicide rate was the subplot factor.

To determine the critical period for bactericide applications, the timing of the first copper hydroxide (Kocide 2000, Griffin, L.L.C., Valdosta, GA) application was staggered. Whole plots received a weekly application of 0.90 kg a.i./ha copper hydroxide, beginning approximately four weeks pre-bulb initiation to two weeks post-bulb initiation. Subplots were treated with maneb (Maneb 75DF, Cerexagri Inc., King of Prussia, PA) at a rate of 0, 0.42, 0.84, or 1.68 kg a.i./ha initiated on the same day as the copper hydroxide treatment for that whole plot treatment. The untreated plots did not receive any copper hydroxide, but did receive 0, 0.42, 0.84, or 1.68 kg a.i./ha maneb. Once copper hydroxide and maneb treatments were initiated, they were reapplied weekly until approximately 14-days before harvest. In 2002, the four-week pre-bulb initiation, three-week pre-bulb initiation, two-week pre-bulb initiation, one-week pre-bulb initiation, bulb initiation, one-week post-bulb initiation, and two-week post-bulb initiation treatments began 101,108,115,123,130,137, and 144 days after planting, respectively. These treatments received 9, 8, 7, 6, 5, 4, or 3 total applications, respectively. The copper hydroxide untreated plots received 5 applications of maneb at the rates specified previously, beginning 130 days after planting. In 2003, copper hydroxide treatments began 92, 96, 105, 114, 122,128, or 135 days after planting for the various timings, and 9, 8, 7, 6, 5, 4, or 3 total applications were made for each timing, respectively. The copper hydroxide untreated plots received 5 applications of maneb at the rates specified previously, beginning 122 days after planting. In 2004, copper hydroxide treatments began 71, 77, 84, 93, 98, 106, or 112 days after planting, and 14, 13, 12, 11, 10, 9, or 8 total applications were made for each timing, respectively. The copper hydroxide untreated plots received 10 applications of maneb at the rates specified previously, beginning 98 days after

planting. All treatments were applied in 90 liter of water/ ha using a 45-cm long boom equipped with two 8002 even flat-fan nozzles, and was pressurized to 275 kPa using compressed CO_2. Plots were not inoculated with *X. axonopodis* pv. *allii*.

Statistical analyses. Statistical analyses were performed using the PROC MIXED function of SAS v. 9.1 (SAS Institute, Cary, NC). Bacterial population data from growth chamber studies were log transformed to achieve independently and normally distributed experimental errors with a common variance. The area under the bacterial growth curve was calculated from growth chamber studies of *Xanthomonas axonopodis* pv. *allii* epiphytic and *in planta* populations and used as the response variable in statistical analyses. Replication of experimental units and the entire experiment were considered random factors. In field studies, location and replication were considered fixed and random factors in the mixed models analyses, respectively. Relative area under the disease progress curve was used as the response variable in field studies.

RESULTS

Epiphytic population assays. Epiphytic populations of *X. axonopodis* pv. *allii*, as measured by the area under the bacterial population growth curve, were equivalent for cultivars Vantage and Cometa (P=0.088) in response treatments. Cultivar*treatment interactions were not significant (P=0.204). *X. axonopodis* pv. *allii* populations on plants treated with acibenzolar-S-methyl or copper hydroxide-mancozeb treatments differed on sampling days, but the area under the bacterial growth curve was not different among the treatments (P=0.089) (Figure V. 1). Acibenzolar-S-methyl or copper hydroxide-mancozeb treatment reduced epiphytic populations 0.5 to 2.0 logarithmic units per g fresh weight of tissue at each sample time 24 hours after inoculation, as compared to untreated plants.

***In planta* population dynamics.** *In planta* populations of *X. axonopodis* pv. *allii* in leaves of onion cultivars Vantage and Cometa were equivalent as measured by the area under the bacterial population growth curve (P=0.334), and cultivar*treatment interactions were not significant (P=0.137). Treatment with acibenzolar-S-methyl or copper hydroxide-mancozeb reduced *in planta* bacterial populations as compared to untreated plants (Figure V. 2). *X. axonopodis* pv. *allii* populations were reduced on acibenzolar-S-methyl and copper hydroxide-mancozeb plants on most (5 of 6) or all sampling days as compared to the untreated; but this decrease was less than 1 logarithmic unit per g fresh weight of tissue.

Evaluation of acibenzolar-S-methyl and biological control agents. Field trials were conducted over three years in different Colorado onion production regions to compare acibenzolar-S-methyl and biological control agents to conventional copper bactericides. Despite repeated inoculations with *X. axonopodis* pv. *allii* in 2002, an epidemic of Xanthomonas leaf blight did not occur at either location. The lack of disease development allowed us to determine the impact of acibenzolar-S-methyl on onion yield and grade in the absence of disease. Ten weekly applications of acibenozolar-S-methyl reduced total onion yield 9.3 (22%) and 8.7 (27%) t/ha at Rocky Ford and Fort Collins, respectively (Table V. 1).

In 2003, acibenzolar-S-methyl reduced Xanthomonas leaf blight equal (Fort Collins and Yuma) or superior (Rocky Ford) to copper hydroxide and copper hydroxide-mancozeb (Table V. 2). At Rocky Ford, acibenzolar-S-methyl treatment reduced the RAUDPC 43% as compared to the copper hydroxide-mancozeb treatment. Acibenzolar-S-methyl applied alone was as effective as acibenzolar-S-methyl applied in combination with copper hydroxide-mancozeb at all locations. Acibenzolar-S-methyl treatment increased the yield of jumbo grade bulbs at Rocky Ford as compared to copper hydroxide-mancozeb and untreated plots, but a combination of acibenzolar-S-methyl and copper hydroxide-mancozeb reduced jumbo yield 24% as compared to acibenzolar-

S-methyl alone. Total yields at the three locations were not affected by acibenzolar-S-methyl or copper bactericide treatment as compared to untreated plots.

In 2004, acibenzolar-S-methyl provided Xathomonas leaf blight suppression equal (Rocky Ford and Yuma) or superior (Fort Collins) to copper hydroxide and copper hydroxide-mancozeb (Table V. 3). At Fort Collins, the RAUDPC was reduced 29 or 33% with acibenzolar-S-methyl as compared to copper hydroxide or copper hydroxide-mancozeb, respectively. Acibenzolar-S-methyl alone was as effective as the combination with copper hydroxide-mancozeb at all locations. Bulb yield or grade did not differ among treatments at any location.

The combined application of *P. agglomerans* strain C9-1 + *P. fluorescens* strain A506 was as effective as copper hydroxide (all locations) or copper hydroxide-mancozeb (Rocky Ford and Fort Collins) in reducing Xanthomonas leaf blight severity (Table V. 3). The combination of these biological control agents with copper hydroxide-mancozeb at Yuma was superior to the biological control agents alone, and was equivalent to copper hydroxide-mancozeb. Disease severity was equal in *P. agglomerans* strain C9-1 and untreated plots at all locations.

Identification of the critical period for bactericide applications. Natural epidemics of Xanthomonas leaf blight occurred in 2003 and 2004. The disease was not present in 2002, so comparisons among application timing and maneb rates were not possible. Yields were not expected to differ in the absence of disease and plots were not harvested in 2002. In 2003, a late season (within 14 days of harvest) epidemic of Xanthomonas leaf blight occurred, and all treatments that included copper hydroxide reduced the RAUDPC as compared to the untreated (Table V. 4). Plots in which treatments were initiated 2 to 4 weeks before bulb initiation had less disease than those started at bulb initiation or later. Disease severity was equal among treatments initiated four, two, one week before bulb initiation, but two or three less applications achieved

this level of disease suppression when treatments were initiated one to two weeks before bulb initiation. Disease suppression was equal among different timings irrespective of maneb rate. Yields were not estimated because disease pressure was not considered severe enough to cause measurable treatment effects.

In 2004, a longer epidemic (27 days) of Xanthomonas leaf blight occurred, but no application timing or maneb rate significantly improved Xanthomonas leaf blight suppression (Table V. 4). Yields were estimated, but did not differ among treatments.

DISCUSSION

Copper bactericides tank-mixed with EBDC fungicides are a central component of onion production in Colorado for Xanthomonas leaf blight and other bacterial disease control (31), but this strategy for bacterial disease management increases production costs and reliance upon EBDC fungicides. A critical need exists for new disease management strategies that are more effective, economical, and environmentally sound than calendar-based copper/EBDC bactericides. In this study, we demonstrated acibenzolar-S-methyl and biological control agents effectively control Xanthomonas leaf blight, and can replace the bactericides currently used to suppress this disease in Colorado. Additionally, we demonstrated the disease can be managed without early season copper (prior to one to two weeks before bulb initiation) applications or maneb amendment

Acibenzolar-S-methyl appears to be a valuable tool for Xanthomonas leaf blight management in Colorado. Acibenzolar-S-methyl reduced *in planta* and epiphytic *X. axonopodis* pv *allii* populations in two onion cultivars as much as copper hydroxide-mancozeb in growth chamber studies. Under field conditions with inoculation with a copper-sensitive strain of *X. axonopodis*

pv. *allii*, four weekly applications of acibenzolar-S-methyl applied at 39 g a.i./ha were as or more effective than nine to 10 weekly application of either copper hydroxide or copper hydroxide-mancozeb, without reducing bulb yield or grade when Xanthomonas leaf blight was present. In six experiments, disease suppression was not improved by combining acibenzolar-S-methyl with copper hydroxide-mancozeb, suggesting this novel chemical treatment can fully replace copper hydroxide-mancozeb applications for Xanthomonas leaf blight management.

