Relation of Apple Flower Age to Infection of Hypanthium by *Erwinia amylovora*

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ABSTRACT

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Blossom age as related to hypanthial susceptibility to *Erwinia amylovora* is not well established, but is relevant to disease risk assessment. To test this, detached crab apple blossoms were maintained for various periods and at different temperatures before applying inoculum to hypanthia. Inoculum potential on hypanthia due to wetting was evaluated by subjecting detached stigma-inoculated blossoms ($\sim 10^6$ CFU per flower) to varying amounts and durations of simulated rain (or dew) at 14°C. Blossoms of varying age on mature 'Gala' apple trees were inoculated on hypanthia with 10^2 , 10^4 , or 10^6 CFU per flower. In the laboratory, susceptibility decreased with flower age at rates that increased with temperature. Wetness periods up to 12 h resulted in populations on hypanthia of $<10^3$ CFU per flower; 24 h of wetness resulted in $\sim 10^4$ or $\sim 10^5$ CFU. A dose response was shown in the orchard, and regression curves indicated steepest decline of susceptibility during initial days after petal expansion. Disease models incorporating a blossom-age component may be effective because they indicate the potential for infection when temperatures favor rapid bacterial growth on stigmas within a window of high hypanthial susceptibility. Further investigation of these relationships could lead to advancements in determining fire blight risk.

Additional keywords: fire blight, *Malus pumila*, risk assessment

Fire blight, a major disease of apple and pear trees, is most commonly initiated by epiphytic populations of *Erwinia amylovora* on blossoms (36). Under dry conditions in the western United States, *E. amylovora* colonizes flower stigmas and rain or heavy dew facilitates its downward movement to the cup-shaped hypanthium where infection generally occurs through nectarhodes (22,33). Management programs have emphasized risk assessment (2) and suppression of *E. amylovora* on floral parts with antibiotics (17,36). Reliance on streptomycin in particular led to pathogen resistance in many production areas (15,16). Biological control is a viable alternative (9) or complementary measure (13,29) for suppressing *E. amylovora* on flower stigmas.

The dynamics of floral biology in relation to *E. amylovora* and disease development have not been thoroughly investigated. Recent studies gave attention to flower age in relation to bacterial colonization of stigmas (14,21,34), but little has been done to establish the relation of

flower age to infection. In a field experiment focused on apple stigma age and bacterial colonization, Thomson and Gouk (34) noted that incidence of infection was significantly higher in flowers 1 to 3 days old than in flowers 5 to 8 days old. In other research with apple, Taylor et al. (32) observed disease symptoms only when the combined epiphytic and endophytic levels of *E. amylovora* per blossom exceeded 10^6 CFU by 4 days after newly opened flowers were inoculated on stigmas. In the current report, we directly inoculated the hypanthia of blossoms of varying ages. This was done in the laboratory with detached crab apple flowers held at a range of temperatures and in an orchard experiment involving different inoculum doses applied to apple blossoms. Our intention was to assess how these factors interrelate as determinants of infection and how they may fit the Cougarblight disease risk assessment model (27), with its moving temperature evaluation, or other models incorporating a blossom-age component.

MATERIALS AND METHODS

Pathogen strain. *E. amylovora* strain Ea153na1, which is resistant to nalidixic acid, was obtained from K. Johnson (Oregon State University, Corvallis). It was isolated from cankers on 'Gala' apple in Oregon and later used in studies with apple and pear (10,11).

Bacteria were cultured on nutrient yeast dextrose agar (NYDA; nutrient broth, 8 g; yeast, 5 g; dextrose, 5 g; agar, 15 g; and

deionized water, 1 liter) for 24 h at 24°C. Inoculum suspensions were prepared in 10 mM potassium phosphate buffer (pH 7.0) and 0.03% Tween 20, then adjusted to 0.1 optical density at 600 nm with a spectrophotometer (Spectronic 20; Milton Roy Co., Rochester, NY) to approximate a cell density of 1×10^8 CFU/ml. Desired inoculum concentrations were prepared accordingly.

