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Epiphytic Survival of *Xanthomonas axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* on Leguminous Hosts and Onion

David H. Gent, National Forage Seed Production Research Center, USDA-ARS, Corvallis, OR 97331; and Jillian M. Lang and Howard F. Schwartz, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins 80523-1177

ABSTRACT

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Xanthomonas leaf blight of onion (*Allium cepa*), caused by *Xanthomonas axonopodis* pv. *allii*, and common bacterial blight of dry bean (*Phaseolus vulgaris*), caused by *Xanthomonas axonopodis* pv. *phaseoli*, are perennial problems in the Central High Plains of the United States. Onion and dry bean are commonly grown in rotation in Colorado, but it is unknown if *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* survive epiphytically or pathogenically on dry bean and onion, respectively. Under high humidity growth chamber conditions, epiphytic *X. axonopodis* pv. *allii* populations increased on alfalfa, chickpea, dry bean, lentil, and soybean, but the epiphytic populations were at least 10-fold greater on onion. When artificially inoculated under field conditions, epiphytic populations of *X. axonopodis* pv. *allii* were recovered from dry bean, lentil, and onion, but the bacterium did not persist on chickpea or soybean. Epiphytic *X. axonopodis* pv. *phaseoli* was recovered from symptomless onion plants in fields cropped to dry bean the prior year, but not from fields cropped to a host other than dry bean. Close rotation of onion and dry bean may allow *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* to persist epiphytically, and crop rotation schemes may need to be altered to reduce survival of these pathogens in onion and dry bean cropping systems.

Additional keywords: integrated pest management, onion bacterial blight, phyllosphere, *Xanthomonas campestris* pv. *allii*, *Xanthomonas campestris* pv. *phaseoli*

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 (1,12,14,21,25,27,28,34). 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 other *Allium* species (1,6,10,14,26), 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 mm² leaf disk under experimental conditions.

Onion is frequently rotated with leguminous hosts such as dry bean and occasionally soybean in Colorado because few pests in this region are known to attack both onion and these crops, and the production practices of onion and leguminous crops 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 (10), 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 asymp-

tomatically in onion and legume cropping systems, negating some of 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 epiphytic survival on both leguminous hosts and onion.

MATERIALS AND METHODS

Bacterial strains, culture, and DNA isolation. A rifampicin-resistant 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 rifampicin at 200 µg/ml, but selection routinely was performed on nutrient agar amended with 50 µg/ml. 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. DNA was isolated from bacteria using an UltraClean Microbial DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. DNA was stored at -20°C in tris-acetate buffer (40 mM Tris-acetate, pH 8.2).

Pathogenicity assays to confirm isolate identity. 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-h-old nutrient agar culture plate. Each pin-pricked leaf area was inoculated with a droplet of bacterial matrix approximately equal in size to the needle tip. Leaves of plants serving as negative controls were 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 per 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 0.01 M potassium

Corresponding author: H. F. Schwartz
E-mail: howard.schwartz@colostate.edu

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phosphate, pH 7.2). Three- to four-week-old dry bean plants were sprayed (Crown SpraTool, Aerovoe Industries, 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 $\mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$, 100% relative humidity, and daily misting with tap water to runoff. Plants were observed daily for symptom development.

Epiphytic population assays in growth chamber trials. 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 a 14-h photoperiod, with approximately 2 h of supplemental incandescent lighting) until they were 3 to 4 weeks (soybean, lentil, chickpea, dry bean, and alfalfa) or 6 to 8 weeks (onion) old. Three plants of each species were seeded individually in MetroMix 200 potting soil (Grace Sierra Horticultural Products Company, Milpitas, CA) in 1-liter pots.

Twenty pots each containing three plants of each 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 per 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 into individual plastic bags. Another set of four pots (replications) of each plant species was destructively sampled each day for 4 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 $\mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$, 100% relative humidity, and daily misting with tap water to runoff.

Harvested plant samples were weighed, placed in sterile 250-ml flasks containing 100 ml of magnesium sulfate-potassium phosphate buffer, and shaken at 250 oscillations per min for 60 min at room temperature (approximately 22°C). Aliquots (100 μl) were diluted in 10-fold serial dilu-

tions in sterile magnesium phosphate buffer before plating in duplicate onto nutrient agar amended with rifampicin and cycloheximide at 50 $\mu\text{g}/\text{ml}$. Characteristic *X. axonopodis* pv. *allii* colonies were enumerated after 72 h of incubation at 26°C, and a subset of rifampicin-resistant colonies was 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 once over time.

