Rates of Epiphytic Growth of *Erwinia amylovora* on Flowers Common in the Landscape

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ABSTRACT

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We evaluated epiphytic growth of the fire blight bacterium, *Erwinia amylovora*, on flowers of plant species common to landscapes where pears and apples are grown. The plants were from genera regarded as important nectar and pollen sources for pollinating insects: *Acer*, *Amelanchier*, *Brassica*, *Cytisus*, *Populus*, *Prunus*, *Rubus*, *Salix*, *Taraxacum*, *Trifolium*, and *Symphoricarpos*. Floral bouquets were inoculated with *E. amylovora* and incubated in growth chambers at 15°C for 96 h. Regardless of their susceptibility to fire blight, all species from the rose family except *Prunus domestica* (European plum) supported epiphytic populations of *E. amylovora* that exceeded 1×10^6 CFU/flower with relative growth rates for the populations that averaged 7% per hour. Nonrosaceous plants were generally poor supporters of epiphytic growth of the fire blight pathogen with relative growth rates averaging <4% per hour. In two seasons of field inoculations, the rosaceous non-disease-host plants, *Prunus avium* (sweet cherry) and *Rubus armeniacus* (Himalayan blackberry), yielded mean population sizes of *E. amylovora* that exceeded 1×10^6 CFU/flower; in contrast, at 8 days after inoculation, mean population sizes of the pathogen were in the range of 5×10^3 to 5×10^4 CFU/flower on *Cytisus scoparius* (Scotch broom) and $<1 \times 10^2$ CFU on *Acer macrophyllum* (big leaf maple). Because vectors of *E. amylovora*, principally bees, visit many kinds of flowers in landscape areas between pear and apple orchards, flowers of rosaceous, non-disease-host species could serve as potential sites of inoculum increase during their periods of bloom.

Additional keywords: *Malus pumila*, *Pyrus communis*

Fire blight, caused by the bacterium *Erwinia amylovora*, is a serious disease of pear, apple, and other plants of the rose family (21). Commonly, this disease is initiated when *E. amylovora* enters the host via natural openings in the floral nectary (23). Prior to infection, *E. amylovora* grows as an epiphyte on the floral stigma (4,16,17,22), where it can greatly increase its population size. The large population size enhances the likelihood of floral infection and of further movement to noncolonized flowers (4,5).

Knowledge of the epiphytic nature of the fire blight pathogen on host stigmas has been instrumental in the development of models for timing chemical and biological sprays that suppress floral infection (13,14,18). Environmental conditions favorable for floral infection are relatively easy to quantify and index (13,14,18), but index thresholds that trigger a management action generally assume that pathogen inoculum is present. Frequently this as-

sumption is invalid, which contributes to inaccurate forecasts of infection events. Direct monitoring of orchards for epiphytic *E. amylovora* is not practiced widely owing to the cost of sampling and to the rapidity at which epiphytic populations can change (8). Adjustment of thresholds for expected, non-weather-related variation in disease risk, however, has proven useful. Example adjustments are for cultivar susceptibility and the disease history of an orchard (13).

With regard to disease expectations, a consistent observation that we (and others [1,2]) have made in the northwestern United States concerns the association of fire blight with later as opposed to earlier timings of bloom. Factors affecting bloom timing include crop species and cultivar, orchard elevation, and importantly, the presence or absence of secondary types of bloom (2). A higher incidence of fire blight with later bloom timings is explained partially by a greater frequency of warm temperatures as summer approaches; however, this reason for a seasonal effect on fire blight risk is accounted for by current disease warning models. We hypothesized that increasing disease risk is also related to greater abundance of pathogen inoculum, but knowledge of the factors that contribute to inoculum abundance are not well documented.

E. amylovora overwinters in host branches diseased in the previous season, but old cankers are often very difficult to locate and observe once winter pruning and sanitation activities have occurred. Nonetheless, on a spatial scale that is poorly understood, the annual disease cycle is initiated when a small number of cells of the pathogen are moved from a canker to the surfaces of a few flowers. Once introduced to flowers, the pathogen can undergo many cycles of reproduction and dissemination without a requirement to reenter a host. Because many bacterial epiphytes of flowers are not pathogens (16), we became interested in understanding the degree to which *E. amylovora* could be a floral epiphyte of plants that do not normally host the disease. In fruit production areas, plant species with some stage of bloom can be present for periods approaching 10 weeks, and bees and other pollinating insects visit many kinds of flowers in the vicinity of pear and apple orchards. Thus, as the spring season progresses, the diversity and number of floral habitats where an epiphyte could reside expand, which in the case of *E. amylovora* could contribute to a seasonal bias in timing of fire blight infections. The objective of this study was to evaluate the epiphytic capability of *E. amylovora* on flowers of plant species common to landscapes where pears and apples are grown.

