Mechanistic modeling of light-induced chemotactic infiltration of bacteria into leaf stomata
(Supporting Information)

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Biological aspects

The model includes various biological factors related to plant and bacteria, and they are briefly discussed here. These details are only to provide an idea about the underlying subcellular events that have been included in the modeling framework presented in this manuscript.

Leaf

A typical leaf section is shown in Fig. Aa. It includes a mesophyll tissue in the middle and two epidermis layers at top and bottom. The leaf surfaces are covered by a waxy cuticle. Stomatal openings are responsible for selective mass exchange between the leaf tissue and outside environment based on various biotic and abiotic environmental conditions [2-4]. Inside plant cells (Fig. Ab), the cytoplasm is covered by a plasma membrane and a cell wall. The majority of the cytoplasm volume is occupied by vacuoles that mainly contain water. Guard cells and mesophyll cells contain chloroplasts that are responsible for photosynthesis (production of organic nutrients). All cells within the leaf section have mitochondria that are responsible for respiration (production of energy from oxidation of organic nutrients). Water and nutrients can move from one cell to another through plasmodesmata, known as symplastic transport. In contrast, mainly in apoplastic phloem loaders such as spinach leaves, nutrients are also available in the apoplastic region [5]. Here, any water outside of the plasma membrane is called free water whereas intracellular water is called bound water. During photosynthesis (Fig. Ac), light reactions occur within thylakoid membranes in which water is consumed as an electron donor and releases oxygen, hydrogen ions and electrons. The electrons are supplied to two successive electron transport chains that end up with production of ATP and NADPH to contribute in carbon fixation. Calvin cycle uses these molecules to convert carbon dioxide (CO₂) to triose phosphates (TPs) (i.e., three-carbon sugar-phosphate molecule) that are building blocks for sugar production. The enzyme that catalyzes this carbon fixation step is RuBP carboxilase/oxygenase, or RuBisCO. The cycle runs three times to generate one molecule of triose-phosphate that is then provided to the plant to synthesize starch and sugars (e.g., sucrose).
Figure A: a) A leaf cross section with b) an illustration of cell organs. c) A schematic of a chloroplast with various functions that lead to photosynthesis in plant cells. The chloroplast image inspired by Campbell et al. [1]. Note that only organs and functions that are of interest in the present study are shown.
If the concentration of CO₂ declines, RuBisCO can bind oxygen molecules (O₂) in place of CO₂. This process is called photorespiration which does not generate ATP. See Campbell et al. [1] for more details.

Sucrose is synthesized in cytoplasm from TPs coming from chloroplast. In apoplastic phloem loaders, sucrose/glucose diffuse into the apoplast, mediated by SWEET transporter proteins located at the cell plasma membrane [6], and then actively transported into sieve elements via SUT1 sucrose transporter [7,8]. In the appoplast (and also within cells), invertase enzymes hydrolyse sucrose into glucose and fructose where they can be accessible by microorganisms present at the intercellular spaces of the leaf tissue [9].

Bacteria

The underlying pathways related to transport and growth of bacteria (i.e., E. coli) are shown in Fig. B. Note that these detailed subcellular pathways are shown here only to provide an idea about the underlying events that lead to bacterial behaviors (motility, chemotaxis, AI-2 synthesis and glucose uptake) that have been included in the modeling framework presented in this manuscript. Bacterial motility (Fig. Ba) is referred to as the random tumbling motion of a bacterium cell due to rapid changes in the direction of flagellar rotation. At the cytoplasmic membrane of bacteria, several chemoreceptors called methyl-accepting chemotaxis proteins (MCPs) exist that are sensitive to different extracellular molecules (i.e., ligands). While there is no external molecule bound to the MCPs, bacterium continues the tumbling motion. If an external attractant molecule binds to the MCPs (Fig. Bb), bacterium continues to swim toward the attractive chemicals (e.g., glucose, AI-2, O₂) [10]. Bacteria communicate by producing and sensing signaling molecules. The intercellular signaling known as quorum sensing (QS) allows bacteria to regulate production of gene products, such as enzymes, and coordinate behavioral responses at high cell densities [14]. Among various QS systems discovered in E. coli [15], auto-inducer 2 (AI-2) signaling pathway is considered here (Fig. Bc). AI-2 has a key role in quorum sensing and biofilm formation in E. coli. AI-2 is a chemoattractant for E. coli in a process mediated by LsrB binding protein and Tsr chemoreceptor
Figure B: A brief illustration of the underlying pathways leading to a) bacterial cell motility [10], b) chemotaxis [10,11], c) AI-2 synthesis [12], and d) glucose uptake by bacteria [13].
It enhances bacterial chemotaxis toward external attractants [11] like glucose and oxygen.