Phytotoxicity has been reported with acibenzolar-S-methyl treatment on tobacco (6), tomato transplant seedlings (15), and pepper (24), and must be used carefully to avoid phytotoxicity to onion. We observed a significant total yield depression when 10 weekly applications of acibenzolar-S-methyl were made in the absence of disease, but not when four applications were made when low to high levels of disease developed. Cole (6) reported increasing application volumes reduced phytotoxicity to tobacco, but we only evaluated acibenzolar-S-methyl at one application volume simulating a conventional ground-rig application. Future studies should evaluate acibenzolar-S-methyl applications in a range of application volumes, including aerial and chemigation applications. We only evaluated four or more applications of acibenzolar-S-methyl at one rate in these studies, but Xanthomonas leaf blight may be effectively controlled with lower rates or fewer than four applications. In other studies, Xanthomonas leaf blight suppression occurred by two applications of acibenzolar-S-methyl combined with six application of copper hydroxide-mancozeb and was more effective than copper hydroxide-mancozeb alone (7).

We evaluated two onion cultivars susceptible to Xanthomonas leaf blight (Lang and Schwartz, unpubished) in field studies with acibenzolar-S-methyl, but combining moderate levels of host resistance with acibenzolar-S-methyl may contribute additively to disease control. Tomato genotypes susceptible or resistant to early blight (caused by *Alternaria solani*) have different

accumulation patterns of pathogenesis-related (PR) proteins in response to pathogen challenge and induced systemic resistance (13), and higher constitutive and inducible levels of some PR proteins are associated with resistance. Resistant genotypes were also associated with a more rapid induction of PR genes in the early stages of infection (14). Acibenzolar-S-methyl is known to activate a number of physiological, biochemical, and ultrastructural changes in plants (2,9,22), and the level of activation may be greatest in onion genotypes with more rapid induction and higher levels of inducible defense pathways. Acibenzolar-S-methyl may also negatively impact some genotypes (24), and acibenzolar-S-methyl should be evaluatated on a broad collection of onion cultivars to determine potential interactions between this SAR-inducing compound and plant genotype under varying environmental conditions.

Reports of SAR-induction by acibenzolar-S-methyl effective against biotrophic and necrotrophic plant pathogens and insects are found in the literature (1,2,6,9,15,18,22,24), but acibenzolar-S-methyl could potentially aggravate some disease or insect pests on onion. Cross-talk between the salicylic and jasmonic acid pathways is not fully understood, but these pathways appear to mediate resistance to distinct pathogens (35). SAR is energetically costly to plants, and other defense pathways are often down-regulated in response to SAR (10,12,34,37); but the interactions among defense pathways vary depending on plant species, pests attacking a plant, and physiological state of the plant (23). We have evaluated onion treated with acibenzolar-S-methyl for other diseases and pests, and have not observed increases in diseases caused by necrotrophic pathogens (e.g., *Botrytis* spp.) or insects (e.g., *Thrips tabaci*) populations (data not presented). However, treatment of onion with 1 or 10 mM methyl jasmonate triggers induced systemic resistance that reduced *T. tabaci* populations but renders plants hypersensitive to Xanthomonas leaf blight (Gent and Schwartz, unpublished). Evaluation of acibenzolar-S-methyl in a complete onion production system is necessary for intelligent use of this chemical for Xanthomonas leaf blight and other onion diseases.

Biological control of Xanthomonas leaf blight comparable to weekly applications of copper hydroxide-mancozeb was achieved using a commercial formulation of lyophilized cells of a mixture of *P. agglomerans* strain C9-1 and *P. fluorescens* strain A506, but not with *P. agglomerans* strain C9-1 alone. A strain of *P. agglomerans* isolated from the onion phyllosphere in Barbados provided nearly complete suppression of Xanthomonas leaf blight in growth chamber assays (21), but we did not observe any measurable effect of strain C9-1 under field conditions in Colorado. We did not include *P. fluorescens* strain A506 alone in the experiments presented here, so the relative contributions of *P. agglomerans* strain C9-1 and *P. fluorescens* strain A506 to Xanthomonas leaf blight suppression are unclear. Tank-mixes of the biological control agents with copper hydroxide-mancozeb generally did not improve disease suppression as compared to the copper hydroxide-mancozeb alone, presumably because these bacteria are copper sensitive. Future research should evaluate *P. agglomerans* strain C9-1 and *P. fluorescens* strain A506 against other bacterial diseases of onion such as center rot (caused by *Pantoea ananatis*) and sour skin (caused by *Burkholderia cepacia*). In petri-dish bioassays, antibiotics produced by *P. agglomerans* strain C9-1 inhibited the growth of *P. ananatis* (Gent and Ishimaru, unpublished), and these biological control agents may be able to suppress multiple onion bacterial pathogens.

Experiments evaluating the timing of copper hydroxide with varying rates of maneb indicated two or more copper hydroxide applications may be eliminated without compromising Xanthomonas leaf blight suppression when disease pressure is low. Disease did not develop in 2002, and disease pressure was relatively light in 2003 and 2004. Preventative copper hydroxide applications beginning one to two weeks prior to bulb initiation in 2003 were as effective as those beginning during early- to mid-vegetative growth (three to four weeks before bulb initiation). In 2004, a late-season epidemic of Xanthomonas leaf blight occurred and no copper hydroxide application timing significantly improved onion yield or grade. Late-season application of copper

hydroxide may not be necessary under low disease pressure because onion yield and grade are unlikely to be affected as plants near maturity.

The critical period for Xanthomonas leaf blight control appears to be near bulb initiation. Schwartz et al. (32) reported weather patterns before bulb initiation are not associated with the appearance or severity of Xanthomonas leaf blight in Colorado, but copper hydroxide applications initiated after bulb initiation result in poor disease control and yield depression (31). Bartolo et al. (3) simulated different levels of hail damage to onion at various growth stages in Colorado, and found onion yield and grade to be most negatively impacted if defoliation occurred near bulb initiation. Yield losses became less as plants neared maturity. A similar phenomenon may exist for Xanthomonas leaf blight since the disease reduces the photosynthetic area of onion similar to mechanical damage. Late-season epidemics, as observed in 2003 and 2004, are likely to impact bulb yield and grade negligibly, and copper hydroxide applications for Xanthomonas leaf blight are probably not necessary in the one to two weeks preceding onion harvest.

In this study we have identified effective and economical means of reducing the use of copper bactericides and EBDC fungicides for Xanthomonas leaf blight suppression. Copper bactericide and EBDC fungicide application frequency may be reduced by only applying them during the critical periods for disease control, or by relying upon acibenzolar-S-methyl and biological control agents. Integration of these chemical and biological controls within an integrated pest management program that emphasizes the planting of pathogen-free seed (25,27), sanitation of weeds, leguminous crops, infested crop debris, and volunteer onion, avoids reuse of irrigation water, and follows a two-year or longer rotation to nonhosts such as small grains should greatly reduce the need for class B2 carcinogen to manage Xanthomonas leaf blight in Colorado and perhaps elsewhere.

LITERATURE CITED

1. Anith, K. N., Momol, M. T., Kloepper, J. W., Marois, J. J., Olson, S. M., and Jones, J. B. 2004. Efficacy of plant growth–promoting rhizobacteria, acibenzolar-*S*-methyl, and soil amendment for integrated management of bacterial wilt on tomato. Plant Dis. 88:669-683.

2. Babu, R. M., Sajeena, A., Vijayasamundeeswari, A., Sreedhar, A., Vidhyasekeran, P., and Reddy, M. S. 2003. Induction of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) resistance in rice by treatment with acibenzolar-S-methyl. Annals of Applied Biol. 143:333-340.

3. Bartolo, M. E., Schwartz, H. F., and Schweissing, F. C. 1994. Yield and growth-response of onion to simulated storm damage. HortScience 29:1465-1467.

4. Bender, C. L., and Cooksey, D. A. 1986. Indigenous plasmids in *Pseudomonas syringae* pv. *tomato*: Conjugative transfer and role in copper resistance. J. Bacteriol. 165:534-541.

5. Bowen, P., Gibbs, H. A., and O'Garro, L. W. 1996. Garlic, chives, shallot, and leek are alternative hosts to *Xanthomonas campestris*, the pathogen of leaf blight of onion. Proc. Int. Congress of Plant Pathol. 7:696.

6. Cole, D. L. 1999. The efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance, against bacterial and fungal diseases of tobacco. Crop Protection 18:267-273.

7. Gent, D. H., and Schwartz, H. F. 2004. Managing Xanthomonas leaf blight of onion with copper bactericides and acibenzolar. Phytopathol. 94:S34.

8. Gent, D. H., Schwartz, H. F., Ishimaru, C. A., Louws, F. J., Cramer, R. A., and Lawrence, C. B. 2004. Polyphasic characterization of *Xanthomonas* strains from onion. Phytopathol. 94:184-195.

9. Gorlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K. H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., and Ryals, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 8:629-643.

10. Hammond-Kosack, K.E., and Parker, J.E. 2003. Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. Curr. Opin. Biotechnol. 14:177-193.

11. Kadota, I., Uehara, K., Shinohara, H., and Nishiyama, K. 2000. Bacterial blight of Welsh onion: A new disease caused by *Xanthomonas campestris* pv. *allii* pv. *nov.* J. Gen. Plant Pathol. 66:310-315.

12. Kunkel, B. N., and Brooks, D. M. 2002. Cross talk between signaling pathways in pathogen defense. Curr. Opin. Plant Biol. 5:325-331.

13. Lawrence, C. B., Joosten, M. H. A. J., and Tuzun, S. 1996. Differential induction of pathogenesis-related proteins in tomato by *Alternaria solani* and the association of a basic chitinase isozyme with resistance. Physiol. and Mol. Plant Pathol. 48:361-377.