MATERIALS AND METHODS

Bacterial strains. Spontaneous selections derived from *E. amylovora* strain 153 (Ea153) were used in all experiments. Ea153 was first isolated in 1989 from a diseased apple branch from Milton-Freewater, OR. Growth chamber studies in 2002 and 2003 were conducted with strain Ea153N, which is resistant to nalidixic acid. Growth chamber studies in 2004 and field studies in 2004 and 2005 were conducted with strain Ea153S, which is resistant to streptomycin sulfate. Both Ea153N and Ea153S have shown growth characteristics and pathogenicity similar to the parental strain (K. B. Johnson, *unpublished*). Bacteria were prepared for experiments by culturing lawns of each strain on nutrient agar (Difco Laboratories, Detroit, MI) amended with 1% (wt/vol) glycerol. After 5 days incubation at 20 to 24°C, the bacteria were harvested with a spatula and lyophilized (5,15). Dried cultures were ground with a mortar and pestle and

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screened to a final mesh size of 0.3 mm². Preparations of lyophilized bacteria were stored at -50°C; titers of these preparations averaged 1 to 2 × 10¹¹ CFU/g and were reevaluated periodically by serial dilution plating.

Selection of plant species. The reference *Plants for Beekeeping in Canada and the Northern United States* (11), was used as a guide to the selection of flowers that were investigated in growth chamber and field experiments. This book lists important nectar and pollen sources for bees in the major ecoregions across the North American continent. Plant genera listed for ecoregions of northwestern Canada and the United States included *Acer*, *Amelanchier*, *Brassica*, *Salix*, *Cytisus*, *Malus*, *Populus*, *Prunus*, *Rubus*, *Salix*, *Symphiocarpus*, *Taraxacum*, and *Trifolium*. Floral bouquets of species from these genera were collected from managed and natural landscapes near Corvallis, OR (Table 1). Flowers from four additional genera that grow commonly in western Oregon also were collected: *Crataegus*, *Rhododendron*, *Rosa*, and *Vaccinium*.

Growth chamber experiments. Flowers of selected species were sampled as each plant bloomed during the spring seasons of 2002 to 2004. At the time of collection, blossom-bearing branches were placed in plastic pails with the proximal end of the branch submerged in water. Newly opened blossoms were harvested with the pedicel (or in some cases, the peduncle) intact and placed onto 80-well, polypropylene microcentrifuge tube racks (Fisher Scientific, Pittsburgh, PA) with the proximal end of the pedicel submerged into a well that contained 2 ml of 10% (wt/vol) sucrose (6).

Inoculation of flowers followed a standard protocol, although variation in size and morphology among the species resulted in differences in the sample unit used to measure the potential for epiphytic growth of *E. amylovora* (Table 1). Suspensions of Ea153N or Ea153S were prepared by resuspending a freeze-dried preparation in distilled water to achieve a concentration of 1 × 10⁷ CFU/ml. These suspensions were misted onto blossoms to near runoff using a reagent atomizer (Kontes Glass Co., Vineland, NJ). Racks holding inoculated flowers were transferred to 6-liter plastic, lidded boxes (Rubbermaid, Wooster, OH), the floors of which were flooded with 500 ml of a 40% glycerol solution (v/v) to maintain a relative humidity of 93 to 94% (6). Boxes containing the racks of blossoms were then incubated for 96 h in nonlighted growth chambers (VWR Scientific Model 2105, Sheldon Mfg., Inc., Cornelius, OR) set to 15°C. Two or three replicates of each plant species were inoculated each season; 8 (2002 and 2003) or 12 (2004) flowers per replicate were washed at each sampling time. In 2004, half of the blossoms were inoculated with Ea153S suspended in water. For the other half, the pathogen inoculum was resuspended in a solution containing 100 µg/ml streptomycin sulfate. The purpose of this amendment was to suppress potential competition from indigenous bacterial epiphytes that were residing on the field-grown flowers (7,16).

The population size of *E. amylovora* on the sampled floral organs was determined by dilution plating at sampling times of 0 and 96 h after inoculation. Floral pistils from individual flowers (Table 1) were removed and placed into 1 ml of sterile 10

mM phosphate buffer, pH 7.0. After sonication (2 min) to dislodge the bacteria from surfaces, the wash buffer and two serial 100-fold dilutions were spread onto CCT medium (defined by Ishimaru and Klos, 3) amended with nalidixic acid (50 µg/ml) for selective recovery of Ea153N or streptomycin sulfate (100 µg/ml) for recovery of Ea153S. Colonies of *E. amylovora* were counted after 3 or 4 days incubation at 20 to 24°C. The detection limit of the dilution plating procedure was 1 × 10² CFU/flower.