Bacterial growth is often limited by availability of nutrients. In *E. coli*, the growth limiting nutrients [16] can be sources of carbon (e.g., glucose), nitrogen (e.g., ammonium), phosphorus (inorganic phosphate), etc. Oxygen can also affect the growth of *E. coli* as a facultative anaerobe [17]. Most of the microorganisms, including *E. coli*, prefer glucose as their primary carbon source [18]. If sufficient glucose is present in the growth medium, synthesis of the enzymes needed for transport and metabolism of the less favorable sugars will be repressed. This phenomena is called carbon catabolite repression (CCR) [13]. Glucose (and many other carbohydrates) is assimilated by bacteria through the phosphotransferase system (PTS) (Fig. Bd) [17].

**Model overview**

An overview of the model, including all involved species and their interconnections are shown in Fig. C.

**Derivation of transport equations for CO₂ and O₂**

Transport of species, *i*, (i.e., CO₂ and O₂) in the gas and water phases are governed by:

\[ S_y \phi \frac{\partial c_{i,g}}{\partial t} = \nabla \cdot (S_y \phi D_{i,g} \nabla c_{i,g}) \]  
(S1)

\[ (S_{wf} + S_{wb}) \phi \frac{\partial c_{i,w}}{\partial t} = \nabla \cdot ((S_{wf} + S_{wb}) \phi D_{i,w} \nabla c_{i,w}) + (S_{wf} + S_{wb}) \phi R_{i,w} \]  
(S2)

where \( R_{i,w} \) is an arbitrary source term (mol/m³ · s). Assuming the equilibrium between gas and water phases to be described by Henry’s law [19]:

\[ c_{i,w} = RTK_{H,i} c_{i,g} \]  
(S3)
and plugging into Eq. 4, the total concentration of each species in the REV is defined as:

\[ c_i = (S_g + (S_{wf} + S_{wb})RTK_{H,i}) \phi c_{i,g} \]  

(S4)

Using Eq. S4, Eq. S2 can be rewritten as:

\[
\begin{align*}
(S_g + (S_{wf} + S_{wb})RTK_{H,i}) \phi \frac{\partial c_{i,g}}{\partial t} &= \nabla \cdot \left( (S_g D_{i,g} + (S_{wf} + S_{wb})D_{i,w}RTK_{H,i}) \phi \nabla c_{i,g} \right) \\
&+ (S_{wf} + S_{wb}) \phi R_{i,w}
\end{align*}
\]  

(S5)

Finally, by adding Eq. S1 and Eq. S5 and applying Eq. S4, the combined transport equation in the REV is obtained as:

\[
\frac{\partial c_i}{\partial t} = \nabla \cdot (D_{i,eff} \nabla c_i) + (S_{wf} + S_{wb}) \phi R_{i,w}
\]  

(S6)

where \( D_{i,eff} \) is effective diffusivity of species \( i \) in the porous media:

\[
D_{i,eff} = \frac{S_g D_{i,g} + (S_{wf} + S_{wb})D_{i,w}RTK_{H,i}}{S_g + (S_{wf} + S_{wb})RTK_{H,i}}
\]  

(S7)
Input parameters of the model

Input data for the simulations are shown in Table A. Details of some of the input parameters are discussed here.

**CO₂ assimilation parameters**

**RuBisCO-limited**: Temperature dependence of kinetics parameters of RuBisCO (Eq. 16) including $V_{c,max}$, $K_{m,co₂}$ and $K_{m,o₂}$ can be described by Arrhenius equations [45]. The activation energy for the maximum carboxilation capacity of RuBisCO, $V_{c,max}$, in spinach leaves was 64900 J/mol. Based on the data of Yamori et al. [46] at 25°C, the maximum carboxilation capacity (mol/m³ · s) was calculated as:

$$V_{c,max} = 49.9 \times 10^{-6} \exp \left( \frac{64900(T - 298)}{298RT} \right) \alpha_t$$  \hspace{1cm} (S8)

Here, $\alpha_t$ is the reciprocal of the leaf thickness (1/m), which is used to get a volumetric value for $V_{c,max}$. The temperature dependence of the Michaelis-Menten constants of RuBisCO (Pa), within a range of 5-40 °C, were calculated as [47]:

$$K_{m,co₂} = 27 \exp \left( \frac{80990(T - 298)}{298RT} \right)$$  \hspace{1cm} (S9)

$$K_{m,o₂} = 16500 \exp \left( \frac{23720(T - 298)}{298RT} \right)$$  \hspace{1cm} (S10)