Epiphytic population studies in field plots. 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 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. The soil type was a Fort Collins loam. A plot of each plant species consisted of four contiguous beds 7.6 m in length. 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 of 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 and 23 July 2004. 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 spectrophotometrically to 10^8 CFU/ml ($\text{OD}_{600} = 0.12$) and then diluted 100-fold in sterile magnesium phosphate buffer.

Approximately 20 to 40 g of asymptomatic leaf material was collected arbitrarily 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 g 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, Norwood, MA). Representative colonies were confirmed as *X. axonopodis* pv. *allii* by physiological and

biochemical tests, and pathogenicity on onion was confirmed 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 asymptotically on dry bean and onion, respectively. In 2003, 14 dry bean and onion fields were monitored, including three onion fields planted to a nonhost (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. In 2004, epiphytic xanthomonad populations were monitored in 20 fields, including five onion fields planted to a nonhost in 2003 (field corn, potato, sugar beet, or winter wheat), nine onion fields planted to dry bean in 2003, and five dry bean fields planted to a nonhost (winter wheat or field corn) in 2003. The number of fields surveyed varied among crop rotations and was low for some crop rotation patterns because we were unable to identify growers in the region following all of the cropping rotation patterns we were interested in monitoring.

Approximately 20 to 40 g 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 in resealable plastic bags and promptly transported to the laboratory on ice for epiphyte enumeration. Four bulked samples (each 5 to 10 g) 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, Norwood, MA) onto modified MXP medium (9) containing 50 $\mu\text{g}/\text{ml}$ kasugamycin, 50 $\mu\text{g}/\text{ml}$ cephalixin, and 50 $\mu\text{g}/\text{ml}$ 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 as specified by the manufacturer (Biolog, Hayward, CA), and pathogenicity to onion or dry bean.

Recovered isolates were characterized further in polymerase chain reaction (PCR) assays with primers specific to most pathogenic xanthomonads (16) or specific to *X. axonopodis* pv. *phaseoli* (3). The

highly conserved putative ATPase gene *hrpB6* was amplified from genomic DNA with the oligonucleotide primers RST2 (5'-AGGCCCTGGAAGGTGCCCTGGA-3') and RST3 (5'-ATCGCACTGCGTACCGC-GCGCA-3'), which direct the amplification of an approximately 840-bp fragment (16). A PCR product for this gene is expected from most phytopathogenic xanthomonads, but is not expected from non-pathogenic xanthomonads that lack the *hrp* gene cluster. Isolates were identified as pathogenic *X. axonopodis* pv. *phaseoli* by PCR with primers X3k (5'-GCTGTTGATCGCGCCGCCGTACC-3') and X4e (5'-CGCCGGAAGCACGATCCTCGAAG-3'), which under the stringent conditions described by Audy et al. (3) direct the amplification of an approximately 1,150-bp fragment only from *X. axonopodis* pv. *phaseoli*. DNA was amplified in reaction mixtures containing 12.5 µl of Promega (Madison, WI) PCR Master Mix, 1 µM of each primer, 50 ng DNA, and PCR-grade water to a total volume of 25 µl. PCR amplification cycle conditions were as described by Leite et al. (16) or Audy et al. (3) using a three-step thermal profile. 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, 1 mM EDTA, pH 8.2), stained with ethidium bromide (0.5 µg/ml), and visualized with a UV transilluminator (Fotodyne, Hartland, WI).

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). The area under the disease progress curve was calculated for each treatment and used as the response variable. Replication of the entire experiment was considered a fixed effect in the model, but was not significant ($P = 0.176$). Therefore, means are pooled over both replications of the experiment. 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 analyzed as a completely randomized design, where an entire field following the same crop rotation pattern was considered an experimental unit.

RESULTS

Epiphytic population assays in growth chamber trials. Epiphytic populations of *X. axonopodis* pv. *allii* strain R-O177 increased on all hosts evaluated in warm, humid conditions favorable to the pathogen (Fig. 1). Host and sample date affected the culturable epiphytic populations ($P < 0.0001$). Epiphytic populations (CFU/g fresh weight) 4 days after inoculation were 1.67×10^4 , 3.03×10^5 , 4.32×10^5 , 5.5×10^5 , 7.90×10^5 , and 2.17×10^6 for chickpea, lentil, soybean, dry bean, alfalfa, and onion, respectively. Epiphytic populations increased 3.05 logarithmic units per gram fresh weight of plant tissue on onion over the time of the experiment, which was at least 1.0 logarithmic unit greater than for the leguminous plants evaluated. This re-

sult suggested that 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 per gram fresh weight of tissue on soybean and chickpea, respectively, despite favorable temperatures and high relative humidity.