In 2004 experiments, for each replicate, the wash from three blossoms that received Ea153S in water also was dilution plated onto *Pseudomonas* agar F (PAF, Difco Laboratories, Detroit, MI). This medium was used to measure the total population size of culturable bacteria on the floral surfaces (Ea153N and indigenous bacterial epiphytes).

Field inoculations. Based on results of the growth chamber experiments, sweet cherry, big leaf maple, Scotch broom, and Himalayan blackberry were selected to evaluate their ability to support epiphytic growth of *E. amylovora* under field conditions at sites near Corvallis, OR. In 2004 and 2005, as each species bloomed, a total of 9 stems (on one to several plants) were flagged; newly open flowers on these stems were marked by securing a small piece of colored tape around the pedicel. Suspensions of Ea153S (1 × 10⁷ CFU/ml) were prepared as described above and misted onto blossoms to near runoff with a handheld, adjustable trigger sprayer (0.5-liter capacity). Three stems were inoculated with Ea153S resuspended in distilled water, and three received the same inoculum resuspended in a solution of strepto-

Table 1. Plants species selected to evaluate their potential to support epiphytic growth of *Erwinia amylovora* on stigmatic surfaces

Plant family	Botanical name ^a	Common name ^a	Inflorescence type	Flowers per inflorescence	Part(s) of individual flower washed
Aceraceae	<i>Acer macrophyllum</i> Pursh.	Big leaf maple	Raceme	~65	Pistil
Caprifoliaceae	<i>Symphoricarpos albus</i> (L.) Blake	Common snowberry	Raceme	8–11	Stigma, style
Compositae	<i>Taraxacum officinale</i> G. H. Webber ex Wiggers	Common dandelion	Capitulum	~100	Whole flower
Cruciferae	<i>Brassica rapa</i> L.	Field mustard	Raceme	4–6	Stigma, style
Ericaceae	<i>Vaccinium corymbosum</i> L.	Highbush blueberry	Raceme	7–10	Pistil, hypanthium
Ericaceae	<i>Rhododendron macrophyllum</i> D. Don ex G. Don	Pacific rhododendron	Corymb	1–14	Stigma, style
Fabaceae	<i>Cytisus scoparius</i> (L.) Link	Scotch broom	Solitary	1	Pistil
Leguminosae	<i>Trifolium repens</i> L.	White clover	Raceme	~50	Whole flower
Rosaceae	<i>Amelanchier alnifolia</i> (Nutt.) Nutt. ex M. Roemer	Saskatoon serviceberry	Raceme	10–15	Pistils, hypanthium
Rosaceae	<i>Crataegus douglasii</i> Lindl.	Black hawthorn	Corymb	12	Pistil, hypanthium
Rosaceae	<i>Malus pumila</i> P. Mill.	Apple	Cyme	5	Pistils
Rosaceae	<i>Prunus avium</i> (L.) L.	Sweet cherry	Corymb	5–7	Pistil
Rosaceae	<i>Prunus persica</i> (L.) Batsch	Peach	Solitary	1	Pistil
Rosaceae	<i>Prunus domestica</i> L.	European plum	Fasicle	2–5	Pistil, hypanthium
Rosaceae	<i>Rosa nutkana</i> K. Presl	Nootka rose	Solitary	1	Stigmas, styles
Rosaceae	<i>Rubus armeniacus</i> Focke	Himalayan blackberry	Cyme	3–6	Pistils
Salicaceae	<i>Salix hookeriana</i> Barratt ex Hook.	Dune willow	Catkin	~100	Pistil
Salicaceae	<i>Salix × sepulcralis</i> Simonkai [alba × ?pendulina]	Weeping willow	Catkin	~80	Pistil
Salicaceae	<i>Populus deltoides</i> Bartr. ex Marsh.	Eastern cottonwood	Catkin	~30	Stigmas, styles

^a USDA PLANTS database (20).

mycin sulfate (100 µg/ml). The last three stems were sprayed with distilled water only. Dates of field inoculations in 2004 were 29 March for sweet cherry and big leaf maple, 10 May for Scotch broom, and 25 May for Himalayan blackberry. In 2005, the inoculation dates were 31 March, 11 and 28 April, and 14 June for cherry, maple, broom, and blackberry, respectively. For an inoculation event, the relative favorability of the environment for epiphytic growth of *E. amylovora* was summarized by estimating the total degree-hours base 10°C (°h) over the 8-day period. Degree-hours were estimated from daily minimum and maximum temperatures following a method we developed previously (6).