Plant material. Laboratory experiments were performed with detached blossoms from crab apple trees (*Malus* sp. 'Manchurian' on M26 rootstock), 1.6-cm minimum stem diameter, received from a local nursery (Van Well Nursery, Wenatchee, WA). Trees were induced to bloom in a greenhouse as previously described (18). Newly opened flowers were collected and each maintained with cut pedicle in 10% sucrose in a 2-ml vial, then supported by plastic holders in a 4-liter plastic container. Relative humidity was established at 90% by flooding the bottom of the container with 1 liter of a glycerol solution (392 ml of glycerol and 808 ml of deionized water) and closing the container with a lid (8).

In 2005 and 2006, a field experiment was performed with apple (*Malus pumila* P. Mill. 'Gala' on M7 rootstock). Trees were part of a research block planted in 1994 at Columbia View near Wenatchee, WA.

Laboratory experiments. Detached crab apple blossoms were held for various periods (0, 1, 2, 3, 4, 5, 6, 8, and 10 days) at different temperatures (10, 14, 18, and 22°C) prior to inoculation. A 2.5- μ l volume of bacterial suspension (10^9 CFU/ml) was directed into the hypanthium of each flower with a micropipet, entering between stamen filaments while avoiding injury to floral tissues. The inoculated flowers were incubated at 24°C for 5 days and evaluated for disease on the basis of a necrosis progression indicated by a darkening of tissue away from the hypanthial surface; 0 = no necrosis, 1 = necrosis barely detectable, 2 = up to half of ovary necrotic, 3 = over half of ovary necrotic, and 4 = necrosis in pedicel. If bacterial ooze was detected in advance of discoloration, flowers were rated according to the location of the ooze. When symptoms were questionable, presence of *E. amylovora* was verified by surface-sterilizing flowers with 70% ethanol and streaking tissue sections on CCT medium (6).

To evaluate the transfer of *E. amylovora* from stigmas to the hypanthium during wetting, detached flowers were inoculated

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on stigmas as described previously (18) before artificial wetting. Approximately 0.1 to 0.2 μl of inoculum suspension (10^7 CFU/ml) was applied per flower by touching the aqueous meniscus from a micropipette tip to each stigma (five per flower) to form a thin film of moisture. Flowers were incubated at 24°C and 90% relative humidity to accelerate growth and obtain a near-maximum population level on stigmas during a 24-h period prior to simulating rain with sterile water. Water was applied with a hand-pump pressure sprayer delivering 0.1- to 2.0- μl droplets for 0.4, 2.0, or 4.0 min to simulate approximate rain accumulation of 2, 10, or 20 mm, respectively. The 2-mm accumulation was meant to simulate dew, as well as light rain. Flowers were held at 100% relative humidity and 14°C (near average temperature during apple bloom in central Washington) for 0, 3, 6, 12, or 24 h, then allowed to air dry at room temperature (approximately 22°C) in a laminar flow hood for 45 min. This eliminated free moisture from more visible flower parts (i.e., petals, calyx, and anthers), restricting wetness to the hypan-

thium, which is more protected from air movement. This was thought to simulate what frequently occurs in the orchard and facilitate isolation of bacteria from only the hypanthial surface. Population sizes of *E. amylovora* on prewetting stigmas and hypanthia, and hypanthia of all other flowers after wetting, were estimated by placing respective tissues in sterile microcentrifuge tubes containing 1 ml of sterile buffer (10 mM potassium phosphate, pH 7.0). Stigmas were collected with portions of supporting styles, and hypanthia were partially isolated by removing stigmas, styles, corolla, calyx, and pedicels. Tubes were briefly vortexed and placed in a sonication bath for 60 s. Samples were again vortexed and serial dilutions spread on CCT medium amended with nalidixic acid (100 $\mu\text{g}/\text{ml}$). After 3 or 4 days of incubation at 24°C, bacterial colonies were counted to estimate population size.

Each laboratory experiment involved 10 replicate flowers per treatment and was performed twice.