Epiphytic population studies in field plots. Crop species and sampling date were significant effects on epiphytic populations of *X. axonopodis* pv. *allii* in the mixed model ($P = 0.029$ and <0.0001 , respectively) where replication and year were considered random factors. In 2003, culturable epiphytic populations 33 days after inoculation (239 day of year) varied among crop species. Epiphytic populations decreased at least 0.8 logarithmic units per gram fresh weight on leaves of leguminous species and increased 2.72 logarithmic units per gram fresh weight on onion leaves (Fig. 2A). *X. axonopodis* pv. *allii* populations on onion increased on six of seven sampling dates, and 3.21×10^7 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 8.29×10^2 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 plant species except onion (Fig. 2B), which did not change significantly. Fifty-three days after inoculation (258th day of the year), epiphytic populations varied from undetectable (chickpea and soybean) to greater than 2.10×10^5 CFU/g fresh weight of leaves (onion). Final bacterial populations on dry bean and lentil did not differ from those on the first sampling date, suggesting that the bacterium persisted but did not multiply on these species. *X. axonopodis* pv. *allii* populations on onion were greater on all subsequent sampling dates than on the first sampling date, but varied from 2.10×10^5 to 3.66×10^7 CFU/g fresh weight of leaves among sampling dates and tended to decrease throughout the season after the 220th day of the year.

Recovery of epiphytic *X. axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* from commercially produced onion and dry bean. In 2003, epiphytic *X. axonopodis* pv. *phaseoli* was recovered on at least one sampling date in all five dry bean fields preceded by dry bean, both dry bean fields preceded by winter wheat, and all four onion 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. axono-*

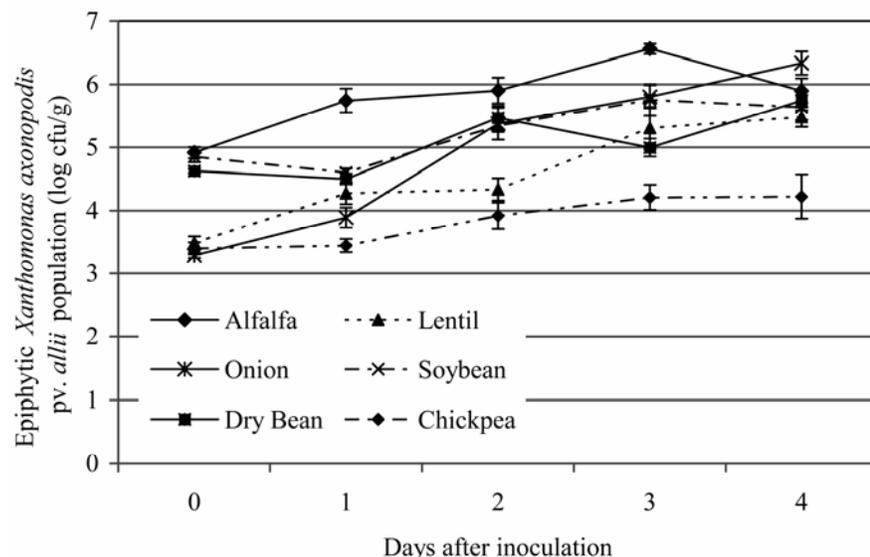


Fig. 1. Epiphytic population dynamics of *Xanthomonas axonopodis* pv. *allii* strain R-O177 on leguminous hosts and onion under high humidity and temperature growth chamber conditions. Data are means of four replications repeated over two experiments ($n = 8$) \pm standard error of the mean.

podis pv. *allii* and nonpathogenic xanthomonads were not identified from isolates recovered from any fields sampled.