Flowers were sampled from the field sites at 0, 4, and 8 days after inoculation to measure the population size of Ea153S and associated indigenous epiphytes on the surfaces of pistils. Ten flowers per stem replicate were sampled at each sampling time. The flowers were processed and washed as described above and then diluted onto PAF and onto CCT amended with streptomycin sulfate (100 µg/ml).

Weather data were obtained from a station located at an experimental field site near Corvallis, OR. The station is part of the AgriMet Pacific Northwest Cooperative Agricultural Weather Network (U.S. Bureau of Reclamation, Boise, ID); the data are available at their website.

Data summary and analysis. Mean population sizes of strains of Ea153 were

summarized for each sample date. Prior to averaging, estimates of bacterial population size on individual blossoms were transformed to log₁₀ (6). Blossoms sampled from the field sites onto which *E. amylovora* was inoculated but could not be detected were assigned a value of one-half of the detection limit (i.e., 50 CFU/flower).

For comparisons among plant species, a relative growth rate for *E. amylovora* (proportional increase in population size per hour, *r*) was computed for each replication of the growth chamber experiments:

$$r = 1/96 \cdot [\ln(y_{96}) - \ln(y_{zero})]$$

where *y*_{zero} and *y*₉₆ are bacterial population sizes near the time of inoculation and after 96 h, respectively (6). Replicate values of the relative growth rate were subjected to analysis of variance (ANOVA) with plant species as the main effect. Ea153N replicates from 2002 (two) and 2003 (three) were combined for this analysis. For the 2004 experiment, a two-way ANOVA was performed initially to test the effects of plant species and the presence of streptomycin on the relative growth rate of Ea153S; when no effect of streptomycin was observed, a second ANOVA was conducted with plant species as the only main effect. Means for relative growth rate were compared among species with Fisher's protected least significant difference at *P* = 0.01.

For the field studies, environmental conditions following the inoculations were summarized for each plant species by tabu-

lating the frequency of recovery and population size of indigenous bacteria on the water-treated flowers as well as rainfall amount and degree-hours accumulated during the 8-day postinoculation periods. Within each plant species, the null hypothesis that treatment with streptomycin had no effect on frequency of recovery or population size of Ea153S was evaluated by ANOVA. Separate ANOVA analyses were conducted to determine if the main effect of plant species affected frequency of recovery or population size of the pathogen.

RESULTS

Growth chamber experiments. Inflorescence type and flower size varied among the plant species (Table 1), and this variation resulted in initial population sizes of *E. amylovora* on each species that ranged from 5 × 10² to 5 × 10⁴ CFU/flower. After 96 h of incubation, all plant species hosted higher population sizes of *E. amylovora* compared with the populations measured immediately after inoculation (Table 2). Among plant species, final mean population sizes of *E. amylovora* varied from 8 × 10³ (on weeping willow) to 8 × 10⁷ CFU/flower (on Himalayan blackberry in 2002 and 2003 and Nootka rose in 2004). Plants of the rose family—apple, Himalayan blackberry, Saskatoon serviceberry, sweet cherry, black hawthorn, peach, and Nootka rose—each supported mean populations of the fire blight pathogen that exceeded 1 × 10⁶

Table 2. Initial population size (log₁₀[CFU/flower])^a, final population size after incubation at 14°C for 96 h, and estimated relative growth rate of *Erwinia amylovora* strain 153N (2002–2003) or strain 153S (2004) on detached flowers of 19 common plant species sampled from areas near Corvallis, OR, during spring seasons of 2002–2004