Field experiment. Fifteen 'Gala' apple trees with sufficient numbers of blossom clusters were selected within an orchard block. On each of nine successive days (15 to 23 April 2005 and 24 April to 2 May 2006), six newly opened flowers per tree were tagged. Each tagged flower was in a separate cluster and all other flowers (or buds) in that cluster were removed; in 2006, one extra flower was tagged in two of the six designated clusters (per tree and date) for later estimation of bacterial population size with the procedure described for laboratory experiments. As flowers aged, general physical characteristics were noted and recorded. On the ninth day, all tagged flowers were inoculated by directing 10 μl of cell suspension into the hypanthium as described for laboratory experiments. Three suspension concentrations (10^4 , 10^6 , and 10^8 CFU/ml) were used, and each concentration was applied to five trees selected randomly from the larger group of fifteen.

Irrigation was applied below the tree canopy prior to inoculation to raise soil moisture and maximize water potential in the trees. In 2005, trees were irrigated from 0700 h on 22 April to 0700 h on 23 April. Inoculation was performed on 23 April during overcast conditions between 0400 and 1900 h. In 2006, irrigation occurred between 0700 and 1900 h of 1 May and inoculations were made between 1730 and 2030 h on 2 May. Flowers were inoculated on the shaded east side of trees while the sun was still above the horizon and on the west side of trees after the sun disappeared behind an eastern-facing slope. Between 16 and 18 h after inoculation in 2006, the extra inoculated flowers in clusters were collected, and population size of *E. amylovora* in hypanthia was estimated as already described. Weather data within the orchard were collected with a Watch-

Dog Data Logger (Model 450; Plainfield, IL).

Flowers in the orchard were evaluated for disease symptoms on 5 May 2005 and 15 May 2006. Each tagged flower was removed and examined in full sunlight for disease symptoms. A positive result was recorded whenever ooze or dark necrotic tissue was visible, as described for the laboratory experiment, between the hypanthial surface and the pedicel base. Flowers were evaluated for symptoms only once since prior experience indicated that disease incidence from direct inoculations of hypanthia changes little after 12 to 14 days. Accumulated degree days $>12.7^\circ\text{C}$ from time of inoculation to disease evaluation was close to 57, the value established in the MARYBLT model (35) for predicting early symptom development.

Statistical analyses. Single-flower replications were used in the laboratory; in the field, subsamples of flowers per treatment and sampling date were averaged for each single-tree replication before obtaining means and standard errors. Data were subjected to nonlinear regression analysis with SigmaPlot 9.0 (SYSTAT Software, Inc., Point Richmond, CA). Data sets from separate trials of laboratory experiments were tested for homogeneity using Hartley's *F*-max test and then pooled prior to further analysis.

RESULTS

Laboratory experiments. Severity and incidence of infection initiated through the hypanthium decreased with flower age at a rate dependent on temperature (Fig. 1). Inoculation of newly opened flowers resulted in maximal severity ratings (Fig. 1A) and an infection incidence of 100% (Fig. 1B). At the low temperature of 10°C, incidence decreased by 40% when flowers were inoculated at 10 days of age. In contrast, flowers held at 22°C for 8 or 10 days prior to inoculation exhibited no disease symptoms.

In a second experiment, quantity of bacterial cells transferred from stigmas to the hypanthium during wetting was evaluated. Prior to wetting, population size on the inoculated stigmas was almost 10^6 CFU per flower and no bacteria were detected in the hypanthia (Fig. 2). Simulated rain at levels from 2 to 20 mm followed by incubation at 14°C and wetness periods extending as much as 12 h (plus time during transition from wet to dry within 45 min) resulted in bacterial levels of nearly 3.0 log CFU or less per flower hypanthium. In both trials of the experiment, bacterial populations were comparatively smaller in the hypanthia of flowers treated with only 2 mm of water followed by 3 or 6 h of wetness, but appeared to recover during 12 h of wetness; water absorption by the plant, as indicated by swollen anthers, may have temporarily reduced surface wetness to a level marginally sufficient for bacterial

