Report

Current Biology

# Visual Detection and Avoidance of Pathogenic Bacteria by Aphids

## Highlights

- Pea aphids avoid feeding on leaves with pathogenic bacteria on their surface
- Pea aphids use vision in the presence of ultraviolet light to avoid pathogenic bacteria
- Avoidance is mediated by visual detection of fluorescence from bacterial pyoverdine

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## In Brief

*Pseudomonas syringae* is a common plant epiphyte and aphid pathogen. Hendry et al. report that pea aphids avoid feeding when virulent *P. syringae* bacteria are present on leaves. This avoidance behavior is mediated by visual detection of fluorescence from the bacterial compound pyoverdine.

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# Visual Detection and Avoidance of Pathogenic Bacteria by Aphids

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## SUMMARY

**Aphids are diverse sap-sucking insects [1] that can be serious agricultural pests and vectors of plant disease [2]. Some species, including pea aphids (*Acyrthosiphon pisum*), are susceptible to infection by epiphytic bacteria that are commonly found on plant surfaces [3–5]. Pea aphids appear unable to recover from these infections, possibly because pea aphids are missing apparent orthologs of some immune response genes [6], and these aphids exhibit relatively low immune responses after pathogen exposure [7]. We therefore tested the ability of pea aphids to use avoidance as a non-immunological defense against *Pseudomonas syringae*, a widespread plant epiphyte and aphid pathogen [8, 9]. Pea aphids avoided highly virulent strains of *P. syringae*, but not all strains, and avoidance led to a significant reduction in infection among aphids. We found that aphids can use visual cues to detect the ultraviolet (UV)-based fluorescence of the bacterial siderophore pyoverdine [10] produced by virulent strains. Avoided epiphytic bacteria caused light leaving the surface of leaves to be richer in wavelengths that were tightly linked to both aphid visual sensitivities and the fluorescent emission spectra of pyoverdine, suggesting that pyoverdine fluorescence mediates avoidance and may be a visual cue used by aphids to detect epiphytic pathogens. Although pyoverdine production in *Pseudomonas* species may be a broadly reliable indicator of bacterial virulence within the phyllosphere, it was not directly responsible for virulence to aphids. Aphids may be under selection to avoid fluorescence on leaves, a phenomenon with potential use for the control of agricultural pest insects.**

## RESULTS AND DISCUSSION

### Avoidance of Virulent Bacteria

Epiphytic bacteria can be highly infective and virulent to pea aphids [3–5, 9, 11, 12]. For instance, *P. syringae* strains can cause death with fewer than ten cells ingested [13] and can infect up to 30% of individuals on a plant [11]. Infected individuals may die in as few as 4 days and do not appear able to recover from infections [13]. Evidence suggests that aphids may use non-immunological defense strategies against such pathogens. These include the following: symbiont-mediated immunity, where symbionts protect against pathogens [14, 15]; fecundity compensation, where individuals increase reproduction after infection [13]; or avoidance [16]. Given the high infectivity, virulence, and incidence of potential bacterial pathogens on plants, we hypothesized that the ability to detect and avoid these pathogens in the phyllosphere before exposure should be beneficial to aphids, and therefore be selected for. To test this, we performed experiments using multiple strains of *P. syringae* that varied in virulence to pea aphids. We observed the preference of aphids given a choice between leaves with or without epiphytic bacteria. Additionally, we sought to determine which cues aphids used to detect the presence of bacterial pathogens.

In choice assays on plants, aphids significantly avoided some, but not all, epiphytic *P. syringae* strains (Figure 1A). Individual leaflets within broad bean (*Vicia faba*) leaf pairs were painted with either a control solution or bacterial suspension. Pea aphids were introduced at the base of the plant and observed daily. The majority of aphids settled to feed on a leaf within a few hours, and numbers feeding on leaves stayed relatively constant for several days (Figure S1A). Many bacterial strains elicited no significant difference in the numbers of aphids feeding on control leaves versus leaves with bacteria. Some bacterial strains, however, caused aphids to significantly avoid leaves. For instance, on average, the strains *P. syringae* pv. *syringae* B278a (referred to as *Psy* B728a for ease) and *P. syringae* Cit7 caused 65% and 60% of aphids to choose control leaves after 4 hr on plants, with up to 81% of aphids preferring control leaves on some plants treated with *Psy* B728a. These two strains are thought to be representative of strains that persist well epiphytically [17, 18], demonstrating that common epiphytic bacteria can decrease the likelihood that aphids will feed on leaves.

Aphid avoidance was positively correlated with the virulence of strains to aphids (Figure 1B, One-tailed Pearson's product-moment correlation: $t = 2.09$, $df = 6$, $p = 0.04$, $r = 0.65$), suggesting that avoidance may benefit aphids by decreasing their risk of infection by deadly bacteria. Supporting this possibility, we found that aphids with a choice between control and bacterial leaves had lower infection rates than when all available leaves were painted with virulent bacteria (5% versus 14% infected, respectively; GLMM; $\chi^2_1 = 6.04$, $p = 0.01$, $n = 240$) despite