14. Lawrence, C.B., Singh, N.P., Qiu, J., Gardner, R., and Tuzun, S. 2000. Constitutive hydrolytic enzymes are associated with polygenic resistance of tomato to *Alternaria solani* and may function as an elicitor release mechanism. Physiol. and Mol. Plant Pathol. 57:211-220.

15. Louws, F. J., Wilson, M., Campbell, H. L., Cuppels, D. A., Jones, J. B., Shoemaker, P. B., Sahin, F., and Miller, S. A. 2001. Field control of bacterial spot and bacterial speck of tomato using a plant activator. Plant Dis. 85:481-488.

16. Marco, G. M., and Stall, R. E. 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper. Plant Dis. 67:779-781.

17. Minsavage, G. V., Canteros, B. I., and Stall, R. E. 1990. Plasmid-mediated resistance to streptomycin in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathol. 80:719-723.

18. Momol, M. T., Olson, S. M., Funderburk, J. E., Stavisky, J., and Marois, J. J. 2004. Integrated management of tomato spotted wilt on field-grown tomatoes. Plant Dis. 88:882-890.

19. O'Garro, L. W., and Paulraj, L. P. 1997. Onion leaf blight caused by *Xanthomonas campestris*: Alternative hosts and resistant onion genotypes. Plant Dis. 81:978-982.

20. Paulraj, L., and O'Garro, L. W. 1992. Aspects of the epidemiology of a leaf blight of onion in Barbados. Pp. 89-96 in: Proc. of the Tenth Annual Conference of the Barbados Society of Technologists in Agriculture. St. Micheal, Barbados, Nov 11-13, 1992.

21. Paulraj, L., and O'Garro, L. W. 1993. Leaf blight of onion in Barbados caused by *Xanthomonas campestris*. Plant Dis. 77:198-201.

22. Resende, M. L. V., Nojosa, G. B. A., Cavalcanti, L. S., Aguilar, M. A. G., Silva, L. H. C. P., Perez, J. O., Andrade, G. C. G., Carvalho, G. A., and Castro, R. M. 2002. Induction of resistance in cocoa against *Crinipellis perniciosa* and *Verticillium dahliae* by acibenzolar-S-methyl (ASM). Plant Pathol. 51: 621-628.

23. Rojo, E., and Solano, R. 2003. Interactions between signaling compounds involved in plant defense. J. Plant Growth Regulation 22:82-98.

24. Romero, A. M., Kousik, C. S., and Ritchie, D. F. 2001. Resistance to bacterial spot in bell pepper induced by acibenzolar-*S*-methyl. Plant Dis. 85:189-194.

25. Roumagnac, P., Gagnevin, L., and Pruvost, O. 2000. Detection of *Xanthomonas* sp., the causal agent of onion bacterial blight, in onion seeds using a newly developed semi-selective isolation medium. Eur. J. Plant Path. 106:867-877.

26. Roumagnac, P., Gagnevin, L., Gardan, L., Sutra, L., Manceau, C., Dickstein, E. R., Jones, J. B., Rott, P., and Pruvost, O. 2004. Polyphasic characterization of xanthomonads isolated from onion, garlic and Welsh onion (*Allium* spp.) and their relatedness to different *Xanthomonas* species. Int. J. Syst. Evol. Microbio. 54:15-24.

27. Roumagnac, P., Pruvost, O., Chiroleu, F., and Hughes, G. 2004. Spatial and temporal analyses of bacterial blight onion caused by *Xanthomonas axonopodis* pv. *allii*. Phytopathol. 94:138-146.

28. Schaad, N.W., Jones, J.B., and Chun, W. eds 2001. Laboratory Guide for the Identification of Plant Pathogenic Bacteria. 3rd ed. The American Phytopathological Society Press, St. Paul, MN. 398 pp.

29. Scheck, H. J., and Pscheidt, J. W. 1998. Effect of copper bactericides on copper-resistant and -sensitive strains of *Pseudomonas syringae* pv. *syringae*. Plant Dis. 82:397-406.

30. Schwartz, H.F., and Bartolo, M.E. eds. 1995. Colorado Onion Production and Integrated Pest Management. Bull. 547A Colorado State University, Fort Collins, CO. 39 pp.

31. Schwartz, H. F., and Otto, K. J. 1998. Onion bacterial disease management in Colorado. Pp. 214-218 in: Proc. of the 1998 National Onion (and other *Allium*) Research Conference, Dec. 10 – 12, 1998, Sacramento, CA.

32. Schwartz, H. F., Otto, K. L., and Gent, D. H. 2003. Relation of temperature and rainfall to development of Xanthomonas and Pantoea leaf blights of onion in Colorado. Plant Dis. 87:11-14.

33. Stall, R. E., Loschke, D. C., and Jones, J. B. 1986. Linkage of copper resistance and avirulence loci on a self-transmissible plasmid in *Xanthmonas campestris* pv. *vesicatoria*. Phytopathol. 76:240-243.

34. Traw, M. B., Kim, J., Enright, S., Cipollini, D. F., and Bergelson, J. 2003. Negative cross-talk between salicylate- and jasmonate-mediated pathways in the Wassilewskija ecotype of *Arabidopsis thaliana*. Molecular Ecol. 12:1125-1135.

35. Thomma, B. P. H. J., Eggermont, K., Penninckx, I. A. M. A., Mauch-Mani, B., Vogelsang, R., Cammue, B. P. A., and Broekaert, W. F. 1998. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. USA 95:15107-15111.

36. Weller, D. M., and Saettler, A. W. 1978. Rifampin-resistant *Xanthomonas phaseoli* var. *fuscans* and *Xanthomonas phaseoli*: Tools for field study of bean blight bacteria. Phytopathol. 68:778-781.

37. Zhao, Y. F., Thilmony, R., Bender, C. L., Schaller, A., He, S. Y., and Howe, G. A. 2003. Virulence systems of *Pseudomonas syringae* pv. *tomato* promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. Plant J. 36:485-499.

Table V. 1. Onion bulb yield and grade in response to chemical treatment with copper hydroxide and acibenzolar-S-methyl in the absence of Xanthomonas leaf blight at two locations in Colorado in 2002.

	Yield and grade (t/ha)[x]				
	Rocky Ford[y]			Fort Collins[y]	
Treatment[z]	Medium	Jumbo	Total	Medium	Total
Untreated	26.1a	8.3a	42.7a	15.4a	31.8a
Copper hydroxide	22.8a	6.5a	39.9ab	9.2a	28.7ab
Acibenzolar-S-methyl	20.8a	5.6a	33.4b	10.9a	23.1b

[x] Treatments within a column followed by the same letter are not significantly different based on Fisher's F-protected least significant difference. Data are mean of four replications.

[y] Cultivar X-201 was used in experiments at Rocky Ford, and cultivar Vantage was used in experiments at Fort Collins.

[z] Ten weekly applications of each treatment were applied to plots beginning approximately two weeks before bulb initiation; copper hydroxide (Kocide 2000, Griffin, L. L. C., Valdosta, GA), and acibenzolar-S-methyl (Actgard 50WG, Syngenta Crop Protection, Greensboro, NC).

Table V. 2. Xanthomonas leaf blight severity, yield, and grade of onion in relation to copper bactericide and acibenzolar-S-methyl applications at three locations in Colorado in 2003.

	Location, disease severity, and yield (t/ha)[v]							
	Rocky Ford[w]				Fort Collins[wx]		Yuma[wx]	
Treatment[y]	**RAUDPC**	**Medium**	**Jumbo**	**Total**	**RAUDPC**	**Total**	**RAUDPC**	**Total**
Untreated	0.17a	10.6ab	12.1a	24.4a	0.25a	7.8a	0.37a	7.3a
Copper hydroxide	0.15b	8.3ab	16.1abc	25.7ab	0.18b	9.3a	0.24ab	7.7a
Acibenzolar-S-methyl	0.08c	11.1bc	20.0c	32.1ab	0.15b	9.0a	0.16b	6.4a
Copper hydroxide-mancozeb subplot								
Untreated	0.14b	16.1c	15.7b	33.8b	0.18b	7.3a	0.28ab	6.8a
Copper hydroxide	0.14b	4.6a	17.1abc	22.2ab	0.14b	8.7a	0.25ab	7.7a
Acibenzolar-S-methyl	0.09c	12.3bc	15.3ab	29.5ab	0.14b	8.5a	0.18b	6.8a
Factor[w]								
Treatment	0.0022	0.0597	0.5732	0.4616	0.0970	0.0784	0.2913	0.6331
Copper hydroxide-mancozeb	0.3033	0.3972	0.9504	0.4494	0.0989	0.4464	0.1331	0.9513
Treatment*copper hydroxide-mancozeb	0.0774	0.0290	0.0048	0.0097	0.0178	0.8957	0.0120	0.3627

[v] RAUDPC= relative area under the disease progress curve. RAUDPC was calculated according to $\{\sum^{n}_{i=1}[(x_{i+1}+x_i)/2][t_{i+1}-t_i]\} / t_n - t_i$. Treatments within a column followed by the same letter are not significantly different based on Fisher's F-protected least significant difference (P=0.05). Data are mean of four replications.

[w] Cultivar X-201 was used in experiments at Rocky Ford, and cultivar Vantage was used in experiments at Fort Collins and Yuma.

[x] Total yield at Fort Collins and Yuma is weight (kg) of 10 bulbs harvested randomly from each plot.