Fig. 1. Age of crab apple flowers in the laboratory related to severity (A) or incidence (B) of infection by *Erwinia amylovora* through the hypanthium. Detached flowers were maintained at temperatures of 10, 14, 18, or 22°C for various periods before direct inoculation of hypanthia with 2.5×10^6 CFU per flower. Severity rating was based on extent of necrosis away from the hypanthium; 0 = none, 1 = detectable, 2 = up to half of ovary, 3 = over half of ovary, and 4 = necrosis in pedicel. Each point represents mean of 20 replicate flowers and vertical bars represent standard error. Regressions, based on quadratic equation, had adjusted R^2 values of 0.91 to 0.99 and significance of $P \leq 0.0004$.

cells to survive. After a 24-h wetting period, the population size of *E. amylovora* in the hypanthium ranged from 3.8 to 5.2 log CFU per flower, depending on the amount of water applied.

Field experiment. During the 8 days of tagging new flowers in the orchard experiment, temperatures averaged 13.0°C in 2005 and 14.5°C in 2006 (Fig. 3A and E). Rate of flower development and senescence was similar in the 2 years. When flowers were newly opened, stigmas appeared light green and changed to light yellow by 4 days, yellow to brown through 7 days, and brown or black by 8 days. Stamens were bright yellow and none dehiscent on newly opened flowers; all were brown and fully dehiscent by 4 days. Petals began dropping from flowers at 4 days of age and were absent by 7 days.

Irrigation prior to inoculation did not significantly affect relative humidity in tree canopies, as judged by levels before and after irrigation (Fig. 3B and F). Soil moisture in 2005 approached saturation, resulting in muddy conditions. Rain began near the end of inoculation and lasted 1.5 h, with an accumulation of 3.8 mm (Fig. 3C). This resulted in 6 h of wetness in tree canopies (Fig. 3D). A prolonged dew was detected the evening of the first day newly opened flowers were tagged (Fig. 3D). In 2006, the soil moisture levels appeared lower than in the previous year, and no rain or surface wetness was recorded (Fig. 3G

and H). Although the sky was clear during inoculation in 2006, flowers were not directly exposed to sunlight from the time bacteria were applied until the next day. Considering also that apple hypanthia are narrow and shielded by hairs (unlike exposed pear hypanthia), moisture loss during initial hours after applying inoculum suspensions was likely minimal. There was essentially no air movement and relative humidity peaked above 50% during the night.

Size of pathogen populations in the hypanthia was estimated once after inoculation in 2006, but not in 2005. After 16 to 18 h, population size of *E. amylovora* was similar for all flower ages, averaging >1.0 log unit below inoculum levels. With the detection limit at 10^2 CFU per flower, 4.8 ± 0.22 (standard error) CFU were recovered per flower inoculated with 10^6 CFU (applied as 10 µl of 10^8 CFU/ml), and 2.9 ± 0.13 CFU were recovered per flower inoculated with 10^4 CFU. Bacteria were detected in only 19% of flowers inoculated with 10^2 CFU.

During both years of the experiment, there was no detectible background incidence of fire blight in the orchard block. In 2005, newly opened blossoms (day 0) inoculated with 10^6 CFU of *E. amylovora* per flower showed a disease incidence of 88% (Fig. 4A). On the basis of a curve ($R^2 = 0.93$), the incidence decreased to approximately 58 and 34% when flowers were inoculated at 4 and 8 days old, respectively. Inoculation with 10^4 CFU per flower resulted in a disease incidence of 38% in flowers inoculated at day 0, decreasing on a curve ($R^2 = 0.73$) to 16 and

8% in flowers inoculated at day 4 and 8, respectively. Incidence for inoculations with 10^2 CFU per flower were negligible, not exceeding 6.7%.

In 2006, inoculation with 10^6 CFU per flower at day 0 resulted in a disease incidence of 72%, based on curve ($R^2 = 0.93$), decreasing to 36% at day 4 and 28% at 8 days (Fig. 4B). For inoculations with 10^4 CFU, fit of data with quadratic equation was marginal ($R^2 = 0.63$); actual mean disease incidence in flowers inoculated at day 0 was 36% and incidence for flowers of all other ages ranged from 0 to 15%. None of the flowers inoculated with 10^2 CFU exhibited disease symptoms.