The CO₂ compensation point without dark respiration (Pa), $Γ^*$, is defined as the partial pressure of CO₂ at which no net assimilations occur [47]. The temperature dependence of $Γ^*$ was reported by Medlyn et al. [48] for spinach leaves:

$$Γ^* = 0.101325 \left( 42.7 + 1.68(T - 298.15) + 0.0012(T - 298.15)^2 \right)$$  \hspace{1cm} (S11)
Figure C: A big picture of the model showing the interconnection between all species via reaction terms. Note that free water refers to the intercellular water, and bound water refers to intracellular water.
**Electron transport-limited:** The volumetric rate of electron transport (in Eq. 17) (mol/m$^3$.s), $J$, can be described in terms of light limited, $J_{ll}$, and light saturated, $J_{ls}$, rates of electron transport in PS II [49]:

$$J = \frac{J_{ll} + J_{ls} - \sqrt{(J_{ll} + J_{ls})^2 - 4\theta J_{ll}J_{ls}}}{2\theta}$$

where $\theta = 0.97$. The light limited rate of electron transport (mol/m$^2$·s), $J_{ll}$, is determined from the amount of the available light (mol/m$^2$·s), $I$, to be absorbed by the chlorophyll pigments that can vary by the light wavelength [20]:

$$J_{ll} = \alpha_{PSII}\Phi_{PSII}I$$

where $\alpha_{PSII} = 0.5$ is the fraction of absorbed photons driving PS II electron transport, and $\Phi_{PSII} = 0.85$ mol/mol is the maximum quantum efficiency of PS II in electron transport. The distribution of light within the leaf tissue was calculated using Beer-Lambert’s law:

$$\frac{\partial I}{\partial z} = a_{chl}I$$

where $a_{chl}$ is the absorption coefficient of chlorophyll $a$ which depends on the specific absorption (m$^2$/mol) [20,34], $a_{chl}^*$, and the density of chlorophyll $a$ within the leaf tissue (mol/m$^3$), $\rho_{chl}$:

$$a_{chl} = a_{chl}^*\rho_{chl}$$

The profile of chlorophyll density within spinach leaves was obtained from Vogelmann and Evans [20] who measured the chlorophyll fluorescence profiles within spinach leaves (Fig. Da).

The light saturated rate of electron transport (mol/m$^2$·s) is defined as:

$$J_{ls} = \beta J_{max}$$

where $\beta$ is defined here as the relative photosynthetic capacity whose profile was obtained from
Figure D: Profiles of a) chlorophyll density and b) relative photosynthetic capacity within spinach leaves, inspired from Vogelmann and Evans [20] and Evans and Vogelman [50], respectively.

Evans and Vogelman [20] (Fig. Db), and $J_{max}$ is the maximum rate of electron transport (mol/m$^2$·s), and can be calculated by [51]:

$$J_{max} = 1 \times 10^6 \frac{\exp\left(37.08 - \frac{79500}{RT}\right)}{1 + \exp\left(\frac{650T - 201000}{RT}\right)}$$  \hspace{1cm} (S17)

**TPU-limited:** A value of $9.19 \times 10^{-6}$mol/m$^2$·s was adopted for the TPU rate (in Eq. 18) (mol/m$^2$·s), $T_p$ [19]. So, the volumetric value of TPU rate is:

$$T_p^* = T_p \alpha_t$$ \hspace{1cm} (S18)
| Parameter                          | Symbol   | Value                   | Units | Source                          |
|-----------------------------------|----------|-------------------------|-------|---------------------------------|
| **Dimensions**                   |          |                         |       |                                 |
| Typical values for stomatal size | $D_{stoma}$ | $4 \times 10^{-6}$, in dark | m     | Assumed from measurements       |
|                                   |          | $16 \times 10^{-6}$, in blue light | m     | Assumed from measurements       |
|                                   |          | $14 \times 10^{-6}$, in white light | m     | Assumed from measurements       |
|                                   |          | $12 \times 10^{-6}$, in red light | m     | Assumed from measurements       |
|                                   |          | $9 \times 10^{-6}$, in green light | m     | Assumed from measurements       |
| Depth of leaf section            | $H_{leaf}$ | $700 \times 10^{-6}$ | m     | Vogelmann and Evans [20]        |
| Depth of spongy mesophyll layer  | $H_{spg}$ | $370 \times 10^{-6}$ | m     | Assumed                        |
| Depth of palisade mesophyll layer| $H_{pls}$ | $270 \times 10^{-6}$ | m     | Assumed                        |
| Depth of epidermis layer         | $H_{stoma}$ | $30 \times 10^{-6}$ | m     | Assumed                        |
| Width of the domain              | $W_{leaf}$ | $80 \times 10^{-6}$ | m     | Assumed                        |
| **Density**                      |          |                         |       |                                 |
| Carbon dioxide                   | $\rho_{CO_2}$ | Ideal gas | kg/m$^3$ | Assumed                        |
| Oxygen                            | $\rho_{O_2}$ | Ideal gas | kg/m$^3$ | Assumed                        |
### Diffusivity