[y] Copper hydroxide (Kocide 2000, Griffin, L. L. C., Valdosta, GA), acibenzolar-S-methyl (Actigard 50WG, Syngenta Crop Protection, Greensboro, NC), and copper hydroxide-mancozeb (ManKocide, Griffin, L. L. C.). Ten weekly applications of copper hydroxide or copper-hydroxide-mancozeb, or four weekly applications of acibenzolar-S-methyl were applied to plots beginning approximately two weeks before bulb initiation. The experiment was arranged as a randomized split-block design, where the whole plot treatments (designated treatment) were untreated, copper hydroxide, or acibenzolar-S-methyl treatments; and the subplot treatment (designated copper hydroxide-mancozeb) was the presence or absence of weekly applications of copper hydroxide-mancozeb.

[z] Timing, maneb, and timing*maneb interaction were analyzed using the PROC MIXED procedure of SAS v 9.1 (SAS Institute, Cary, NC). Replications were considered random in the mixed model.

Table V. 3. Xanthomonas leaf blight severity, yield, and grade of onion in relation to copper bactericide, acibenzolar-S-methyl, and biological control agents at three locations in Colorado in 2004.

	Location, disease severity, and yield[wx]										
	Rocky Ford				Fort Collins			Yuma			
Treatment[y]	RAUDPC	Medium	Jumbo	Total	RAUDPC	Medium	Total	RAUDPC	Medium	Jumbo	Total
Untreated	0.48a	28.2a	16.0a	47.4a	0.29a	29.3a	39.5a	0.06a	15.0a	14.8a	32.7a
Copper hydroxide	0.29bc	23.6a	14.7a	41.9a	0.21b	28.1a	36.9a	0.03bc	16.1a	15.5a	34.9a
Acibenzolar-S-methyl	0.23c	25.4a	20.4a	48.8a	0.15d	32.7a	47.8a	0.03bc	14.4a	16.6a	33.0a
Pa C9-1	0.36a	28.4a	14.2a	46.7a	0.30a	26.1a	39.0a	0.04ab	14.6a	19.0a	35.5a
Pa C9-1/Pf A506	0.26bc	25.2a	14.0a	42.8a	0.22bc	30.0a	39.5a	0.04b	13.7a	15.6a	31.1a
Copper hydroxide-mancozeb subplot											
Untreated	0.34b	27.0a	17.4a	48.8a	0.24ab	24.2a	35.9a	0.03c	11.9a	20.8a	34.7a
Copper hydroxide	0.24c	23.8a	16.5a	43.7a	0.20bc	24.2a	41.2a	0.02c	11.3a	20.0a	33.9a
Acibenzolar-S-methyl	0.21c	25.0a	19.7a	47.9a	0.15d	29.3a	41.7a	0.02c	12.1a	16.7a	31.5a
Pa C9-1	0.31bc	28.2a	10.8a	45.1a	0.23bc	30.0a	39.3a	0.02c	11.1a	21.9a	36.2a
Pa C9-1/Pf A506	0.21c	27.9a	15.8a	44.9a	0.19cd	33.4a	41.2a	0.02c	12.0a	15.4a	29.3a
Factor[z]											
Treatment	0.0026	0.5102	0.2426	0.3507	0.0001	0.6406	0.4219	0.1115	0.8658	0.4261	0.1498
Copper hydroxide-mancozeb	0.0129	0.8997	0.9241	0.6775	0.0552	0.4122	0.7093	0.0065	0.1286	0.2742	0.9125
Treatment* Copper hydroxide-mancozeb	0.0055	0.9672	0.8626	0.8474	0.2517	0.0837	0.1930	0.0090	0.4655	0.5929	0.8886

[w] RAUDPC= relative area under the disease progress curve. RAUDPC was calculated according to $\{\sum^{n}_{i=1} [(x_{i+1} + x_i)/2][t_{i+1} - t_i]\} / t_n - t_i$. Treatments within a column followed by the same letter are not significantly different based on Fisher's F-protected least significant difference. Data are mean of four replications.

[x] Cultivar X-201 was used in experiments at Rocky Ford, and cultivar Vantage was used in experiments at Fort Collins and Yuma.

[y] Copper hydroxide (Kocide 2000, Griffin, L. L. C., Valdosta, GA), acibenzolar-S-methyl (Actigard 50WG, Syngenta Crop Protection, Greensboro, NC), copper hydroxide-mancozeb (ManKocide, Griffin, L. L. C.), Pa C9-1=*Pantoea agglomerans* strain C9-1 (BlightBan C9-1, NuFarm, Laverton, Australia), and Pa C9-1/Pf A506= *P. agglomerans* strain C9-1 + *Pseudomonas fluorescens* strain A506 (BlightBan C9-1/A506, NuFarm). Ten weekly applications of copper hydroxide or copper hydroxide-mancozeb, four weekly applications of

acibenzolar-S-methyl, or two weekly applications of *P. agglomerans* strain C9-1 + *Pseudomonas fluorescens* strain A506 were applied to plots beginning approximately two weeks before bulb initiation. The experiment was arranged as a randomized split-block design, where the whole plot treatments (designated treatment) were untreated, copper hydroxide, acibenzolar-S-methyl, *P. agglomerans* strain C9-1, or *P. agglomerans* strain C9-1 + *Pseudomonas fluorescens* strain A506 treatments; and the subplot treatment (designated copper hydroxide-mancozeb) was the presence or absence of weekly applications of copper hydroxide-mancozeb.

[z] Timing, maneb, and timing*maneb interaction were analyzed using the PROC MIXED procedure of SAS v 9.1 (SAS Institute, Cary, NC). Replications were considered random in the mixed model.

Table V. 4. Effect of copper hydroxide application timing and maneb rate on Xanthomonas leaf blight severity and bulb yield and grade of onion cultivar X-201 at Rocky Ford, CO in 2003 and 2004.

	2003		2004				
					Yield (t/ha)y		
Treatment[w]	Total sprays[x]	RAUDPC[y]	Total sprays[y]	RAUDPC	Medium	Jumbo	Total
Untreated	5	0.06a	10	0.05a	10.4a	41.3a	52.3a
4-weeks prebulb	9	0.01c	14	0.04a	13.6a	37.3a	51.5a
3-weeks prebulb	8	0.02bc	13	0.04a	11.4a	41.3a	52.8a
2-weeks prebulb	7	0.01c	12	0.05a	11.7a	42.8a	53.9a
1 week prebulb	6	0.02bbc	11	0.04a	11.8a	40.9a	55.3a
Bulb intiation	5	0.03b	10	0.05a	10.9a	44.2a	58.7a
1 week postbulb	4	0.03b	9	0.05a	11.5a	42.3a	54.3a
2 weeks post bulb	3	0.03b	8	0.06a	13.7a	39.1a	52.9a
Factor[z]							
Timing	--	<0.0001	--	0.0761	0.6199	0.8102	0.4919
Maneb	--	0.3755	--	0.8685	0.6565	0.1052	0.0695
Timing*maneb	--	0.3250	--	0.3089	0.5476	0.3427	0.4906

[w] Treatments consisted of copper hydroxide (Kocide 2000, Griffin, L. L. C., Valdosta, GA) applications, where the timing of the initial spray was staggered weekly. The 4-week prebulb treatment was applied approximately four weeks before bulb initiation. Treatments were applied weekly once a spray program was initiated for a given timing. The experiment was arranged as a randomized split-block design, where the whole plot treatment (designated timing) was the initial timing when the spray program began; and the subplot factor was maneb (Maneb 75DF, Cerexagri Inc., King of Prussia, PA) rate at four levels (0, 0.42, 0.84, or 1.68 kg a.i./ha).

[x] Untreated plots did not receive any copper hydroxide, but did receive maneb at 0, 0.42, 0.84, or 1.68 kg a.i./ha depending on the subplot treatment. Maneb applications on the untreated plots began at bulb initiation.

[y] RAUDPC= relative area under the disease progress curve. RAUDPC was calculated according to $\{\sum^{n}_{i=1} [(x_{i+1} + x_i)/2][t_{i+1} - t_i]\} / t_n - t_i$. Treatments within a column followed by the same letter are not significantly different based on Fisher's F-protected least significant difference. Data are mean of three replications.

[z] Timing, maneb, and timing*maneb interaction were analyzed using the PROC MIXED procedure of SAS v 9.1 (SAS Institute, Cary, NC). Replications were considered random in the mixed model.

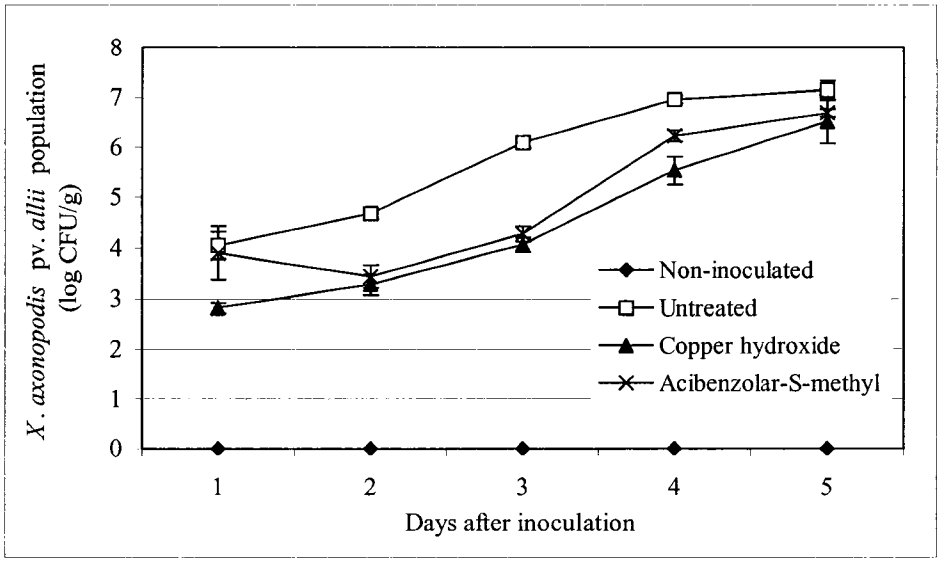


Figure V. 1. Epiphytic multiplication of *Xanthomonas axonopodis* pv. *allii* on onion treated with copper hydroxide, acibenzolar-S-methyl, or water (untreated). Onion cultivars Vantage and Cometa were used in these growth chamber studies, but epiphytic *X. axonopodis* pv. *allii* populations did not differ among cultivars (*P*-value=0.088). Data are mean of four replications averaged over cultivar and two replications of the entire experiment (n=16) ± standard error of the mean.