DISCUSSION

This study demonstrated that pomaceous blossoms become less susceptible to invasion of hypanthial tissues by *E. amylovora* as flowers age. Temperature and dose effects were also significant and will be discussed.

Controlled-environment experiments with detached flowers have enhanced our understanding of variables affecting bacterial colonization on the stigma (12,20,21) and bacterial infection through the hypanthium (3,20). This approach may have less value for investigating disease in relation to flower age, since over time the physiology of detached flowers is expected to increasingly diverge from that of blossoms in the field. Nevertheless, detached flowers appear to go through the same late development and senescence stages as those observed in the field (21; P. L. Pusey, unpublished). When such flowers were inoculated in the current study, infection

Fig. 2. Quantity of *Erwinia amylovora* transferred from stigmas to the hypanthium during wetting. Detached crab apple flowers were inoculated on stigmas and incubated at 24°C and 90% relative humidity for 24 h, resulting in population size on stigmas of $\log 5.83 \pm 0.15$ (standard error) CFU per flower and no detectible bacteria in the hypanthium prior to wetting. Sterile water was applied with a hand sprayer to simulate rain accumulation of 2, 10, or 20 mm (2 mm was meant to simulate dew as well as light rain), and then flowers were held at 14°C (close to average temperature during apple bloom in central Washington) and 100% relative humidity for 3, 6, 12, or 24 h before air drying in laminar flow hood for 45 min. Detection limit was 10 CFU per flower. Each bar represents mean population size in hypanthia for 20 replicate flowers immediately after drying. Vertical lines above bars represent standard error.

Fig. 3. Weather information for a 'Gala' apple orchard in 2005 (A to D) and 2006 (E to H). Data were recorded every 30 min for temperature (A and E), relative humidity (B and F), rainfall (C and G), and vegetative wetness (D and H). Rain indicated in 2005 is for three successive 30-min periods, totaling an accumulation of 3.8 mm (C). Time of inoculation in each year is indicated with an arrow (A and E).

initiated through hypanthial tissues decreased as flowers aged and the rate of declining susceptibility increased with temperature. It is reasonable to assume that the effect of temperature occurs with intact plants as well, though this was not tested. Other critical determinants of disease development in the controlled environment, such as relative humidity and water potential, were held constant at levels shown to be highly conducive for disease (20).

One cell of *E. amylovora* can potentially infect pomaceous flowers through the hypanthium (3,5); however, the minimum infective dose generally depends on environmental conditions (20), pathogen aggressiveness (3), and likely also cultivar susceptibility (36). In our field experiment, the inoculum level of 10^2 CFU per flower hypanthium caused a very low disease incidence in 2005 and resulted in no detectable disease symptoms in 2006.

The quantity of epiphytic bacteria transferred to the flower hypanthium during wetting is limited by populations on the stigma, which reach maximal levels between 10^6 and 10^7 CFU per flower (9). When crab apple stigmas bearing nearly 10^6 CFU per flower were subjected to simulated rain (or dew) and a wetness period as long as 12 h, bacterial numbers in the hypanthium were generally 3 to 4

log units below those of prewetting stigmas. In a pear field study involving natural wetness periods not exceeding 5 h (33), populations of *E. amylovora* in the hypanthia were only 1 to 2 log units smaller than populations on stigmas. These different findings are not surprising, considering that the hypanthia of pear are more open compared with apple and crab apple, and thus, likely to receive and accumulate more bacterial cells moving via water from stigmas. When wetness on crab apple flowers was extended for 24 h, bacterial populations in crab apple hypanthia increased to within 1 or 2 log units of stigma populations, perhaps due more to cell multiplication than further migration. Given the above laboratory results with crab apple and that bacterial populations on apple stigmas can be as large as 10^7 CFU per flower (21), the inoculum dose of 10^4 CFU per flower hypanthium in our field test appears to be near the upper limit of bacterial numbers transferred from apple stigmas to hypanthia during a few hours of wetness. The dose of 10^6 CFU may represent a level that can be reached or exceeded when wetting is prolonged and temperatures favor significant multiplication of bacteria in the hypanthium (20,37,38). A return to dry conditions will generally lead to increased nectar concentration and osmotic pressure in young hypanthia, causing a slowing or cessation of bacterial growth and eventual decline in viable cell numbers (7,19).