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

In 2004, epiphytic *X. axonopodis* pv. *phaseoli* was recovered on at least one sampling date from seven of nine onion fields planted to dry bean the previous season, and culturable xanthomonad populations ranged from 0 to 9.51×10^3 CFU/g of leaves among fields and sampling dates. Crop rotation was again a significant effect in the mixed model (Table 1), indicating epiphytic xanthomonad populations on onion and dry bean varied among crop rotation patterns. *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 (Fig. 4). Epiphytic xanthomonads were recovered from onion preceded by nonhosts in two fields sampled (5.01×10^2 and 1.00×10^2 CFU/g leaves), but these xanthomonads were not pathogenic to dry bean or onion. In PCR experiments, these strains did not yield DNA products with primers RST2/RST3 or X3k/X4e, indicating they lack an essential element of the Type III secretion system and are nonpathogenic to dry bean, respectively. Therefore, we assumed these strains were nonpathogenic xanthomonads.

Epiphytic xanthomonad populations on dry bean and onion varied among years. These populations on onion preceded by dry bean were not significantly different from those on onion preceded by winter wheat in the first year of this study, but were greater in 2004 (Table 2). In 2003, but not in 2004, epiphytic populations on dry bean preceded by winter wheat were greater than 0 ($\alpha = 0.1$).

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*

dis 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 United States do not incite common bacterial blight on leguminous crops (1,10,26), and *X. axonopodis* pv. *phaseoli* does not

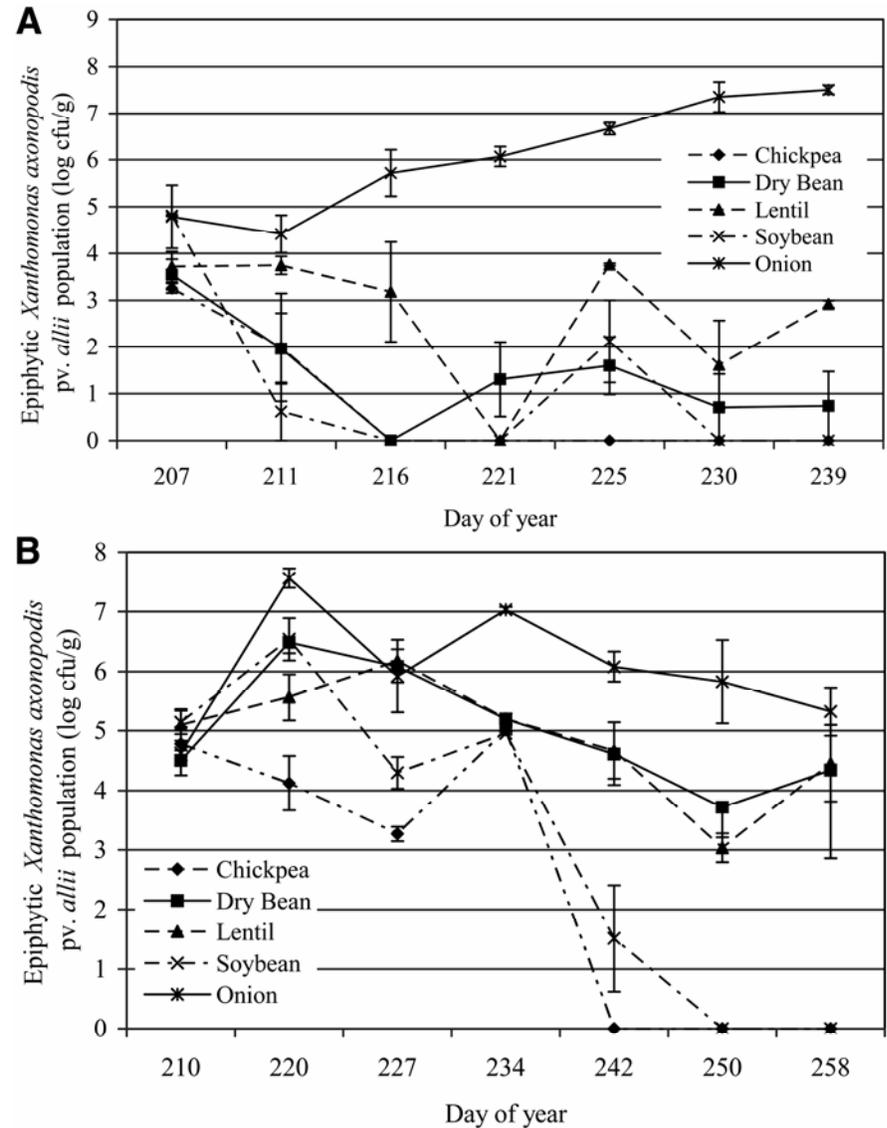


Fig. 2. Epiphytic population dynamics of *Xanthomonas axonopodis* pv. *allii* strain R-O177 on leguminous hosts and onion in inoculated experimental plots near Fort Collins, CO, in 2003 (A) and 2004 (B). Plots were inoculated with a 10^6 CFU/ml suspension of *X. axonopodis* pv. *allii* strain R-O177 on 25 July 2003 or 23 July 2004. Data are means of four replications ($n = 4$) \pm standard error of the mean.