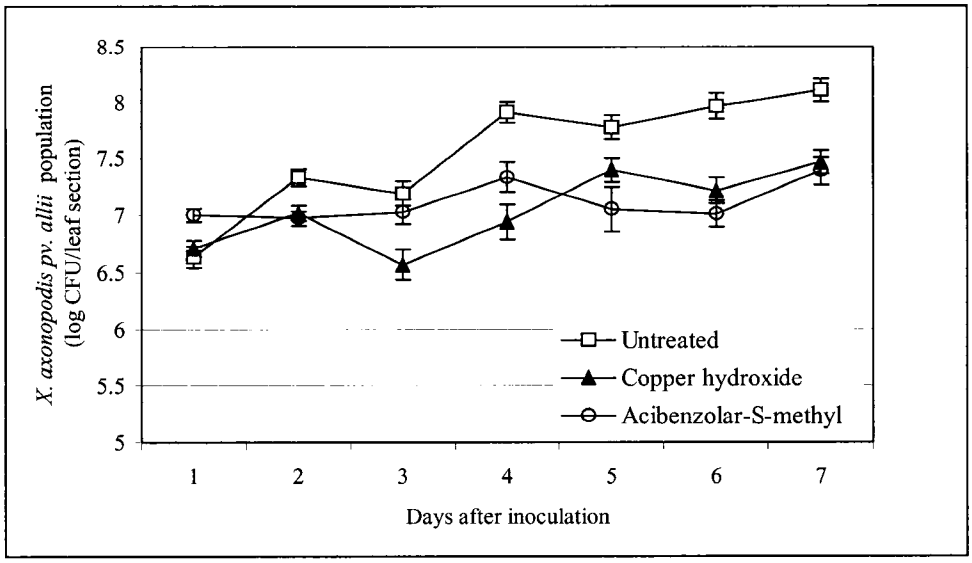


Figure V. 2. *In planta* multiplication of *Xanthomonas axonopodis* pv. *allii* in onion in relation to treatment with copper hydroxide, acibenzolar-S-methyl, or water (untreated). Onion cultivars Vantage and Cometa were used in these growth chamber studies, but *in planta X. axonopodis* pv. *allii* populations did not differ among cultivars (*P*-value=0.334). Data are mean of four replications averaged over cultivar and two replications of the entire experiment (n=16) ± standard error of the mean.

CHAPTER VI

INFLUENCE OF NITROGEN FERTILZATION AND SEED CONTAMINATION ON EPIPHYTIC XANTHOMONAS AXONOPODIS PV. ALLII AND XANTHOMONAS LEAF BLIGHT DEVELOPMENT

INTRODUCTION

Xanthomonas leaf blight of onion (*Allium cepa*), caused by the bacterium *Xanthomonas axonopodis pv. allii*, is a yield limiting disease in Colorado. The disease first appears as small, lenticular-shaped chlorotic lesions, but lesions quickly enlarge and become water-soaked in humid or rainy weather. The pathogen is not known to attack bulbs, but yield losses associated with foliage blighting, plant stunting, and reduced bulb size can be significant (11,20,22). The host range of *X. axonopodis pv. allii* appears limited to a few *Allium* species (1,3,6,7,16).

Few management strategies are available for Xanthomonas leaf blight management, but crop rotation (12), host resistance (12), copper and zinc applications (13,20), biological control (14), and the planting of pathogen-free seed (17) have been suggested. Most of these management strategies have not been evaluated in Colorado, and consequently growers rely largely upon copper-based bactericides for disease suppression (20).

The impact of cultural practices, such as nitrogen fertilization and planting pathogen-free seed, on disease development in semi-arid production environments are unknown. Therefore, we sought

to address these deficits in our knowledge and develop new strategies for managing Xanthomonas leaf blight.

METHODS AND MATERIALS

Bacterial strain and culture. A rifampicin mutant of *X. axonopodis* pv. *allii* strain O177 (ATCC 508) was generated as previously described (24), and is referred to as R-O177. Strain R-O177 is resistant to greater than 200 μg/ml rifampicin, but selection routinely was performed on nutrient agar amended with 50 μg/ml of rifampicin. To prepare inoculum of strain R-O177, loopfuls of bacteria were streaked onto rifampicin-amended nutrient agar medium before incubating culture plates at 29°C for 72 h in the dark. Cells were harvested from plates by flooding with deionized water and gently scraping the plates with a small, flame-sterilized spatula. The cell suspension was adjusted to 10^8 CFU/ml spectrophotometrically (OD_{600}=0.12) before adjustment to the desired concentration for an experiment. The bacterial strain was preserved in 15% nutrient glycerol broth at -80°C for long-term storage.

Nitrogen fertility studies. Field studies were established at the Agricultural Research, Development, and Education Center (ARDEC) near Fort Collins, CO to determine the effect of nitrogen fertilization on *X. axonopodis* pv. *allii* epiphytic and subsequent Xanthomonas leaf blight disease development. Onion (cv. Vantage) was planted into plots in May 2003 and April 2004. Plots were established from seed planted approximately 0.1 m apart in beds on 0.76 m centers. Each bed contained two rows of onion spaced 0.15 m apart. The field was furrow irrigated once to twice weekly and did not receive any fertilizer. A plot consisted of four contiguous 0.76 m wide beds 7.6 m in length replicated four times in a randomized complete block design. A different area of the same field was used for studies in 2003 and 2004.

Soil major and micronutrients, pH, and soluble salts were measured (MDS Harris, Lincoln, NE) from bulked soil samples consisting of 6 randomly collected soil cores (2.5 cm in diameter) taken from a depth of 15 cm. Soil nitrate nitrogen content was determined, and used to adjust application rates to achieve the desired nitrogen level for a treatment. The field contained 17 and 70 ppm nitrate nitrogen in 2003 and 2004, respectively. Nitrogen fertilizer (urea, 46-0-0) was applied to each plot by gently loosing the soil between onion rows on the center two beds with a hoe and placing the fertilizer onto the soil surface. The outer rows of the plot did not receive any fertilizer to reduce nitrogen movement among plots and confounding plot interference. The fertilizer was mechanically incorporated approximately 2.5 cm deep by shallow hoeing. Treatments consisted of untreated (no additional fertilizer applied), 112, 224, or 448-kg/ha nitrogen that was applied in two equal (half) applications at the four-leaf stage and bulb initiation.

The center two rows of each plot were inoculated to runoff with 10^6 CFU/ml strain R-O177 amended with 0.1% Silwet L-77 (Loveland Industries, Greeley, CO) using a CO_2-pressurized backpack sprayer 106 and 107 days after planting (approximately bulb initiation) in 2003 and 2004, respectively. Inoculum was prepared by harvesting cells of strain R-O177 from 72-h old rifampicin-amended nutrient agar plates grown at 29°C. The culture was adjusted spectrophotometically to 10^8 CFU/ml (OD_{600}=0.12) and then diluted 100-fold in sterile magnesium phosphate buffer.

Approximately 20 to 40 grams of asymptomatic leaf material were arbitrarily collected from each plot weekly after inoculation, placed into resealable plastic bags, and promptly transported to the laboratory on ice for epiphyte enumeration. Five to 10 grams of this bulked leaf sample was weighed, placed into sterile 250 ml flasks containing 100 ml of magnesium sulfate-potassium phosphate buffer (0.01 M magnesium sulfate and 0.01 M potassium phosphate, pH 7.2), and

shaken at 250 oscillations/min for 60 min at room temperature (approximately 22°C). Aliquots (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium phosphate buffer or spiral plated (Autoplate 4000, Spiral Biotech, Inc., Norwood, MA) directly onto nutrient agar amended with 50 µg/ml rifampicin and cycloheximide. Dilutions and samples were plated in duplicate. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 29°C, and a subset of rifampicin-resistant colonies were confirmed as *X. axonopodis* pv. *allii* by standard physiological and biochemical tests (19); including Gram stain reaction, pigmentation on yeast dextrose carbonate medium, fluorescence on King's medium B, indole test, growth on 0.1% tetrazolium chloride, oxidase test, starch hydrolysis, oxidative utilization of glucose, catalase test, production of H_2S from cysteine, presence of arginine dihydrolase, and casein hydrolysis test. Disease severity was estimated weekly from plots using a modified Horsfall-Barrett scale after visible Xanthomonas leaf symptoms developed. At maturity, 20 bulbs or a 3 m subsection of each plot was mechanically topped and harvested, graded, and weighed to estimate yields.