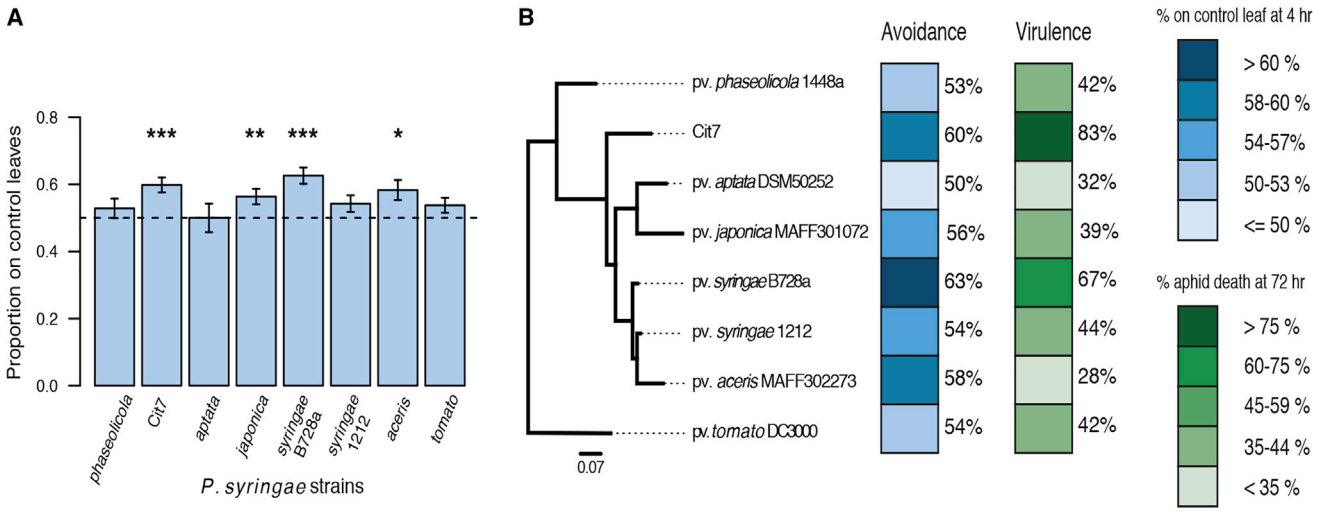


**Figure 1. Pea Aphids Avoid Some, but Not All, *P. syringae* Strains**

(A) Avoidance 4 hr after initial placement on whole plants (GLMM; $\chi^2_7 = 18.3$, $p = 0.01$). Aphids were able to choose between settling on leaves painted with bacteria or leaves painted with 10 mM $MgCl_2$ buffer solution (control leaves). If aphids have no preference, we expect the proportion of aphids on control leaves to be 0.5 (equal to the proportion on leaves with bacteria), whereas a significant increase over that proportion on control leaves indicates significant aphid preference for control leaves over bacterial leaves. The dashed line indicates this avoidance threshold. Those strains where the probability of aphids avoiding bacteria-coated leaves was significantly greater than 0.5 are denoted by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Sample sizes per strain are as follows: *phaseolicola* (n = 557), Cit7 (n = 776), *aptata* (n = 444), *japonica* (n = 552), *syringae* B728a (n = 601), *syringae* 1212 (n = 583), *aceris* (n = 525), and *tomato* (n = 580). Errors bars represent the standard error.

(B) Aphid avoidance was positively correlated with the virulence of bacterial strains (One-tailed Pearson's product-moment correlation: $t = 2.09$, $df = 6$, $p = 0.04$, $r = 0.65$). Mean avoidance values are based on results in (A), and virulence means were previously published [9].

See also Table S2.

both treatments having equal numbers of leaves painted with bacteria. Aphids avoided all strains previously shown to be highly virulent [9], but some strains were avoided disproportionately for their virulence, suggesting that aphids may overestimate, but not underestimate, the virulence of a strain. However, it was initially unclear how aphids are able to make these discriminations. This effect is unlikely to be plant-mediated because many aphids chose control leaves within 1 hr of bacteria being placed on plants, giving fairly little time for the bacteria to elicit a plant defense response (Figure S1B).

## Visual Detection of Bacterial Pathogens

An important part of the aphid life cycle is dispersal—finding and discriminating among potential host plants, and ultimately settling to feed. Aphids rely heavily on visual information in finding and selecting host plants [19]. To test if aphids could use visual cues to avoid potential pathogens, we compared behavioral avoidance of strain *Psy* B728a in the dark with avoidance under normal lighting conditions. In the dark, aphids were no longer able to distinguish between control and bacterial leaves and showed no significant preference compared to aphids under normal light conditions (Figure 2A, light versus dark GLMM: $\chi^2_1 = 28.55$, $p < 0.001$; probability of avoiding bacterial leaves in the dark: $p = 0.54$, 95% CI = 0.43 – 0.64). Again, this response is likely not plant-mediated, as similar avoidance patterns under normal light or dark conditions were seen in the absence of plants when experiments were performed using bacteria suspended in artificial aphid diet (Figure S1B). This result is also not driven by altered feeding rates under different conditions, as feeding rates in the dark did, after a slight lag, match rates under normal light (Figure S1C). These findings demonstrate that aphid vision plays a role in detecting virulent epiphytic bacteria.

Aphids are known to respond differentially to different wavelengths of light [20], and a common feature of many *Pseudomonas* species is that they produce fluorescent compounds that absorb ultraviolet (UV) light and emit visible light [21]. We therefore sought to determine if virulent bacteria could be altering the appearance of leaves due to fluorescence and if aphids could visually detect the presence of bacteria. We repeated choice assays under UV-blocking plastic using strain *Psy* B728a, which was highly avoided under normal lighting conditions. Without the presence of UV light, aphids no longer avoided leaves with these epiphytic bacteria (Figure 2B; light versus UV block GLMM: $\chi^2_1 = 37.90$, $p < 0.001$; probability of avoiding bacterial leaves under UV block: $p = 0.52$, 95% CI = 0.49 – 0.55). Again, this result was not an artifact of decreased feeding rates, as aphids actually fed at higher rates without UV light (Figure S1D). These findings demonstrate that avoidance requires UV light and suggests that avoidance behavior could be dependent on fluorescent molecules produced by *P. syringae*.