Host	2002–2003			2004		
	Initial population size (log ₁₀ [CFU/flower])	Final population size (log ₁₀ [CFU/flower])	Growth rate (%/h)	Initial population size (log ₁₀ [CFU/flower])	Final population size (log ₁₀ [CFU/flower])	Growth rate (%/h)
Apple	3.60 ± 0.05 ^b	7.59 ± 0.11	9.6 ^c ± 0.2	4.30 ± 0.05	7.42 ± 0.13	7.5 ± 0.8
Saskatoon serviceberry	3.26 ± 0.30	7.19 ± 0.14	9.4 ± 0.4	4.65 ± 0.10	7.28 ± 0.25	6.3 ± 1.6
Common snowberry	2.46 ± 0.15	6.13 ± 0.08	8.9 ± 0.2	3.81 ± 0.13	4.57 ± 0.16	4.3 ± 0.4
Himalayan blackberry	4.21 ± 0.18	7.81 ± 0.11	8.6 ± 0.5	4.46 ± 0.22	7.20 ± 0.26	6.6 ± 0.6
Black hawthorn	3.24 ± 0.01	6.63 ± 0.23	8.1 ± 0.5	4.17 ± 0.12	7.14 ± 0.15	7.1 ± 1.2
Sweet cherry	3.26 ± 0.11	6.35 ± 0.05	7.4 ± 0.3	3.59 ± 0.06	6.24 ± 0.09	6.4 ± 0.8
Scotch broom	3.89 ± 0.05	6.87 ± 0.15	7.1 ± 0.4	3.55 ± 0.07	6.94 ± 0.25	8.1 ± 1.5
Peach	3.70 ± 0.06	6.64 ± 0.21	7.0 ± 0.6	3.77 ± 0.05	6.60 ± 0.20	6.8 ± 1.2
Nootka rose	4.14 ± 0.03	6.91 ± 0.17	6.7 ± 0.4	4.14 ± 0.04	7.75 ± 0.04	8.2 ± 0.5
European plum	3.75 ± 0.11	5.82 ± 0.17	5.0 ± 0.4	3.80 ± 0.02	5.99 ± 0.10	5.3 ± 0.3
Highbush blueberry	2.76 ± 0.07	4.72 ± 0.19	4.7 ± 0.3	3.81 ± 0.12	5.59 ± 0.10	4.2 ± 0.5
Dune willow	3.62 ± 0.17	5.40 ± 0.34	4.3 ± 0.6	3.59 ± 0.26	4.71 ± 0.31	2.7 ± 1.1
Eastern cottonwood	3.91 ± 0.06	5.66 ± 0.07	4.2 ± 0.3	3.92 ± 0.06	6.08 ± 0.29	5.2 ± 0.8
Big leaf maple	3.74 ± 0.05	5.45 ± 0.40	4.1 ± 0.8	4.43 ± 0.10	6.22 ± 0.13	4.3 ± 0.4
Field mustard	2.93 ± 0.16	4.53 ± 0.43	3.8 ± 0.7	3.20 ± 0.03	4.37 ± 0.29	2.8 ± 0.8
Weeping willow	2.95 ± 0.09	3.75 ± 0.42	1.9 ± 0.8	3.24 ± 0.10	3.89 ± 0.28	1.6 ± 0.8
Pacific rhododendron	4.10 ± 0.08	4.69 ± 0.30	1.4 ± 0.9	4.03 ± 0.07	5.29 ± 0.24	3.0 ± 0.6
Common dandelion	3.61 ± 0.26	4.15 ± 0.72	1.1 ± 1.3	3.18 ± 0.05	5.36 ± 0.10	5.2 ± 0.3
White clover	4.04 ± 0.07	4.38 ± 0.20	0.9 ± 0.5	3.86 ± 0.10	5.47 ± 0.11	3.8 ± 0.4
LSD <i>P</i> = 0.01 ^d			2.3			1.6

^a For each season, flowers were misted to near runoff with cells of *E. amylovora* (1 × 10⁷ CFU/ml) resuspended from the same freeze-dried culture of the pathogen; individual dates of inoculation corresponded to the bloom time of each species.

^b Standard error of mean; based on 5 (2002–2003) or 6 (2004) replicates of 6 (2004) or 8 (2002–2003) individual flowers per replication.

^c Growth rate calculated as: $r = \{1/96[\ln(y_{96}) - \ln(y_{zero})]\} \cdot 100$, where *y*_{zero} and *y*₉₆ were the replicate means for population size of *E. amylovora* (CFU/flower) near the time of inoculation and after 96 h, respectively.

^d Fisher's protected least significant difference at *P* = 0.01.

CFU/flower during each experiment. For nonrosaceous plants, high mean populations of *E. amylovora* ($>1 \times 10^6$ CFU/flower) were recovered from Scotch broom in each season of experimentation, and from flowers of common snowberry, big leaf maple, and eastern cottonwood in one of the inoculation experiments. In 2004, relative to inoculum prepared by resuspending streptomycin-resistant strain

Ea153S in water, the addition of streptomycin sulfate (100 µg/ml) to inoculum of Ea153S had no effect on the number of colony-forming units of the pathogen recovered at either 0 or 4 days after inoculation. For flowers that received Ea153S in water, faster growth of recovered colonies of the pathogen on the nonselective PAF resulted in colony numbers that averaged 95% of those obtained on the selective, colony-size-restricting CCT medium. Also on PAF, the incidence of bacteria other than Ea153S was observed only rarely.