Seed transmission studies. Experiments were conducted to determine the seed contamination threshold necessary for Xanthomonas leaf blight development under standard production practices in Colorado. One hundred seven onion seed lots of commercial origin were screened for *X. axonopodis* pv. *allii* contamination as described previously (15) using a modified MXP medium (4) (containing 50 µg/ml kasugamycin, 50 µg/ml cephalexin, and 50 µg/ml cycloheximide to reduce growth of other bacteria and fungi), but the bacterium was not detected. In the absence of naturally-infested seed, we inoculated seed with varying concentrations (0, 10^3, 10^5 or 10^7 CFU/g) of *X. axonopodis* pv. *allii*. Seed of the yellow onion cultivar Vantage free of detectable *X. axonopodis* pv. *allii* was rinsed in running tap water for 30 min to reduce pesticides residues. Seed was allowed to dry overnight on paper towels before delivering inoculum by vacuum infiltration. Approximately 20 g of rinsed seed was submersed in a suspension of *X. axonopodis* pv. *allii* strain R-O177 at different concentrations (0, 10^5 or 10^7 CFU/ml sterile

distilled water) and placed under a 15 mm Hg vacuum for 3 min. The vacuum was rapidly removed, and the seed was soaked in the suspension for an additional 4 h at room temperature (approximately 22°C) before air-drying overnight. Seed was assayed by a seed rinse assay (15) to ensure the proper inoculum concentration was delivered to the seed.

Inoculated seed was planted into plots at ARDEC as described for the nitrogen fertility studies. The center two beds of the four row wide plot were planted with inoculated seed, and the outer two rows were planted with seed free from detectable *X. axonopodis* pv. *allii*. Plots were monitored weekly for disease development, and epiphytic populations of *X. axonopodis* pv. *allii* strain R-O177 were recovered and enumerated approximately every three weeks as described previously. Yields were not estimated.

Statistical analyses. Statistical analyses were performed using the PROC MIXED function of SAS v. 9.1 (SAS Institute, Cary, NC). Bacterial population data were log transformed to achieve independently and normally distributed experimental errors with a common variance. The area under the bacterial growth curve and relative area under the disease progress curve (RAUDPC) were calculated, and used as response variables in analysis of nitrogen fertility experiments. Replications of experimental units were considered random factors.

RESULTS

Nitrogen fertility studies. Epiphytic populations of *X. axonopodis* pv. *allii*, as measured by the area under the bacterial growth curve, varied among years (P=0.0187), but did not vary among nitrogen fertilization treatment in 2003 or 2004 (P=0.2914 or 0.2998, respectively). In 2003, epiphytic populations increased nearly 5 logarithmic units per g fresh weight 27 days after inoculation, but decreased one to two logarithmic units in the following 7 days (Figure VI. 1). In

DISCUSSION

In this study we have evaluated the effect of nitrogen fertilization and seed contamination levels on epiphytic *X. axonopodis* pv. *allii* populations and subsequent Xanthomonas leaf blight development. Excessive nitrogen fertilization (greater than 112 kg/ha) increased the severity of Xanthomonas leaf blight in one year, but did not influence epiphytic populations of the pathogen, bulb yield or grade. No relation between seed contamination level and subsequent epiphytic or pathogenic development of *X. axonopodis* pv. *allii* was observed under standard production practices during this study. Knowledge of cultural practices such as fertilization and seed selection are essential to the development of ecologically-based and sustainable management of this pathogen and its associated disease.

Little is known about how nitrogen fertilization levels influence epiphytic bacterial populations. On tomato (*Lycopersicon esculentum*), *X. axonopodis* pv. *vesicatoria* populations and defoliation from bacterial spot are greatest on plants fertilized lightly with nitrogen, but other nutrients may influence epiphytic populations and disease severity as well (10). Abundant nitrogen fertilization can exacerbate bacterial blight of rice (*Oryza sativa*) caused by *X. oryzae* pv. *oryzae* and reduce grain yield (2,5), but high levels of nitrogen can impede invasion of *X. campestris* pv. *campestris* in cabbage (*Brassica oleracea*). In the studies presented here, we observed relatively large differences in Xanthomonas leaf blight severity among plants receiving low and high rates of nitrogen. Similar trends were observed in both years of the study, although statistical significance was only observed in 2004.

Interestingly, neither bulb yield nor grade was affected by nitrogen rate. Approximately 38 and 155 kg/ha of nitrogen was available to plants in 2003 and 2004, respectively. Soil residual nitrogen, combined with mineralization of organic nitrogen sources, apparently supplied the

entire crop demand in this study, and increasing levels of nitrogen beyond 224 kh/ha tended to increase disease severity without increasing yield.

Contaminated seed is known to be an important inoculum source for many diseases caused by xanthomonads (8,18,23,25), including Xanthomonas leaf blight of onion (17), but under natural conditions we were unable to incite a disease epidemic, even with very high levels of seed-borne inoculum. The seed contamination thresholds for development of carrot (*Daucus carota*) bacterial blight caused by *Xanthomonas campestris* pv. *carotae* is 10^4 to 10^5 CFU/g seed when abundant moisture is available (23). Similarly, Roumagnac et al. (17) reported as little as 0.04% contamination of onion seed by *X. axonopodis* pv. *allii* was sufficient to incite an epidemic of Xanthomonas leaf blight in a sub-tropical environment. But they provided 30 min of supplemental irrigation each morning and evening to ensure disease development. In our studies under natural conditions typical of most Colorado onion production, epiphytic populations of *X. axonopodis* pv. *allii* did not persist throughout the season. Very high levels (10^7 CFU/g) of seed-borne inoculum did not produce visible disease symptoms under the low moisture conditions of these studies, which are typical of Colorado onion production regions. Contaminated seed apparently has little importance to Xanthomonas leaf blight epidemics in Colorado and perhaps other semi-arid production regions where onion is grown without sprinkler irrigation. However, we used artificially inoculated seed in this study, and naturally infested seed may be a more important inoculum source under natural conditions. More study is needed before specific management recommendations can be made regarding seed contamination thresholds in Colorado and other semi-arid and arid production regions.

Nonetheless, onion production practices that limit nitrogen fertilization and rely upon pathogen-free seed and transplants where available should reduce losses from Xanthomonas leaf blight in Colorado and elsewhere.

LITERATURE CITED

1. Alvarez, A. M., Buddenhagen, I. W., Buddenhagen, E. S., and Domen, H. Y. 1978. Bacterial blight of onion, a new disease caused by *Xanthomonas* sp. Phytopathol. 68:1132-1136.

2. Awordev, V. A., Bangura, N., and John, V. T. 1991. Incidence, distribution and severity of bacterial diseases on rice in West Africa. Trop. Pest Manag. 37:113-117.

3. Bowen, P., Gibbs, H. A., and O'Garro, L. W. 1996. Garlic, chives, shallot, and leek are alternative hosts to *Xanthomonas campestris*, the pathogen of leaf blight of onion. Proc. Int. Congress of Plant Pathol. 7:696.

4. Claflin, L. E., Vidaver, A. K., and Sasser, M. 1987. MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathol. 77:730-734.

5. Elings, A., Reddy, P. R., Marimuthu, T., Rossing, W. A. H., Jansen , M. J. W., and Teng, P. S. 1997. Rice bacterial leaf blight: Field experiments, systems analysis and damage coefficients. Field Crops Res. 51:113-131.

6. Gent, D. H., Schwartz, H. F., Ishimaru, C. A., Louws, F. J., Cramer, R. A., and Lawrence, C. B. 2004. Polyphasic characterization of *Xanthomonas* strains from onion. Phytopathol. 94:184-195.

7. Kadota, I., Uehara, K., Shinohara, H., and Nishiyama, K. 2000. Bacterial blight of Welsh onion: A new disease caused by *Xanthomonas campestris* pv. *allii* pv. *nov.* J. Gen. Plant Pathol. 66:310-315.

8. Kuan, T. L., Minsavage, G. V., and Gabrielson, R. L. 1985. Detection of *Xanthomonas campestris* pv. *carotae* in carrot seed. Plant Dis. 69:758-760.

9. McElhaney, R., Alvarez, A. M., and Kado, C. I. Nitrogen limits *Xanthomonas campestris* pv. *campestris* invasion of the host xylem. Physiol. and Mole. Plant Pathol. 52:15-24.

10. McGuire, R. G., Jones, J. B., Stanley, C. D., and Csizinsky, A. A. 1991. Epiphytic populations of *Xanthomonas campestris* pv. *vesicatoria* and bacterial spot of tomato as influenced by nitrogen and potassium fertilization. Phytopathol. 81:656-660.

11. Nunez, J .J., Gilbertson, R. L., Meng, X., and Davis, R. M. 2002. First report of Xanthomonas leaf blight of onion in California. Plant Dis. 86:330.

12. O'Garro, L. W., and Paulraj, L. P. 1997. Onion leaf blight caused by *Xanthomonas campestris*: Alternative hosts and resistant onion genotypes. Plant Dis. 81:978-982.

13. Paulraj, L., and O'Garro, L. W. 1992. Aspects of the epidemiology of a leaf blight of onion in Barbados. Pp. 89-96 in: Proc. of the Tenth Annual Conf. of the Barbados Society of Technologists in Agriculture. St. Micheal, Barbados, Nov 11-13, 1992.

14. Paulraj, L., and O'Garro, L. W. 1993. Leaf blight of onion in Barbados caused by *Xanthomonas campestris*. Plant Dis. 77:198-201.

15. Roumagnac, P., Gagnevin, L., and Pruvost, O. 2000. Detection of *Xanthomonas* sp., the causal agent of onion bacterial blight, in onion seeds using a newly developed semi-selective isolation medium. Eur. J. Plant Path. 106:867-877.

16. Roumagnac, P., Gagnevin, L., Gardan, L., Sutra, L., Manceau, C., Dickstein, E. R., Jones, J. B., Rott, P., and Pruvost, O. 2004. Polyphasic characterization of xanthomonads isolated from onion, garlic and Welsh onion (*Allium* spp.) and their relatedness to different *Xanthomonas* species. Int. J. Syst. Evol. Microbio. 54:15-24.