## Pyoverdine Mediates Pathogen Detection

Within *P. syringae*, the dominant fluorescent molecule is pyoverdine, a siderophore used for acquiring iron from the environment, which also produces blue or blue-green fluorescence [10]. To determine if pyoverdine could be responsible for the observed avoidance, we performed choice assays using a pyoverdine-deficient mutant of *Psy* B278a on plants. Compared to the wild-type

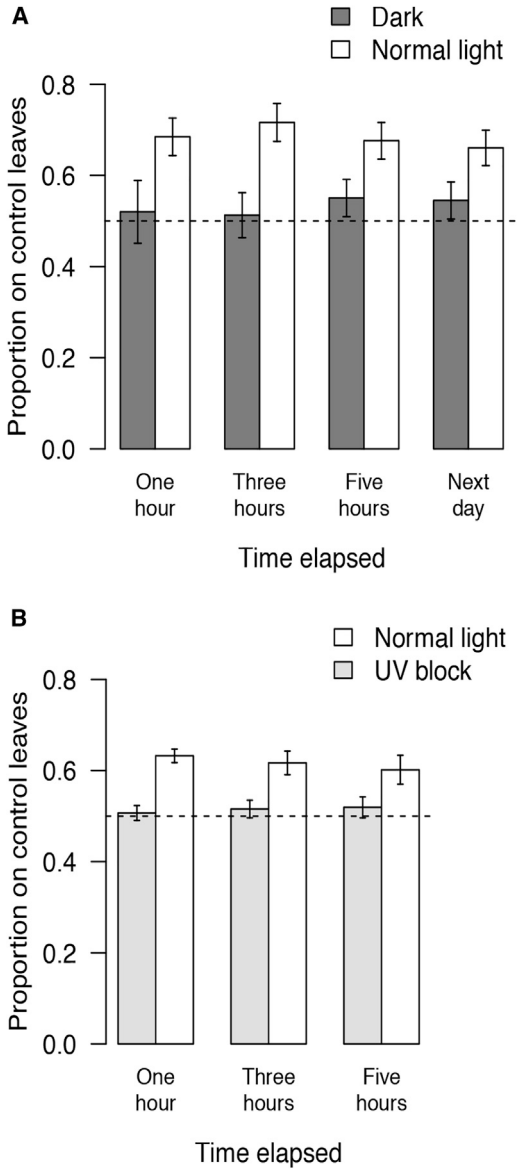


**Figure 2. UV Light Is Necessary for Aphids to Avoid Virulent Strains**

(A) Avoidance of the highly virulent strain *Psy* B278a was dramatically reduced when choice experiments were conducted in the dark (GLMM: $\chi^2_1 = 28.55$, $p < 0.001$), as aphids no longer avoided the leaves painted with bacteria (probability of avoiding bacterial leaves in the dark: $p = 0.54$, 95% CI = 0.43 – 0.64). Time elapsed since the start of the experiment had no effect on the aphids' choice whether in the dark or normal light (GLMM: $\chi^2_3 = 2.03$, $p = 0.57$). Experiments were done with excised leaves and total number of aphids (n) of n = 208 for the dark treatment and n = 196 for the normal light treatment. Errors bars represent the standard error.

(B) This result was mirrored when choice experiments were then conducted under UV-blocking plastic as compared to normal light (effect of UV block: GLMM: $\chi^2_1 = 37.90$, $p < 0.001$; probability of avoiding bacterial leaves under UV block: $p = 0.52$, 95% CI = 0.49 – 0.55). Again, aphid choices did not change over time in either treatment (GLMM: $\chi^2_2 = 0.85$, $p = 0.65$). Experiments were done with excised leaves and n = 737 aphids for the UV block treatment and n = 664 aphids for the normal light treatment. Errors bars represent the standard error.

See also Figures S1, S2, and S3.

strain, aphids showed no avoidance of the mutant, which did not produce visible fluorescence (Figures 3A–3C; wild-type versus mutant GLMM: $\chi^2_1 = 45.37$, $p < 0.001$; probability of avoiding mutant: $p = 0.51$, 95% CI = 0.48 – 0.54). Pyoverdine production appears to be necessary to cause visual avoidance of bacteria by pea aphids, at least within the strain *Psy* B728a.

Because avoidance and virulence were correlated among *P. syringae* strains, we hypothesized that pyoverdine production might be directly responsible for virulence to pea aphids. We performed virulence assays using oral infection in artificial diet by the pyoverdine-deficient mutant. Pea aphids infected with the pyoverdine-deficient mutant showed statistically indistinguishable death rates compared to those infected with the wild-type *Psy* B278a strain, demonstrating that pyoverdine is not responsible for aphid death in this strain (Figure 3D; log rank test of Kaplan-Meier survival curves: $\chi^2_1 = 0.50$, $p = 0.46$). Genes required for pyoverdine production have been identified in most *P. syringae* genomes; however, expression levels have been found to vary by strain and environmental context [21, 22]. In some strains, expression has been linked to epiphytic growth ability, and therefore, aphids may commonly encounter pyoverdine producing strains on plants [23]. Levels of pyoverdine production, or the specific molecule produced, may not be sufficient in all strains to cause avoidance. However, pyoverdine production may correlate with virulence to aphids, as they are both linked to epiphytic growth ability and may be a useful visual cue [9].

## Pyoverdine Mediated Avoidance is Consistent Across Light Conditions

We hypothesized that for aphids to use pyoverdine as an indication of pathogens, aphids should be able to detect pyoverdine across lighting conditions, including varied levels of overall brightness and amounts of UV light, as they might encounter naturally. To test this hypothesis, we performed choice assays using wild-type *Psy* B728a and the pyoverdine-deficient mutant of this strain under both UV-blocking and UV-transmitting plastic and varied lighting conditions. We found that brightness did affect avoidance, as aphids avoided wild-type *Psy* B728a under medium, high, and shaded (high but indirect light) light conditions, but not low light conditions. Increasing brightness increased the mean avoidance even under shaded conditions with no direct light, suggesting that although the amount of available light may impact avoidance, light does not need to directly illuminate leaves for aphids to detect virulent bacteria (Figure S2). Importantly, these effects were dependent upon the amount of UV light available, rather than overall light intensity, and upon the presence of the fluorescent molecule pyoverdine, as avoidance only occurred under UV-transmitting plastic when the wild-type strain was present, and not in any other treatment (Figure S2; UV-transmitting versus UV-blocking plexiglass GLMM: $\chi^2_1 = 24.68$, $p < 0.001$). Moreover, the addition of UV light to the medium lighting condition increased the mean avoidance to similar values seen under high light, but again only under UV-transmitting plastic. These results support our conclusions that both UV light and pyoverdine production are required for avoidance of virulent *P. syringae* and further suggest that the amount of UV light present can influence levels of avoidance. Although light levels can influence avoidance, avoidance occurred under varying levels of UV light (Figure S3) even when light was only