As for final population size, the relative growth rate of *E. amylovora* on flowers varied greatly among the plant species (Table 2), and ranged from 0.9 %/h (on white clover in 2002 and 2003) to 9.6 %/h (on apple in 2002 and 2003). As a group, rosaceous plants supported significantly higher ($P < 0.01$) relative growth rates of the fire blight pathogen on flowers (mean 7.3 %/h) than did nonrosaceous plants (mean 4.0 %/h) (Fig. 1). For nonrosaceous plants, relative growth rates of *E. amylovora* comparable to the mean growth rate on rosaceous plants were observed only on Scotch broom (7.1 and 8.1 %/h in 2002 and 2003, respectively, and 8.1 %/h in 2004) and snowberry (8.1 %/h in 2002 and 2003).

Field inoculations. Field inoculations of Ea153S onto newly open flowers of sweet cherry, big leaf maple, Scotch broom, and Himalayan blackberry were timed to coincide with periods when temperatures were expected to allow for epiphytic growth of bacteria on floral surfaces. For 7 of 8 inoculations, we concluded these timings were successful with total degree-hour accumulations (base 10°C) over the 8-day postinoculation periods that ranged from 193 °h after inoculation of sweet cherry in 2005 to 1002 °h after inoculation of Himalayan blackberry in 2005 (Table 3). In contrast, after inocu-

lation of big leaf maple in 2005, only 68 °h were accumulated over the 8-day period. Rain events occurred during each of the postinoculation periods, with larger amounts of rain recorded in 2005 than in 2004; the most rain (28 mm) occurred after the inoculation of big leaf maple in 2005.

Averaged over host and sampling time, indigenous bacteria were recovered from 60% of sampled flowers with mean population sizes (per host per sample date) that ranged from 1.0×10^2 to 1.5×10^4 CFU/flower (Table 3). Compared with flowers inoculated with Ea153S resuspended in water, the addition of streptomycin sulfate to inoculum of the pathogen generally resulted in a 10 to 20% decrease in both the incidence of recovery and the population size of indigenous bacteria and a corresponding small (5 to 10%) but generally insignificant ($P \geq 0.05$) increase in the recovery and population size of Ea153S (data not shown). An exception was Scotch broom in 2005, where at 8 days after inoculation, the addition of streptomycin to the initial inoculum increased the incidence of recovery of Ea153S by 11-fold (from 8 to 92%) and the mean population size of the pathogen by a similar magnitude (from 1.81 to 3.11 $\log_{10}$ (CFU/flower)). As a consequence of this result, pathogen incidence and population size data from the treatment of Ea153S resuspended with streptomycin sulfate were used to make comparisons among the inoculated host species.

As in the growth chamber experiments, large populations of Ea153S developed on floral stigmas of the rosaceous plants, sweet cherry and Himalayan blackberry (Fig. 2). For these hosts, initial populations of Ea153S were in the 2.7 to 4.9 $\log_{10}$ (CFU/flower) range with the estimated population size increasing by approximately 100-fold over the 8-day period. The incidence of recovery of Ea153S

Fig. 1. Estimates of relative growth rates (%/h) of **A**, *Erwinia amylovora* strain 153N (2002–2003 experiments) and **B**, *E. amylovora* strain 153S (2004 experiments) on detached flowers of 8 plant species from the rose family (solid bars) and 11 plant species from other families (hatched bars) (see Table 1). Growth rates were estimated after a 96-h incubation period in growth chambers held at a constant temperature of 14°C. Vertical line on top of each bar indicates one standard error of the mean.

Table 3. Summary of postinoculation weather conditions and of recovery of indigenous culturable bacteria from individual, water-treated^a flowers of sweet cherry, big leaf maple, Scotch broom, and Himalayan blackberry sampled from field sites near Corvallis, OR, in 2004 and 2005

Host flower	Degree-hours during post-inoculation period (base 10°C)	Total rainfall during post-inoculation period (mm)	Days from water application					
			0 days		4 days		8 days	
			Incidence of recovery ^b (%)	Population size ^c (log ₁₀ [CFU/flower])	Incidence of recovery (%)	Population size (log ₁₀ [CFU/flower])	Incidence of recovery (%)	Population size (log ₁₀ [CFU/flower])
2004								
Sweet cherry	366	2.5	0	—	100	2.0	100	4.1
Big leaf maple	366	2.5	83	2.3	17	2.2	50	2.4
Scotch broom	595	6.4	38	2.7	75	2.9	100	3.0
Himalayan blackberry	852	3.0	67	2.4	67	3.2	67	2.9
2005								
Sweet cherry	193	14.0	0	—	78	2.5	78	3.4
Big leaf maple	68	28.4	33	2.6	78	3.3	100	2.6
Scotch broom	783	11.1	11	2.6	33	2.4	78	2.2
Himalayan blackberry	1002	10.2	22	2.8	100	4.5	78	4.6

^a Water treatment coincided with inoculations of *Erwinia amylovora* strain Ea153S onto flowers on other stems of the same plants.