Table 1. Type 3 tests 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*sampling time	12	40	0.79	0.6592
2004				
Rotation	2	17	5.87	0.0115
Sampling time	3	51	1.07	0.3691
Rotation*sampling time	6	51	0.55	0.7709

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 that *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, suggest-

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

Survival of *X. axonopodis* pv. *allii* on several leguminous plant species under conditions of high humidity in a growth chamber is not surprising. Under greenhouse conditions, *X. axonopodis* pv. *phaseoli* is capable of colonizing field corn, sugar beet, *Chenopodium album* (common lamb's-quarters), *Amaranthus retroflexus* (redroot pigweed), and *Echi-*

nochloa 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 that *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 and 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/g of tissue in an experimental unit. Therefore, it is possible that small, undetected populations of the pathogen were present on chickpea and soybean on sampling dates in which no epiphytes were recovered. Even so, 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 than in 2003, and this may be related to weather conditions. A 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 and 3.5 cm. Mean daily high and low temperatures each month were nearly 5°C and 3°C higher in 2003 than in 2004, respectively. The role of temperature and moisture in colonization and persistence of *X. axonopodis* pv. *allii* on hosts and nonhosts is

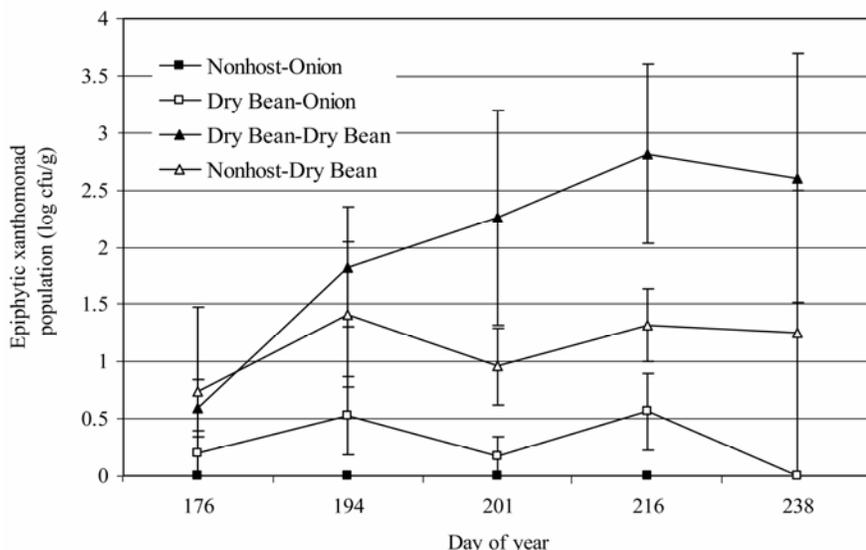


Fig. 3. Epiphytic xanthomonad populations in 2003 on dry bean and onion in relation to cropping system. Epiphytic populations were recovered by a leaf-rinse assay of bulked leaf samples collected on 25 June, 13 July, 4 August, and 26 August by plating onto modified MXP medium (9). Representative xanthomonad colonies were confirmed as a *Xanthomonas* species by physiological and biochemical tests, Biolog substrate utilization profiles, polymerase chain reaction (PCR) assays with primers specific to most pathogenic xanthomonads (16) or *X. axonopodis* pv. *phaseoli* (3), and pathogenicity to onion or dry bean. Nonhost refers to rotation crops that are not hosts of *Xanthomonas axonopodis* pv. *allii* or *X. axonopodis* pv. *phaseoli*, namely winter wheat. The number of fields assayed for epiphytic populations varied among crop rotations: $n = 3$ for nonhost-onion, $n = 4$ for dry bean-onion, $n = 2$ for nonhost-dry bean, and $n = 5$ for dry bean-dry bean rotations. Data are means \pm standard error.