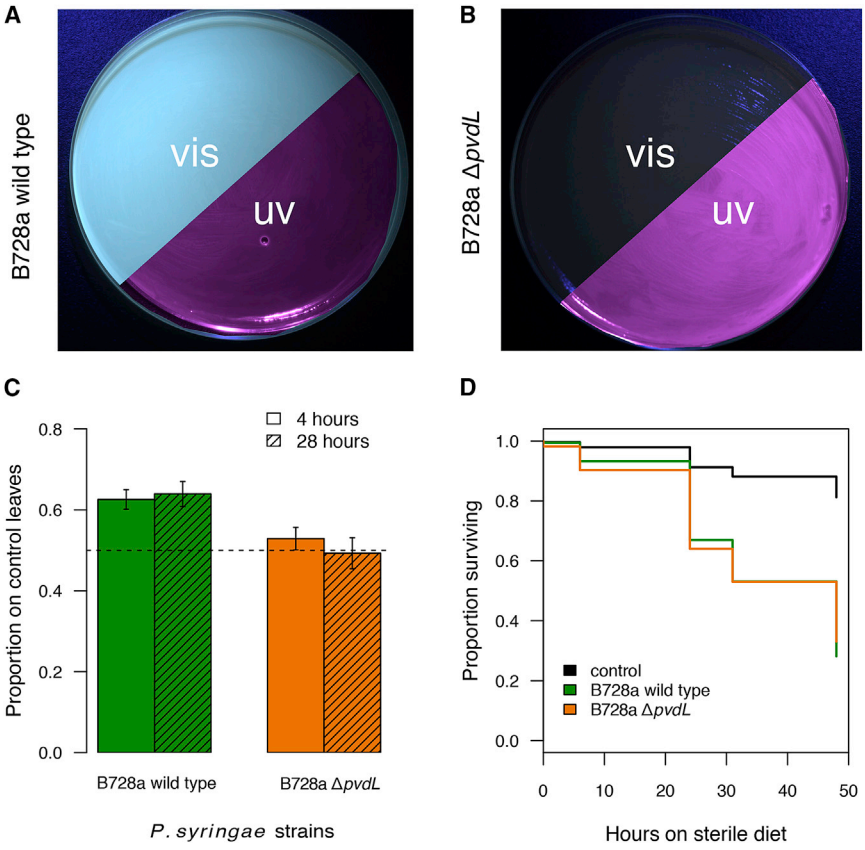


**Figure 3. Pyoverdine Production by Virulent Bacteria Is Necessary for Avoidance but Does Not Kill Aphids**

(A) Composite black-light-illuminated photographs capturing human-visible (vis) and ultraviolet (uv) wavelengths of light illustrate that the wild-type bacterial strain *Psy* B278a which is avoided by aphids is highly fluorescent (vis) as a consequence of the conversion of short-wavelengths of ultraviolet light (uv) being absorbed and re-emitted as longer wavelengths of light.

(B) Conversely, the pyoverdine-deficient mutant of this strain has little/no fluorescence in the human-visual portion of the electromagnetic spectrum (vis) and, correspondingly high levels of reflectance in the ultraviolet (uv). Both (A) and (B) are composite images of bacterial lawns growing on King's B agar plates, illuminated with UV light and photographed using a modified Canon 7D camera with either a 400 nm - 690 nm Baader filter (vis), or a UV only Baader filter (uv).

(C) Knocking out pyoverdine production led to a loss of avoidance in comparison to the wild-type (wild-type versus mutant GLMM: $\chi^2_1 = 45.37$, $p < 0.001$; probability of avoiding mutant: $p = 0.51$, 95% CI = 0.48 – 0.54). These assays were performed on whole plants with n = 1250 aphids for mutant treatments and n = 1205 aphids for wild-type treatments. Errors bars represent the standard error.

(D) Pyoverdine production did not influence the virulence to aphids (log rank test of Kaplan-Meier survival curves: $\chi^2_1 = 0.50$, $p = 0.46$, n = 384 aphids exposed to wild-type, n = 670 aphids exposed to the mutant, and n = 479 control aphids).

indirect (shaded), implying that this phenomenon may be common under varied environmental lighting conditions.

## Pyoverdine Fluorescence as a Potential Visual Cue

To determine if pyoverdine fluorescence could be a visual cue used by aphids, we compared the wavelengths of pyoverdine fluorescence to the visual sensitivities of aphids and to the spectra of leaves coated with epiphytic bacteria either producing or not producing pyoverdine. First, we generated a fluorescence excitation-emission matrix for a suspension of the wild-type pyoverdine-producing strain *Psy* B728a using a spectrofluorometer (Figure 4A). Second, we contrasted this with a matrix of the pyoverdine-deficient knockout strain (Figure 4B). Wild-type *Psy* B728a bacteria has the capacity to re-emit many short wavelengths of light as longer wavelengths (i.e., fluorescence), but differs most strongly in emission spectra from the pyoverdine knockout when excited by light of wavelengths 350 nm and 400 nm, which is consistent with the previously determined peak excitation values of pyoverdine from a related bacterial species, *Pseudomonas fluorescens* [26]. Interestingly, we noted a strong concordance between the cumulative, normalized fluorescence emission curve of wild-type *Psy* B728a and the visual sensitivities of aphids (Figure 4C). The apparent match between aphid visual sensitivities and the cumulative fluorescence emission spectra of the wild-type *Psy* B728a strain supports the results of our behavioral assays, suggesting that aphids are particularly well suited to detecting pyoverdine fluorescence.