^b Proportion of flowers from which indigenous culturable bacteria were recovered; *Pseudomonas* agar F (Difco, Detroit, MI) was used as the recovery medium.

^c Average population size of indigenous bacteria on flowers on which bacteria were detected; the detection limit was 100 CFU.

from cherry and blackberry also remained high through the postinoculation period, with an average of 94% of inoculated flowers harboring a detectable population of the pathogen after 8 days.

On flowers of big leaf maple, in both seasons, the incidence of recovery and the population size of Ea153S declined over the 8-day postinoculation period. For example, immediately after inoculation, incidence of recovery of Ea153S averaged 95%, but at 8 days after inoculation, Ea153S was recovered from only 15% of inoculated flowers; moreover, when Ea153S was detected at 4 or 8 days after inoculation, population size was generally just above the detection limit of 100 CFU/flower. In contrast, Scotch broom flowers provided an environment where Ea153S was detected on 86% of flowers at 8 days after inoculation. Mean population sizes of Ea153S on Scotch broom, however, did not show a net increase from the beginning to the end of the sampling period. Interestingly, however, in both seasons the population size of Ea153S on Scotch broom showed an apparent decrease at the 4-day sample when compared to the 0-day sample but at 8 days after inoculation showed an apparent increase back towards the initial population size estimate. Nonetheless, at 8 days after inoculation, mean population size of Ea153S on flowers of big leaf maple and Scotch broom were significantly ($P < 0.01$) smaller than estimated population sizes on sweet cherry and Himalayan blackberry.

DISCUSSION

Our rationale for this study was to expand on what is known about the epiphytic biology of *E. amylovora* to contribute to understanding availability of inoculum within a production area with fruit trees susceptible to this pathogen. Reviews of this topic (e.g., 18) conclude that *E. amylovora* is a poor epiphyte on host surfaces other than flowers, requiring a resident phase (active or inactive) inside a host to persist for long periods of time. Within the disease cycle, the epiphytic phase on flowers is highly significant because it allows the pathogen, as a prerequisite to infection, to greatly expand its population size from the small number of cells released from cankers produced in the previous season (4). The floral epiphytic phase also facilitates dispersal, which occurs principally via bees and other pollinators. In this regard, several reports in the literature (1,10) implicate common landscape plants that host fire blight (notably hawthorn [*Crataegus* spp.] and firethorn [*Pyracanthus* spp.]) as sources of inoculum for infection of pear and apple. Nonetheless, to our knowledge, the idea that flowers of common plants that do not typically host the disease could contribute to persistence of epiphytic populations of this pathogen has never been investigated. Consequently, owing to

the significance of insects in the fire blight disease cycle, we limited our scope mostly to common plants that are considered important resources for pollinating insects (11).

This study presented several methodological challenges because the evaluated species bloomed at many different times and produced inflorescences that varied widely in size and morphology. We addressed these challenges by using a freeze-dried preparation of bacterial inoculum that provided a high degree of consistency among inoculations (6,15) and by using a flower-size independent, relative growth rate calculation to make comparisons among species (6). In addition, because the flowers were sampled from field sites, we were concerned that contamination by indigenous bacterial populations could influence the observed rates of growth of *E. amylovora*. This concern was addressed with use of a streptomycin-resistant strain Ea153S combined with a streptomycin sulfate amendment to the inoculum suspension. Previously, in a field experiment designed to understand factors influencing dispersal of *Pantoea agglomerans* among flower populations (7), we found that streptomycin was useful as a tool for suppression of growth of indigenous bacterial populations, and that it had no significant effect on growth or dispersal of the streptomycin-resistant strain of *P. agglomerans* that was the subject of the study. For the experiments reported on in this manuscript, we found that streptomycin did not affect growth of Ea153S on the flowers sampled at a young age and incubated in a growth chamber after inoculation. In at least one field inoculation, however, the niche clearing activity of the streptomycin treatment enhanced both incidence of recovery and population size of Ea153S on flowers that had been open for 4 to 8 days. These observations are consistent with the data of Stockwell et al. (16), who found that indigenous bacteria were recovered rarely from newly open flowers of pear, but that high populations of bacterial epiphytes could develop on the flowers as they aged.