Higher moisture conditions in 2005 may explain greater incidences of disease compared with 2006. Rain beginning near the end of inoculation accumulated to 3.8 mm and resulted in a 6-h period of wetness. Higher temperatures on the day of and day before inoculation in 2005 could also have contributed to greater disease incidence according to Billing's risk assessment system (1,2). In addition, the soil was possibly wetter prior to inoculation in 2005 from longer irrigation, resulting in greater water potentials in trees and higher disease susceptibility (20,24,31).

Average temperatures in the field prior to inoculation (13.0°C in 2005 and 14.5°C in 2006) were comparable with the laboratory constant of 14°C . Nonlinear curves resulting from these experiments however, appeared different. In the controlled environment at 14°C , incidence of infection was high for several days before declining significantly, maybe because other conditions were highly conducive to bacterial survival and infection of hypanthial tissues (20). In contrast, decline of disease incidence in the field was generally greatest during the initial days after flower opening. On the basis of the effects of temperature in the laboratory, we would expect higher temperatures in the field to result in even greater rates of declining disease incidence relative to flower age. Daily temperature ranges and averages varied

widely during the experiment in 2005 and 2006, so reference to overall averages is an oversimplification. To determine with any certainty the time limits of flower susceptibility in relation to temperature, it may be necessary to perform tests with trees under controlled or semicontrolled conditions (e.g., growth chambers or tree enclosures).

A general comparison can be made between our field results and those of others (32,34) who used different methods to examine the relationship between flower age and infection by *E. amylovora*. Thomson and Gouk (34) spray-inoculated whole flowers of varying age with suspensions of $>10^7$ CFU/ml and observed a greater incidence of disease symptoms in flowers 1 to 3 days old than in flowers 5 to 8 days old. Taylor et al. (32) inoculated stigmas of newly opened blossoms with various bacterial concentrations, enclosed them with polyethylene for 24 h, and later detected symptoms when total levels of *E. amylovora* per blossom (whole flowers were crushed in the procedure) exceeded 10^6 CFU within 4 days. In both of the previous studies, levels of *E. amylovora* in the hypanthium and time and avenue of infection were undetermined. In the current study, we were interested in determining the susceptibility of apple hypanthia of various ages to pathogen levels resulting from typical wetting events. In 2005, when moisture was high, a pathogen level of 10^4 CFU, which may represent a realistic maximum as discussed, caused a disease incidence that decreased along a quadratic curve ($R^2 = 0.73$) from 38 to 16% for flowers 0 to 4 days old and from 13 to 8% for flowers 5 to 8 days old.

The Cougarblight model was developed in the early 1990s (26,27) for assessing the risk of fire blight in pome fruit production areas of the northwestern United States. An important feature, which was distinctive at its inception, is a moving 4-day temperature evaluation that theoretically accounts for flower age; a running sum of degree hours above 15.5°C 3 days before the day of a wetting event is added to those on the day of wetting. The parameters were established after applying different base temperatures (15.5 and 18.3°C) and periods (1, 2, 3, 4, and 6 days) to past weather data and actual infection events spanning more than 20 years. Other models at the time were based on temperatures of single days or the accumulation of degree hours throughout the bloom period (2). MARYBLT, a model developed in the eastern United States (35), added a blossom-age component that became available with Version 4.3 in 1996 (G. W. Lightner, *personal communication*). According to this system, apple blossoms older than 44.4 degree days, using a base of 4.4°C , are unlikely to be infected upon wetting; this equals approximately 4 days at an average temperature of 15.6°C . A more recent model adopted in Israel in-

Fig. 4. Age of 'Gala' apple blossoms in an orchard related to development of symptoms of infection by *Erwinia amylovora* applied to hypanthia in 2005 (A) and 2006 (B). Results are shown for three inoculum levels distinguished by symbols in the key (B). Each point represents mean of five replicate trees, each with six flowers per flower age, and vertical bars represent standard error. Regressions based on quadratic equation had a significance of $P \leq 0.01$ (*) or $P < 0.001$ (**).

volves a moving 5-day evaluation of temperature and wetness (25); measurements on the previous 3 days are used to assess the likelihood of past infections, and forecasts for the following 2 days are used to determine the risk of future infections.