In addition to examining the fluorescent profile of bacterial suspensions, we also used a UV-VIS reflectance spectrometer to estimate relative excitance from broad bean leaves coated with epiphytic populations of bacteria that differed in both aphid avoidance and virulence to aphids. Excitance values, which capture both reflectance and fluorescence, from leaves with epiphytic bacterial populations were evaluated using an aphid visual model incorporating the known spectral sensitivities of a closely related aphid species, *Myzus persicae* (Figure 4C) [24]. This model revealed significant differences between control leaves and leaves colonized with behaviorally avoided bacterial strains, as well as between leaves coated with differentially avoided bacteria (Table S1, Figure S4). Avoided bacterial strains on leaves showed excitance curves that were blue-shifted (see hue statistics; Table S1) in a range of wavelengths overlapping with the pyoverdine fluorescence emission (gray regions of Figure 4C, Figure 4D), supporting the hypothesis that pyoverdine fluorescence facilitates avoidance (Figure 4D, Figure S4). Additionally, it appears that there may be a threshold color-shift required for avoidance, as leaves coated with unavoided bacterial strains tended to be more blue-photoreceptor-stimulating than leaves painted with a control solution, but less-blue-photoreceptor-stimulating than leaves painted with avoided strains (Figure S4, Table S1). Together with our findings that both UV light and the known fluorescence compound pyoverdine are required for aphid avoidance of virulent *P. syringae* strains, these

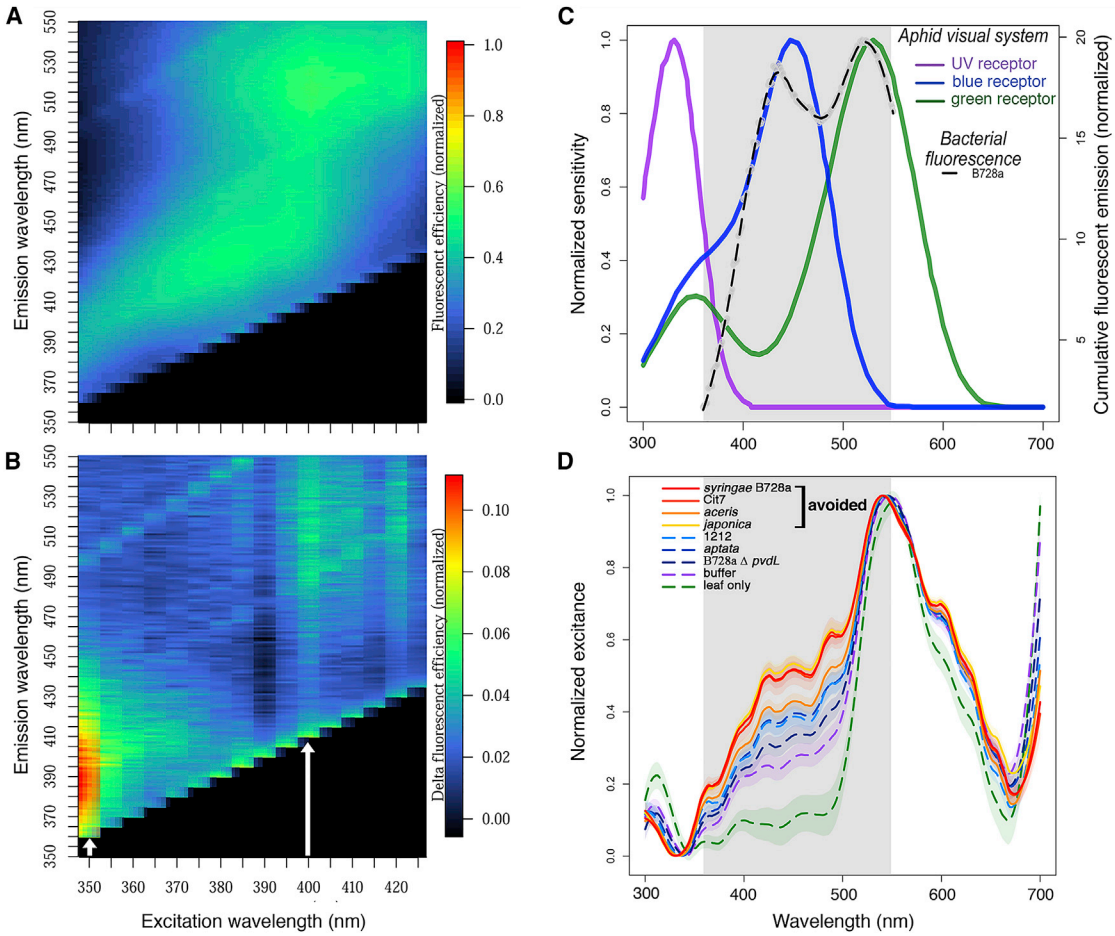


**Figure 4. Pyoverdine Fluorescence Compared to Aphid Visual Sensitivities and Excitance for Leaves with Varied Epiphytic Bacterial Strains**

(A) Excitation/emission matrix illustrating normalized fluorescence efficiency of wild-type *Psy* B728a bacteria.

(B) Differential fluorescence efficiency matrix illustrating the excitation wavelengths most responsible (white arrows) for differences between wild-type and pyoverdine-deficient mutant bacteria.

(C) Normalized sensitivity curves for the green peach aphid *Myzus persicae* [24, 25] with cumulative fluorescence emission from wild-type *Psy* B728a bacteria overlaid, illustrating high concordance between light sensitivity of the aphids and the fluorescence emission of the bacteria.

(D) Idealized excitance (reflectance + fluorescence) spectra for broad bean leaves coated with different bacterial strains. Lines represent mean values from 40 measurements collected from 4 leaves per treatment (10 measurements/leaf), and colored shaded regions represent the standard deviations. The vertical gray bar indicates the region of wavelengths over which fluorescence of *Psy* B728a occurs. The wavelengths showing the most variation in excitance between leaves with different treatments correspond to the wavelengths of highest *Psy* B728a fluorescence, with avoided strains producing higher excitance.

See also Figure S4 and Table S1.

results reinforce the hypothesis that pyoverdine fluorescence mediates aphid avoidance.

## Conclusions

A suite of behavioral assays using genetically diverse bacterial strains, along with visual modeling and fluorescence measurements, reveal how virulent *P. syringae* strains may be visually distinguishable to aphids, supporting our conclusions that aphids can use vision to preferentially avoid feeding on plants colonized with virulent epiphytic bacteria. This work suggests that UV-based fluorescence mediated by the bacterial product pyoverdine may be a reliable indicator of the presence of highly virulent bacteria, and that detection and avoidance of fluorescence may benefit aphids by reducing infection risks. Given the prevalence and high virulence of many bacteria in the phyllosphere, aphids may have evolved this avoidance mechanism due to selection against pathogen infection. This avoidance mechanism has potential applications in biological control of aphid pest species. Fluorescent bacterial strains, or fluorescent compounds, could be used on crops to deter aphid settling. Furthermore, selective pressure imposed by pathogenic bacteria could prevent aphids from evolving to ignore fluorescent deterrents.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING

## SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and two tables and can be found with this article online at https://doi.org/10.1016/j.cub.2018.07.073.