17. Roumagnac, P., Pruvost, O., Chiroleu, F., and Hughes, G. 2004. Spatial and temporal analyses of bacterial blight of onion caused by *Xanthomonas axonopodis* pv. *allii*. Phytopathol. 94:138-146.

18. Schaad, N. W., and Kenkrick, R. 1975. A qualitative method of detecting *Xanthomonas campestris* in crucifer seed. Phytopathol. 65:1034-1036.

19. Schaad, N.W., Jones, J.B., and Chun, W. eds 2001. Laboratory Guide for the Identification of Plant Pathogenic Bacteria. 3rd ed. The American Phytopathological Society Press, St. Paul, MN. 398 pp.

20. Schwartz, H. F., and Otto, K. J. 1998. Onion bacterial disease management in Colorado. Pp. 214-218 in: Proc. of the 1998 National Onion (and other *Allium*) Research Conference, Dec. 10 – 12, 1998, Sacramento, CA.

21. Schwartz, H. F., Otto, K. L., and Gent, D. H. 2003. Relation of temperature and rainfall to development of Xanthomonas and Pantoea leaf blights of onion in Colorado. Plant Dis. 87:11-14.

22. Serfontein, J. J. 2001. Xanthomonas blight of onion in South Africa. Plant Dis. 85:442.

23. Umesh, K. C., Davis, R. M., and Gilbertson, R. L. 1998. Seed contamination thresholds for development of carrot bacterial blight caused by *Xanthomonas campestris* pv. *carotae*. Plant Dis. 82:1271-1275.

24. Weller, D. M., and Saettler, A. W. 1978. Rifampin-resistant *Xanthomonas phaseoli* var. *fuscans* and *Xanthomonas phaseoli*: Tools for field study of bean blight bacteria. Phytopathol. 68:778-781.

25. Weller, D. M., and Saettler, A. W. 1980. Evaluation of seedborne *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* as primary inocula in bean blights. Phytopathol. 70:148-152.

Table VI. 1. Xanthomonas leaf blight severity, yield, and grade of onion in relation to nitrogen fertilization.

Treatment (kg/ha)[x]	Year, disease severity, and yield (t/ha)					
	2003		2004			
				Yield[z]		
	RAUDPC[y]	Yield[z]	RAUDPC[y]	Medium	Jumbo	Total
0	0.18a	13.8a	0.22a	29.0a	3.2a	47.3a
112	0.18a	14.6a	0.24ab	32.2a	10.5a	54.2a
224	0.19a	12.4a	0.28b	34.4a	6.1a	53.0a
448	0.25a	13.3a	0.33c	30.8a	2.2a	46.1a

[x] Nitrogen fertilizer in the form of 46-0-0 was applied in two equal applications by mechanically incorporating it between plants in a row at the four leaf growth stage and bulb initiation. Approximately 38 and 155 kg/ha nitrogen was available to plants in the 0 kg/ha treatment because of soil residual nitrates in 2003 and 2004, respectively.

[y] RAUDPC= relative area under the disease progress curve. RAUDPC was calculated according to $\{\sum^{n}_{i=1} [(x_{i+1} + x_i)/2][t_{i+1} - t_i]\} / t_n - t_i$. Treatments within a column followed by the same letter are not significantly different based on Fisher's F-protected least significant difference (P=0.05). Data are mean of four replications.

[z] Cultivar Vantage was used in experiments in 2003 and 2004. Total yield in 2003 is weight (kg) of 10 bulbs harvested randomly from each plot, and in 2004 is expressed as t/ha.

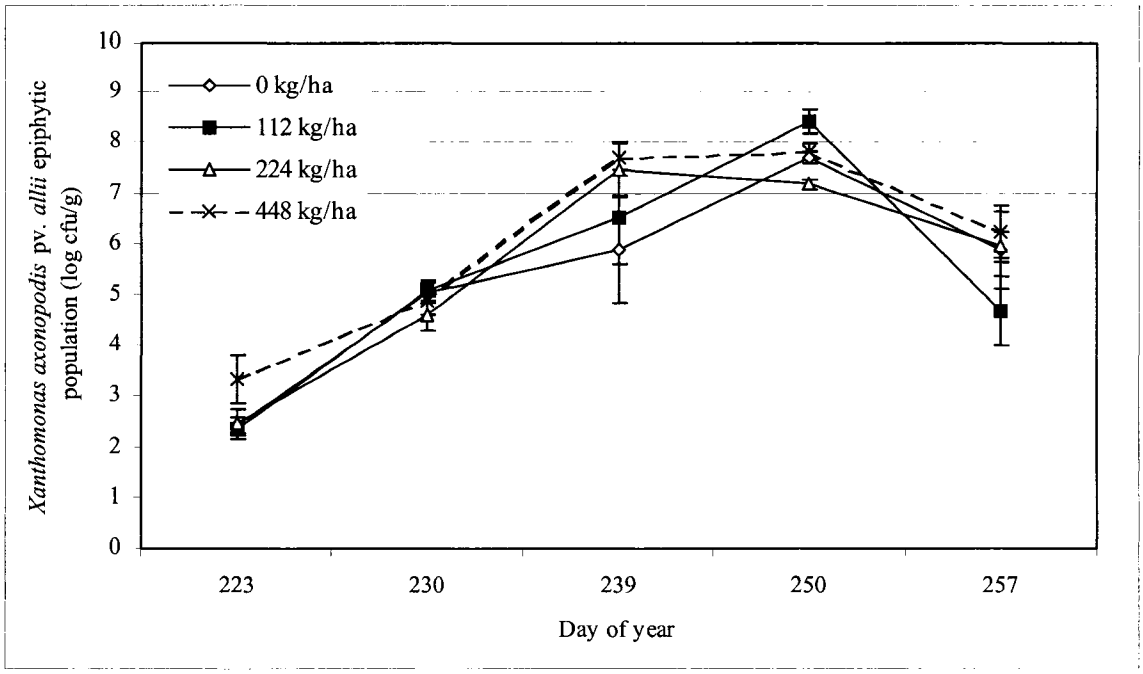


Figure VI. 1. Epiphytic populations of *Xanthomonas axonopodis* pv. *allii* strain R-O177 on onion in relation to nitrogen fertilization rate in 2003. Approximately 38 kg/ha nitrogen was available to plants in the 0 kg/ha treatment because of residual soil nitrates.

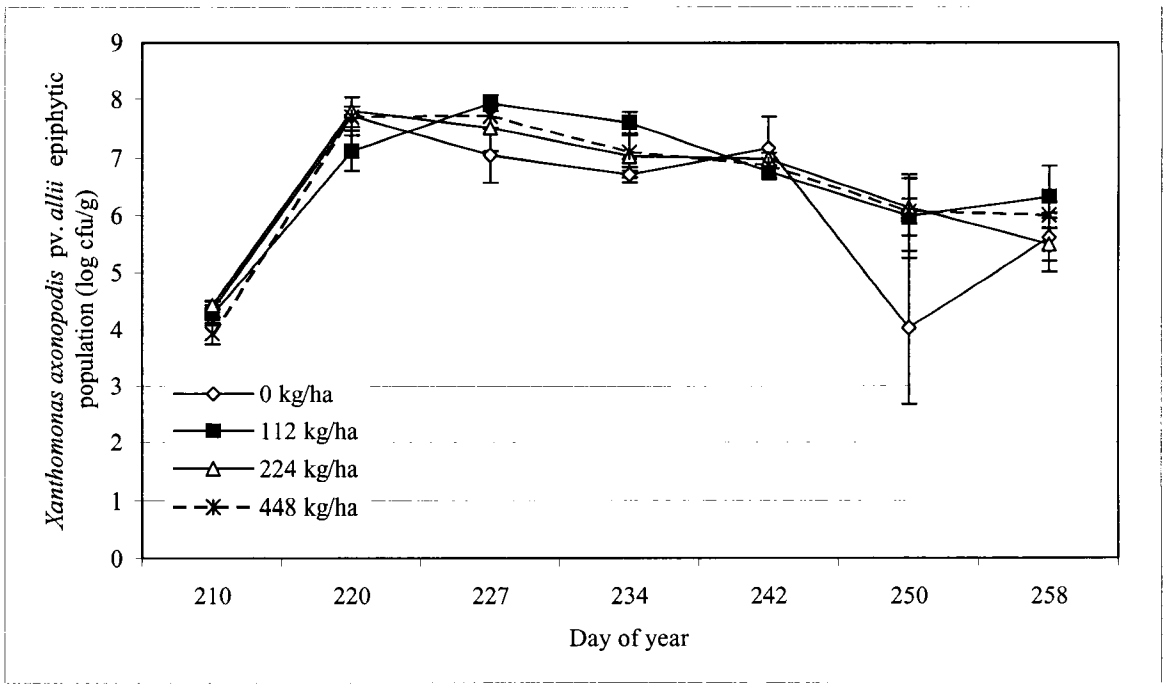


Figure VI. 2. Epiphytic populations of *Xanthomonas axonopodis* pv. *allii* strain R-O177 on onion in relation to nitrogen fertilization rate in 2004. Approximately 155 kg/ha nitrogen was available to plants in the 0 kg/ha treatment because of residual soil nitrates.