An assumption of the Cougarblight model is that flower stigmas support bacterial growth only for a few days (27). The appropriateness of a 4-day temperature evaluation seemed to be confirmed by experiments of Thomson and Gouk (4,27,34), in which apple stigmas inoculated at ages exceeding 3 or 4 days did not support growth of *E. amylovora*. In the same study, however, introduction of the pathogen within 4 days resulted in bacterial growth extending as much as 6 or 8 days from the time of flower expansion. A similar phenomenon occurred with *Pseudomonas fluorescens* strain A506 on pear blossoms (14). Populations of this antagonist increased during 12 or 13 days to approximately 10^6 CFU on flower stigmas inoculated within 5 days of opening and persisted at high levels for even longer periods. Eventual populations were shown to decrease with further increases in flower age at inoculation. The authors suggested possible competition with indigenous organisms because population sizes of strain A506 and indigenous bacteria had an inverse curvilinear relationship. Stockwell et al. (30), who reported increases of indigenous bacteria on pear stigmas up to 15 days after petal expansion, likewise thought indigenous organisms interfere with antagonist establishment, but subsequent indirect analysis (12) did not show this. In experiments with apple, enclosures surrounding trees were used to reduce insect dispersal of test bacteria and indigenous organisms and to manipulate temperature (21). Under low temperatures (average of 13.8°C), blossom stigmas supported growth of *E. amylovora* when inoculated at ages up to 12 days; under high temperatures (average of 22°C), stigmas supported pathogen growth when inoculated up to 5 or 6 days of age. On the basis of the above recent reports, apple and pear blossoms apparently have the capacity to support bacterial colonization and growth at ages much older than 4 days. Therefore, the effectiveness of Cougarblight in assessing disease risk must involve factors in addition to stigma receptiveness to *E. amylovora*.

It seems logical to consider the role of hypanthial susceptibility to disease as related to flower age in the context of epiphytic growth of bacteria and other factors known to be important for infection to occur. Growth rate of *E. amylovora* on flowers, as dependent on temperature, is a primary basis of fire blight risk assessment (23). Also, longevity of floral parts (28) and duration of their receptivity to bacterial colonization (14,21,34) is temperature dependent. On the basis of laboratory re-

sults here, duration of hypanthial susceptibility to infection is likewise affected by temperature. How pathogen and host change in relation to each other in response to temperature has not been addressed, but may be critical. As temperatures increase within the range supporting multiplication of *E. amylovora*, the rate of bacterial growth may increase more dramatically relative to the rate at which flowers senesce and become less vulnerable to colonization and infection. When newly opened detached flowers are routinely inoculated and incubated at a constant 24°C (which exceeds typical temperature averages in the orchard), *E. amylovora* increases from 10^3 CFU to 10^6 or 10^7 CFU per flower within 24 h (18,20,21), and yet flowers appear only slightly changed (P. L. Pusey, unpublished). Petals are still attached and stigmas are light green with papillae that are mostly turgid. Thus, high temperatures can potentially lead to high epiphytic populations of the pathogen before significant flower senescence occurs. The dynamics of host and pathogen are further complicated by the nonsynchronous emergence of flowers and the overall increase of inoculum as bacteria spread from old to new flowers. Nevertheless, while flowers are still emerging, it seems reasonable that higher temperatures will result in higher percentages of blossoms that can potentially be colonized within a period of a few days and then infected when wetting occurs. Cougarblight and similar blossom-age components within other models may be explained by the critical effect of temperature on multiplication of *E. amylovora* within a flower-age window that is highly conducive both to growth on stigmas, and upon wetting, to invasion of hypanthia through nectarthodes. Additional research establishing with greater accuracy the above interrelationships could lead to advancements in fire blight risk assessment.

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