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## AUTHOR CONTRIBUTIONS

T.A.H. and R.L.F. conceived of this work, and T.A.H. designed the research plan. K.R.B. performed choice and infection assays, R.A.L. performed spectral analysis and visual modeling, M.R.S. performed virulence assays, and M.R.S. and R.A.L. conducted data analysis. T.A.H. wrote the manuscript with contributions from R.A.L. and M.R.S., and all authors contributed revisions.

## DECLARATION OF INTERESTS

The authors declare no competing financial interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Bacterial and Virus Strains | | |
| *Pseudomonas syringae* pv. *tomato* | Alan Collmer [27] | strain DC3000 |
| *Pseudomonas syringae* pv. *syringae* | Alan Collmer [28] | strain B728a |
| *Pseudomonas syringae* pv. *syringae* | David Baltrus [29] | strain 1212 |
| *Pseudomonas syringae* pv. *japonica* | David Baltrus [30] | strain MAFF 301072 PT |
| *Pseudomonas syringae* pv. *aptata* | David Baltrus [31] | strain DSM50252 |
| *Pseudomonas syringae* pv. *aceris* | David Baltrus [32] | strain MAFF 302273 PT |
| *Pseudomonas syringae* | David Baltrus [33] | strain Cit7 |
| *Pseudomonas savastanoi* pv. *phaseolicola* | Alan Collmer [34] | strain 1448a |
| *Pseudomonas syringae* pv. *syringae* | Steven Lindow | strain B728a *pvdL* deletion mutant |
| Deposited Data | | |
| data on strain virulence to pea aphids | [9] | N/A |
| Experimental Models: Organisms/Strains | | |
| pea aphids (*Acyrthosiphon pisum*) | Angela Douglas [35] | clone CWR09/18 |
| broad bean (*Vicia faba*) | Johnny's Selected Seeds | Windsor variety |
| Software and Algorithms | | |
| R: A language and environment for statistical computing | [36] | N/A |
| lme4 | [37] | N/A |
| lsmeans | [38] | N/A |
| survival | [39] | N/A |
| survminer | [40] | N/A |
| pavo | [41] | N/A |
| Other | | |
| artificial aphid diet | [42] | N/A |

### CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Tory A. Hendry (th572@cornell.edu).

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

#### Insects

Lab colonies of *A. pisum* were reared at 21°C and a light:dark 16:8 hr cycle. Under long day summer-like conditions pea aphids will reproduce parthenogenetically as clones. Colonies were housed in breeding tents containing several broad bean (*Vicia faba*) plants that were rotated out for fresh plants several times a week to maintain healthy conditions. We used aphid clone CWR09/18, collected by Angela Douglas in Freeville, NY, United States in 2009, which does not harbor any endosymbionts other than *Buchnera aphidicola* [35].

#### Bacteria

Bacterial cultures were grown on King's B (KB) media with rifampicin (50 ng/μL). Culture plates were incubated at 27°C and overnight cultures were grown in an incubator shaker set at 27°C and 300 rpm. Strain designations and origins are listed in Table S2. Strains were obtained from Steven Lindow (*Psy* B728a *pvdL* deletion mutant), Alan Collmer (*Psy* B728a, *P. syringae* pv. *tomato* DC3000, and *P. savastanoi* pv. *phaseolicola* 1448a), and David Baltrus (all others).

## METHOD DETAILS

### Choice assays

Choice assays were performed on either whole plants, excised leaf pairs, or with artificial aphid diet. For assays using whole plants, two week old broad bean plants were used. The apical leaf pair of each plant was removed to encourage aphids to choose among fully expanded leaves. Each plant had three leaf pairs distributed in different orientations around the stem of the plant. Within each leaf pair one leaflet was painted with a 10 mM $MgCl_2$ control solution and the other was painted with bacteria suspended in 10 mM $MgCl_2$ solution. Solution was applied to the leaf surface with a sterile cotton swab until the surface was visibly saturated but not dripping. Bacterial suspensions were made from overnight cultures, which were pelleted, washed and resuspended using 10 mM $MgCl_2$ then adjusted to an optical density of 1.0 ($OD_{600}$) using a WPA CO 8000 Cell Density Meter which measures optical density at 600 nm. Leaves were allowed to dry and then approximately 80 mixed age aphid nymphs (not yet reproductive) were introduced on the soil the base of the plant. Plants were caged individually and incubated at 21°C and 80% relative humidity under a light:dark 16:8 hr cycle with full spectrum cool blue fluorescent grow lights (54W T5 fluorescent with 6400K color temperature approximating daylight) positioned above the plants. Pea aphids feed on the undersides of leaves and on stems and we never observed aphids feeding on the upper surfaces of leaves in these experiments. Because broad bean stems would be difficult to paint with bacteria in a controlled manner we focused on aphids feeding on leaves. Our experimental set up mimicked natural conditions in which aphids would be viewing the undersides of leaves, in that they would be encountering the undersides of leaves that are illuminated from above. Placing aphids at the base of the plant forced them to approach each leaf pair via the stem and petiole, so they must choose one leaflet or the other (control or bacteria) from the petiole. The number of aphids feeding on control and bacterial leaves was observed after 4 hr and then every 7-17 hr for three days for a total of 4 time points. Aphids feeding on stems did not participate in the experiment and were not included in analyses. Eight plants were used in each replicate and assays were replicated three times for each strain. By 4 hr, most aphids had settled to feed and we found that plants had 30-70 aphids feeding on leaves. Additional time points were recorded as well, but we found that numbers of aphids on control versus bacterial leaves stayed consistent across the time frame of the experiment, so except when analyzing change over time (Figure S1), we used data from 4 hr for analyses. At least 444 aphids were included in analyses for each strain.