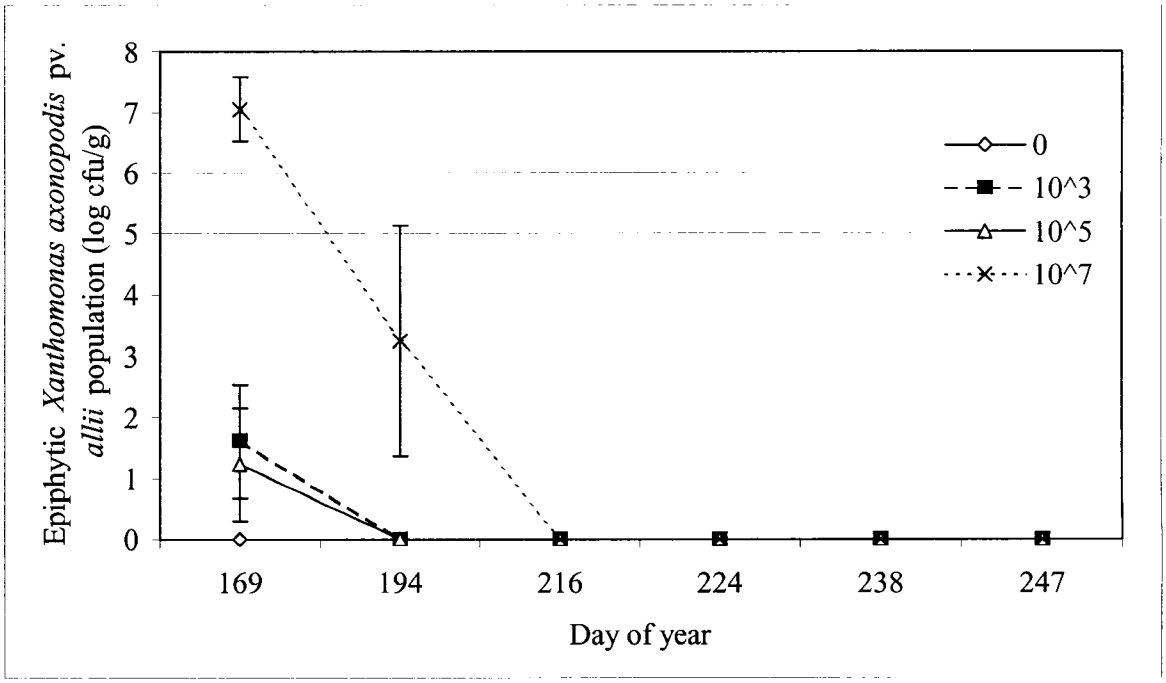


Figure VI. 3. Epiphytic populations of *Xanthomonas axonopodis* pv. *allii* strain R-O177 on onion in relation to seed contamination level in 2003.

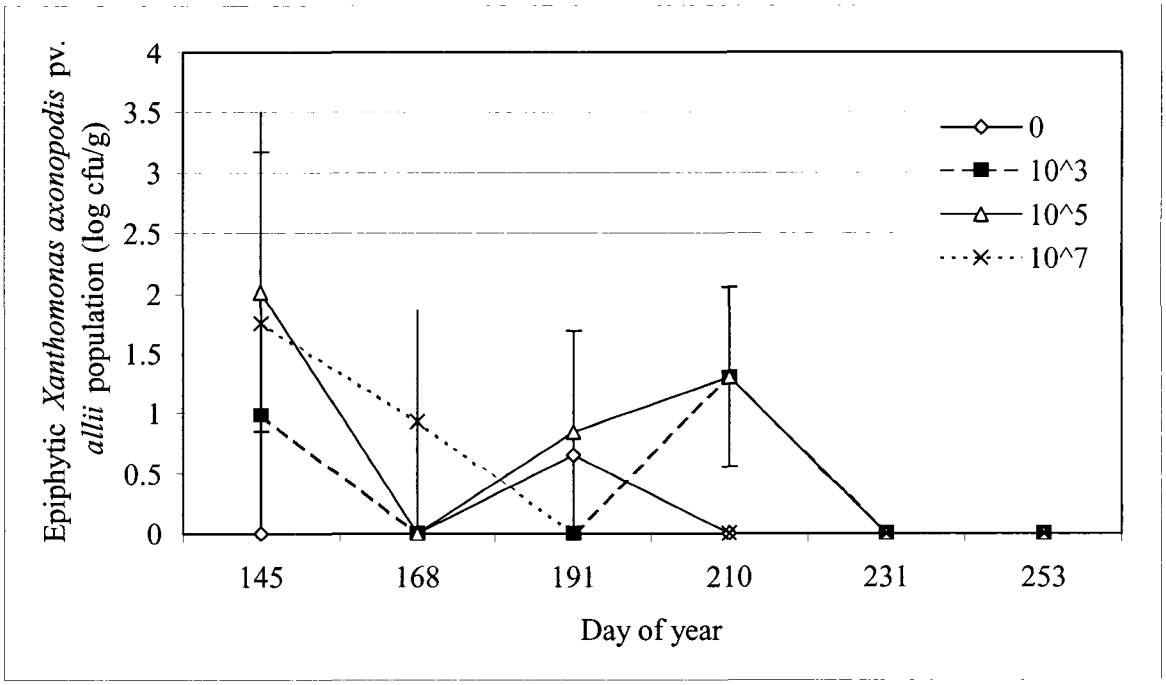


Figure VI. 4. Epiphytic populations of *Xanthomonas axonopodis* pv. *allii* strain R-O177 on onion in relation to seed contamination level in 2004.

CHAPTER VII

OVERALL CONCLUSIONS

Xanthomonas leaf blight is an emerging and potentially damaging disease of onion in Colorado and other onion producing regions of the western U. S. and world. A deficit in basic knowledge and understanding of pathogen and disease biology and epidemiology has limited the development of effective, economical, and sustainable disease management strategies. In the studies presented in this dissertation, we have determined that *Xanthomonas axonopodis* pv. *allii* is genetically and pathogenically diverse, identified and quantifed pathogen overwintering and inoculum sources, and developed rational management strategies for Xanthomonas leaf blight of onion.

Onion strains of Xanthomonas are pathogenically and genetically indistinguishable from *X. campestris* pv. *allii*, but rep-PCR fingerprinting revealed the correct species epithet should be *X. axonopodis* pv. *allii*. The pathogen is genetically and phenotypically diverse, and knowledge of this diversity may be essential for selecting genotypes with durable resistance, conducting epidemiological studies, and developing integrated management strategies. The pathogen is capable of *in planta* multiplication in onion, citrus, and leguminous plants, but disease symptoms are produced only on onion and citrus. Bactericide and antibiotic resistance is not prevalent among strains, but may appear with continued reliance upon conventional chemical controls for disease suppression.

X. axonopodis pv. *allii* and *X. axonopodis* pv. *phaseoli* are capable of epiphytic survival on leguminous hosts and onion, respectively. The implications of this epiphytic survival on other

crop plants for disease management is unknown, but may suggest onion rotational schemes should be critically evaluated in Colorado and elsewhere.

Several inoculum sources of the Xanthomonas leaf blight pathogen exist in Colorado, including infested crop debris, contaminated irrigation water, weeds, volunteer onion, and possibly seed. The pathogen may survive between years in association with infested onion leaves left on the soil surface, but populations of the bacterium decrease linearly in infested leaves buried 25 cm deep. *X. axonopodis* pv. *allii* is readily disseminated in irrigation tail water from fields where Xanthomonas leaf blight was present. Weed and volunteer onion plants can harbor epiphytic and pathogenic populations of *X. axonopodis* pv. *allii*, but weed plants do not appear to be the primary means for bacterial survival in the absence of a suitable host. The role of *X. axonopodis* pv. *allii* contaminated seed in Xanthomonas leaf blight epidemics in Colorado is unclear, but contaminated seed appears to be of little concern under furrow irrigation during years with low to moderate rainfall.

Xanthomonas leaf blight can be managed effectively without copper-based bactericides by using novel chemical and biological control agents such as acibenzolar-S-methyl and combinations of *Pantoea agglomerans* strain C9-1 and *Pseudomonas fluorescens* strain A506, respectively. The critical period of disease control appears to be near or immediately prior to bulb initiation. Bactericides applied early or late in the season apparently contribute little to disease control or yield.

Excessive nitrogen fertilization can exacerbate Xanthomonas leaf blight, and does not improve onion yield or grade. The optimal nitrogen rate to limit disease severity and optimize yield, as determined with one onion cultivar at one location in northern Colorado, appears to be near 112 kg/ha.

Much remains to be learned about the biology, epidemiology, and management of Xanthomonas leaf blight of onion. Genetic determinates of pathogenicity in the bacterium are unknown, but may direct future research efforts toward incorporation of durable resistance. Is host resistance to Xanthomonas leaf blight available within *Allium* species, and what is the nature of this resistance? What is the basis of host specificity among *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *citrumelo*? What role do plasmids have in horizontal gene transfer and pathogen evolution? Is horizontal gene transfer possible among *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli*, and is this increased if both are present epiphytically on the same plant species? What is the relationship among *X. axonopodis* pv. *allii* genotype groups and pathogenic variability or races?

We were able to recover large populations of *X. axonopodis* pv. *allii* from infested crop debris, irrigation water, and weeds, but we did not determine the longevity of the bacterium in and on these media. How long does the pathogen persist in diseased leaves left on the soil surface? How far is the bacterium disseminated down stream by irrigation water? Are weeds an important inoculum source, or is the bacterium only a transient inhabitant of their phyllosphere? How long is a disease epidemic delayed by avoiding these inoculum sources?

Future research should evaluate the effectiveness of multi-tactic management programs on suppression not only of Xanthomonas leaf blight, but other onion bacterial diseases. Are the combined effects of biological control (with bacteria or bacteriophage), systemic acquired resistance, and cultural practices (i.e., irrigation method, planting pattern) additive or synergistic? How does partial host resistance influence acibenzolar-S-methyl-mediated suppression of disease? These and other questions will be essential to the development of truly integrated and

sustainable management of Xanthomonas leaf blight, that ensure high productivity, protect environmental quality and resources, efficiently use resources, and improve grower profitability.