Table 2. Least square mean (LSM) estimates, significance, and confidence limits for culturable epiphytic xanthomonad populations in relation to crop rotation^x

Rotation ^y	df	LSM estimate	<i>t</i> value	Prob <i>t</i> > 0 ^z	LSM 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
Nonhost-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

^x Values were calculated as logarithmic CFU/g fresh weight of leaves.

^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.

unclear, but these factors clearly influence the epiphytic behavior of many phytopathogenic and nonpathogenic bacteria (13,15,17,22). Identification of the conditions enabling or limiting *X. axonopodis* pv. *allii* colonization and persistence in the phyllosphere is necessary to determine if the seasonal population dynamics observed in this study are related to meteorological conditions or other factors such as colony aggregation and stress tolerance (19,20).

In commercial dry bean and onion fields, *X. axonopodis* pv. *phaseoli* was recovered consistently from onion following dry bean in rotation, but not 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 plant species are unlikely to support large epiphytic populations of *X. axonopodis* pv. *phaseoli* under field conditions. Under semi-arid conditions in Colorado, only nonpathogenic xanthomonads were recovered from onion following a nonhost from one field and 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 that the dry bean pathogen was present in

the dry bean crop, overwintered 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 in general, host-specific xanthomonads do not persist epiphytically on a 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 it 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 the infested debris (11). Deep incorporation of onion crop debris would likely prevent *X. axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *allii* 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 suggests the onion 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. This finding is consistent with other reports that indicate that *X. axonopodis* pv. *allii* is nonpathogenic to leguminous plants (1,10,26). 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 require more investigation.

Epiphytic survival of multiple pathovars of *X. axonopodis* on the same host may increase the risk of bactericide resistance developing in these bacteria. 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 (5,18,35). 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.

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 bean and encourage rapid breakdown of crop residues should reduce potential inoculum sources of both pathogens in the Central High Plains.

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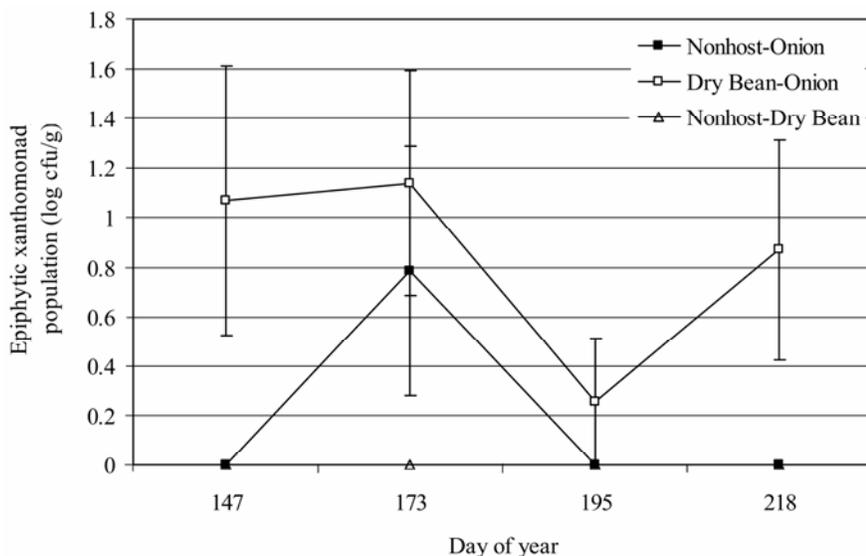


Fig. 4. Epiphytic xanthomonad populations in 2004 on dry bean and onion in relation to cropping system. Epiphytic populations were recovered by a leaf-rinse assay of bulked leaf samples collected on 26 May, 21 June, 13 July, and 5 August by plating onto modified MXP medium (9). Representative xanthomonad colonies were confirmed as a *Xanthomonas* species by physiological and biochemical tests, Biolug substrate utilization profiles, polymerase chain reaction (PCR) assays with primers specific to most pathogenic xanthomonads (16) or *X. axonopodis* pv. *phaseoli* (3), and pathogenicity to onion or dry bean. 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. Xanthomonads recovered from onion preceded by a nonhost were nonpathogenic to onion and dry bean, and did yield PCR products with primers specific to most pathogenic xanthomonads (16) or *X. axonopodis* pv. *phaseoli* (3). The number of fields assayed for epiphytic populations varied among crop rotations: $n = 5$ for nonhost-dry bean, $n = 6$ for nonhost-onion, and $n = 9$ for dry bean-onion rotations. Data are means \pm standard error.

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