Under dark conditions aphids were greatly delayed in settling to feed on whole plants. Therefore, to test aphid preference under dark and no UV light conditions choice assays were done using excised leaves. Methods for assays on excised leaves followed whole plant assays, except that a single leaf pair was removed from a plant and the petiole was embedded in a wedge of water agar in a Petri dish. Mixed age aphid nymphs were introduced into Petri dishes (not onto the leaf directly) and allowed to crawl onto the underside of the leaf. For assays testing preference in the absence of vision, two growth chambers with the same temperature and humidity were used, with lights turned off in one chamber and the other was illuminated with vertical fluorescent lights fixed in the door. For assays testing the importance of UV light on preference, plates were incubated in the same growth chamber but half of the plates were kept under a box constructed out of UV-filtering plexiglass (OP-3/UF-5), which filters out 98% of UV light. Each assay had five plates in each treatment and each treatment was replicated two (dark) or three (UV) times. Approximately 15-25 aphids per dish settled to feed on leaves during experiments in the dark and approximately 30-50 per dish settled during experiments under UV-filtering, for a total of at least n = 196 per dark/light treatment and n = 664 per UV/UV filtered treatment. The highly virulent and highly avoided strain *Psy* B728a was used for these experiments.

In order to determine if aphids would still avoid virulent strains in the absence of a plant, we performed assays testing for aphid preference between strain *Psy* B728a suspended in artificial aphid diet or artificial aphid diet alone. Bacterial suspensions were prepared as described above and corrected to an $OD_{600}$ of 0.8. This suspension was mixed in a 1:5 ratio with artificial diet [42]. Control diet was made with a similar ratio of 10 mM $MgCl_2$. 96-well plates were divided into 4 quadrants with empty rows in between quadrants. Wells in diagonally positioned quadrants were filled with the same treatment, either bacterial suspension or control, with two quadrants for each treatment. Parafilm was stretched across the plate to make a feeding sachet and this was inverted over a plastic box containing approximately 200 mixed age pea aphid nymphs and secured with parafilm so that the aphids could access all of the feeding sachet wells. The number of aphids feeding in each quadrant was recorded every half hour for 4 hr and then once 20-22 hr later. This assay was replicated three times.

### Light controlled assays

To determine the influence of varied lighting conditions on aphid avoidance of virulent *P. syringae*, we performed choice assays with varied light brightness with and without UV light. These experiments were done with excised leaves in Petri dishes as described above and leaves were treated with either wild-type *Psy* B728a or the pyoverdine deficient mutant of this strain. One leaflet in each leaf pair was painted with bacterial suspensions as detailed above or a control solution of 10 mM $MgCl_2$. For each experiment, 7 plates of each bacterial treatment were placed under UV-filtering plexiglass as above, or under UV-transmitting plexiglass (UVT acrylic, EMCO plastics), which allows for transmission of wavelengths in the UV spectrum. Dishes were elevated approximately 10 cm above a diffusely reflecting, spectrally flat polytetrafluoroethylene surface to allow light exposure on the underside of leaves and fluorescent bulbs were suspended approximately 90 cm above them. Experiments were conducted in high-brightness (8 fluorescent bulbs), medium-brightness (4 bulbs) and low-brightness (2 bulbs) light environments. An additional assay was done using medium-brightness with supplemental UV light from two 60 W fluorescent blacklight bulbs (Adkins Professional lighting) to test for the effect of supplemental UV light. Lastly, in an experiment with high-brightness (8 bulbs) we covered Petri dishes with aluminum

foil so that the leaves were shaded. In this treatment the top surface of the Petri dishes was covered and these were illuminated from above. Since the dishes were elevated above a diffusely reflecting surface the undersides of the leaves, where aphids chose to feed, were exposed only to indirect light and no direct light or light transmitted through the leaves from above. These experiments were performed at 21°C and ambient humidity under a light:dark 16:8 hr cycle and replicated twice for each lighting condition. Aphids feeding on leaves were observed at 4 hr after set up and the minimal sample size was 771 aphids per treatment.

### Infection assay

To test if avoidance leads to a decreased rate of *P. syringae* infection in pea aphids we determined infection rates in aphids that either had a choice between bacterial leaves and control leaves or only had access to bacterial leaves. Aphids given a choice were placed on plants treated as above for on plant assays, with one leaflet per leaf pair painted with *Psy* B728a and the other with 10 mM $MgCl_2$ and the top of the plant removed to discourage settling there. Aphids with no choice were placed on plants with all leaves painted with bacteria. However, more of the top of the plant had been removed so that the total number of leaflets with bacteria, and therefore total bacterial leaf surface area, was similar to choice plants. Aphids were left on plants for 48 hr and then 30 aphids were collected from each plant, spread equally across control and bacterial leaves. Each treatment included two plants and this experiment was replicated twice with a total of 240 aphids sampled. Aphids were individually surface sterilized by washing with 70% ethanol, washed in 10 mM $MgCl_2$, and then homogenized in 100 μL of 10 mM $MgCl_2$. The entire homogenate of each whole aphid was plated onto a KB with rifampicin (50 ng/μL) plate and incubated for 48 hr. Colonies were counted and an aphid was considered positive for infection if greater than five colonies were present.

### Virulence assay

*In vitro* oral pathogenicity assays followed the methods in [9] and utilized the wild-type *Psy* B728a strain as well as a pyoverdine-deficient mutant with a deletion of the gene *pvdL* (supplied by Steven Lindow). Briefly, bacterial suspensions were mixed with artificial aphid diet as described above and 200 μL of the suspension or control diet was placed into each well of a 96-well plate to make a feeding sachet. Individual age-controlled (5 days old, approximately third instar) aphids were placed in wells of a second plate and arranged below the feeding sachet. Aphids were allowed to feed on diet with bacterial suspensions for 24 hr under UV-filtering plastic to keep feeding and infection rates consistent across treatments. After 24 hr, the feeding sachet was replaced with another sachet of sterile diet only. The diet was refreshed again after another 24 and 48 hr. Twice daily, at diet changes and time points between them, aphid death was recorded. An aphid was assumed dead if it had turned brown or was at the bottom of the well (not feeding) and did not move when agitated.