The principal finding of this study is that flowers of plants of the rose family can support large epiphytic populations of *E. amylovora* even if the species does not typically host fire blight infections. These non-disease-host species include orchard crops such as sweet cherry (*Prunus avium*), which often grows in close proximity to pear and apple orchards, and also common weeds like Himalayan blackberry (*Rubus armeniacus*), which grows along edges of orchards and blooms coincident with secondary flowering in pear and apple. Although fire blight of both *Prunus* and *Rubus* spp. has been reported (rarely in the case of *Prunus* spp. [9] and with host-specific strains in the case of *Rubus* spp. [10,12]), this is the first study to measure

epiphytic growth rates of *E. amylovora* on flowers of these genera in controlled inoculations and to compare these growth rates to those observed on genera that commonly host the disease (e.g., *Malus* and *Crataegus* spp.). The results of the growth chamber experiment also indicated that *E. amylovora* has some potential to increase its population size on flowers of nonrosaceous plants, but growth rates varied by species and were on average smaller than observed on rosaceous plants (Fig. 1). For the field experiments, the observed temporal dynamics of growth and ultimate population sizes of *E. amylovora* on sweet cherry and Himalayan blackberry flowers were very similar to those we measured previously on pear and apple flowers inoculated at the same location (16). In contrast, the population sizes of *E. amylovora* on field-inoculated flowers of Scotch broom and big leaf maple were significantly smaller than observed on cherry and blackberry. Also, compared to results obtained with Scotch broom in the growth chamber, the measured population sizes of *E. amylovora* on field-inoculated flowers of this species were smaller than expected.

Epidemiologically, the primary implication of this study is that *E. amylovora* may inhabit a larger epiphytic niche than has been recognized previously. In valley production areas of the northwestern United States, fire blight epidemics in pear and apple are sporadic both temporally and

Fig. 2. Population size ($\log_{10}$ [CFU/flower]) of *Erwinia amylovora* strain 153S on flowers of sweet cherry (■), big leaf maple (○), Scotch broom (Δ), and Himalayan blackberry (◆) sampled at 1 h, 4 and 8 days after inoculation at field sites near Corvallis, OR, in A, 2004 and B, 2005. A suspension of Ea153S (1×10^7 CFU/ml) was misted onto the flowers to near runoff. Vertical line drawn through point indicates $\pm$ one standard error of the mean.

spatially, being limited by host species and elevation factors that influence when flowers are open and susceptible to infection. Moreover, fire blight frequently fails to develop during the primary bloom, but many pear and some apple cultivars (e.g., cv. Pink Lady) develop the disease during secondary, rattail blooms that occur in late spring and early summer (2). Because vectors of *E. amylovora*, principally bees, visit many kinds of flowers in the landscape, our results suggest that epiphytic inoculum sources of *E. amylovora* could become more broadly dispersed within a valley or region as a season transitions (e.g., from pear to apple, lower to higher elevation, primary to secondary bloom). Although we highlight *Rubus* and *Prunus* as non-disease hosts on which the fire blight pathogen could reproduce inconspicuously, landscape species (1,10) that host fire blight and/or an orchard of a fire blight-resistant apple cultivar (e.g., cv. Red Delicious) also could be involved in this broadening process. The practical consequence of this broadening phenomenon is that action thresholds within temperature-based disease warning models could signal too many treatments in early spring owing to the rarity of inoculum and too few treatments later in the season, as epiphytic sources of the pathogen become more abundant.

One of the first efforts to develop a prediction model for fire blight incorporated time of season as a factor in assessing disease risk (19), but this concept has been neglected in more recent modeling efforts (13,14). The older temperature-line model (19) characterized the minimum mean daily temperatures at which the introduction and buildup of epiphytic inoculum of *E. amylovora* in healthy pear flowers were observed to occur. The temperature line was seasonally dependent in that a mean daily temperature of 16.7°C was required to expect development of detectable epiphytic population in early March, but with the declining slope of the temperature line, a daily mean temperature of only 14.4°C

was required on May 1. Although Thomson et al. (19) did not comment on the sources of inoculum of *E. amylovora* that led to epiphytic infestations in healthy pear flowers, the negative slope of the temperature line appears to reflect inoculum of the pathogen becoming more abundant as the spring bloom period advances. The results of our study offer one explanation as to why a seasonal dependency could occur.

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