## QUANTIFICATION AND STATISTICAL ANALYSIS

### Avoidance analysis

All statistical analyses were conducted in R version 3.3.1 [36]. For datasets investigating choice assays, response variables were binary counts (choice of a control leaf versus choice of a bacteria-coated leaf; infected or not infected) and so generalized linear mixed-effects models (GLMM) using binomial error distributions were employed from the R package *lme4* [37]. Experimental blocks were included within the GLMMs as random factors. Explanatory variables (in separate models: 1. dark versus natural light, 2. UV block versus natural light, and 3. mutant versus wild-type B728a) were added to null models and their significance tested using likelihood ratio tests of the two models to determine whether or not they had a significant affect on aphids' choices.

To determine whether aphids on plants avoided leaves coated with *P. syringae*, to the extent that significantly more than 50% of the aphids chose the control leaves on the plant, we conducted a GLMM as before, but removed the intercept of the model by including −1 as a variable. This gave us a p value testing the null hypothesis that the probability of avoiding the bacteria was 0.5. To obtain probabilities with confidence intervals from the models, we used the package '*lsmeans*' [38].

In order to demonstrate that pyoverdine is not responsible for aphid death, we conducted survival analysis using log rank tests of pairwise comparisons of Kaplan-Meier survival curves, using the R packages '*survival*' [39] and '*survminer*' [40]. We also conducted a Pearson's product-moment correlation between the virulence of strains and the level of avoidance by aphids (4 hr after introducing them to a plant), with an alternative hypothesis of a positive relationship between the two.

### Reflectance, irradiance, fluorescence analysis

For each of the strains in Table S3, two leaf pairs were painted with a bacterial suspension prepared as described above. Leaves were allowed to dry and the undersides were used for reflectance measurements. We used a UV-VIS spectrometer (FLAME-S-UV-VIS, Ocean Optics, Dunedin, Florida, USA), a pulsed xenon light source (PX2, Ocean Optics), and bifurcated fiber optic measuring probe (which allowed light from the xenon bulb to be directed onto the surface of the leaf and transferring reflected light from the leaf back to the spectrometer) to measure the influence of epiphytic bacterial populations on the wavelengths of light emitted from infected leaves. The spectrometer probe was held at 90° and at a distance of 10mm from the surface of the leaf and measurements were calibrated against a 99% white diffuse reflectance standard (WS-1-SL, Ocean Optics, USA). We averaged 10 scans for each measurement (integration time = 60ms, boxcar width = 20nm), and took 10 measurements per leaf. We used the software OceanView (Ocean Optics) to record reflectance spectra. Additionally, we measured the light environment of our experimental conditions using

a Qstick miniature spectrometer (RGB photonics, Kerlheim, Germany). Five irradiance measurements were collected and averaged per lighting condition to obtain representative irradiance measurements.

Fluorescence excitation/emission matrices were obtained using samples measured in PTI Felix 32 Spectrofluorometer (Photon Technology International) with a LPS-220 lamp power supply in conjunction with FeliX32 Advanced Fluorescence Analysis Software Package. We measured suspensions of wild-type *Psy* B728a bacteria and *pvdL* (pyoverdine-deficient) mutant bacteria suspended in King's B media, as well as a sample containing only media (which we subsequently subtracted from our fluorescence measures of the bacteria). We collected emission data at 1 nm intervals from single excitation wavelengths spanning 340-425 nm, spaced at 5nm intervals (and interpolated our matrix to 1 nm resolution in the excitation wavelength axis).

## Visual modeling

We employed a visual modeling approach to estimate how leaves coated with different strains of epiphytic bacteria might appear to aphids. Specifically, we implemented a noise-receptor model estimate [43] of chromatic contrasts using package *Pavo* in R [41] by first calculating receptor-specific quantum catch values $Q_i$, where $i$ denotes one of the three classes of aphid photoreceptors (UV = ultraviolet, B = blue, G = green; Figure S4) as:

$$Q_i = \int_{300}^{700} I(\lambda) S_i(\lambda) R(\lambda) d\lambda$$

where $I(\lambda)$ is the illumination spectrum, $S_i(\lambda)$ is the spectral sensitivity function of receptor $i$ (Figure S4), and $R(\lambda)$ is the reflectance spectrum of the leaf. In this case, rather than the reflectance spectra, we used normalized excitance curves to estimate the combined influence of reflectance and fluorescence on the appearance of bacterial-coated leaves. Additionally, we used ideal illumination spectra (i.e., irradiance = 1 at all wavelengths) for our primary analyses. After obtaining quantum catch values for each photoreceptor for each color, we then calculated chromatic contrasts between every pairwise combination of measurements in units of Just Noticeable Differences (JNDs) using the equation:

$$\Delta S = \sqrt{\frac{e_{UV}^2(\Delta f_G - \Delta f_B)^2 + e_B^2(\Delta f_G - \Delta f_{UV})^2 + e_G^2(\Delta f_{UV} - \Delta f_B)^2}{(e_{UV}e_B)^2 + (e_{UV}e_G)^2 + (e_B e_G)^2}}$$

where $\Delta f_i$ is the difference in log of quantum catches for receptor $i$ between color pairs and $e_i$ is the internal receptor noise for each receptor class (modeled uniformly as 0.1), implemented via the *coldist* function in *Pavo*. Using the chromatic contrasts among all color measurements, we next calculated the Cartesian coordinates for each color measurement in "aphid color space" using scripts written by Thomas Pike [44], wherein perceptual distances between colors are preserved regardless of directionality and correspond to the Euclidean distance between points. Within this color space, distance from the achromatic origin (white, gray, black) provides a measure of chroma, and the angle ($\theta$) between the vector connecting the achromatic origin and pure UV cone stimulation and the vector connecting the achromatic origin and the Cartesian coordinates of a given color provides a measure of hue (*sensu* [45]; Figure S4).

Following quantification of visual parameters, we compared the appearance of the different strains using linear mixed models with leaves as random variables with the package *lme4* [37] in R [36]. Additionally, we conducted post hoc Tukey tests with the *glht* function in the *multcomp* package [46].