|                  | \( \eta \text{bac,mot} \) | \( 1 \times 10^{-12} \) | m\(^2\)/s | Wu et al. [21] |
|------------------|-----------------------------|--------------------------|----------|----------------|
| Motile-only bacteria in water | \( \eta \text{bac,wt} \) | \( 5 \times 10^{-11} \) | m\(^2\)/s | Wu et al. [21] |
| Wild type bacteria in water | \( D_{\text{CO}_2,g} \) | \( 1.6 \times 10^{-5} \) | m\(^2\)/s | Lide [22] |
| Carbon dioxide in air | \( D_{\text{O}_2,g} \) | \( 1.6 \times 10^{-5} \) | m\(^2\)/s | Lide [22] |
| Oxygen in air | \( D_{\text{CO}_2,w} \) | \( 1.67 \times 10^{-9} \) | m\(^2\)/s | Lide [22] |
| Carbon dioxide in water | \( D_{\text{O}_2,w} \) | \( 2.01 \times 10^{-9} \) | m\(^2\)/s | Lide [22] |
| Oxygen in water | \( D_{\text{HCO}_3,w} \) | \( 1.17 \times 10^{-9} \) | m\(^2\)/s | Geers and Gros [23] |
| HCO\(_3^-\) in water | \( D_{\text{gluc},w} \) | \( 0.67 \times 10^{-9} \) | m\(^2\)/s | Nobel [24] |
| Glucose in water | \( D_{\text{suc},w} \) | \( 0.52 \times 10^{-9} \) | m\(^2\)/s | Nobel [24] |
| Sucrose in water | \( D_{\text{AI2},w} \) | \( 1 \times 10^{-10} \) | m\(^2\)/s | Assumed from Stewart [25] |
| AI-2 in water | \( \phi \) | 0.95 | m\(^3\)/m\(^3\) | Ranjbaran and Datta [26] |
| Porosity | \( \alpha_p \) | \( 1 \times 10^{5} \) | m\(^2\)/m\(^3\) | Calculated from Rah- man [27] |
| Specific surface area of porous zone | \( A_{\text{SWEET}} \) | \( 1 \times 10^{-19} \) | m\(^2\) | Calculated |
| Half saturation constant | \( K_{SWEET} \) | mol/m\(^3\) | Assumed from Chen et al. [30] |
|--------------------------|----------------|-----------|-----------------------------|

**Saturations**

|                      | \( S_{g,spg} \) | m\(^3\)/m\(^3\) | Ranjbaran and Datta [26] |
|----------------------|----------------|----------------|-----------------------------|
| Spongy mesophyll, gas | 0.41           |               |                             |
| Spongy mesophyll, free water | 0.15       | m\(^3\)/m\(^3\) | Ranjbaran and Datta [26] |
| Spongy mesophyll, bound water | 0.44        | m\(^3\)/m\(^3\) | Ranjbaran and Datta [26] |
| Palisade mesophyll, gas | 0.20           | m\(^3\)/m\(^3\) | Ranjbaran and Datta [26] |
| Palisade mesophyll, free water | 0.15        | m\(^3\)/m\(^3\) | Ranjbaran and Datta [26] |
| Palisade mesophyll, bound water | 0.65        | m\(^3\)/m\(^3\) | Ranjbaran and Datta [26] |
| Epidermis layer, gas | 0.20           | m\(^3\)/m\(^3\) | Assumed                     |
| Epidermis layer, free water | 0.15         | m\(^3\)/m\(^3\) | Assumed                     |
| Epidermis layer, bound water | 0.65         | m\(^3\)/m\(^3\) | Assumed                     |
| Stomatal cavity, gas | 0.90           | m\(^3\)/m\(^3\) | Assumed                     |
| Stomatal cavity, free water | 0.10         | m\(^3\)/m\(^3\) | Assumed                     |

**Sub-saturation coefficients**

|                      | \( \gamma_{chl,mes} \) | m\(^3\)/m\(^3\) | Winter et al. [31] |
|----------------------|----------------         |----------------|-----------------------------|
| Chloroplast, mesophyll | 0.254                |               |                             |
| Chloroplast, guard cell | 0.254               | m\(^3\)/m\(^3\) | Assumed                     |
| Chloroplast, epidermis | 0                   | m\(^3\)/m\(^3\) | Assumed                     |
| Mitochondria         | 0.0082                | m\(^3\)/m\(^3\) | Winter et al. [31]          |
| **Gas solubility in water** | $K_{H, CO_2}$ | $0.3876 \times 10^{-3}$ | mol/m$^3$·Pa | Lide [22] |
|-----------------------------|---------------|--------------------------|--------------|-----------|
| Henry’s constant for CO$_2$, at 20 °C | $K_{H, O_2}$ | $0.0137 \times 10^{-3}$ | mol/m$^3$·Pa | Lide [22] |
| Henry’s constant for O$_2$, at 20 °C | | | | |

| **Carbon dioxide hydration** | | | | |
|--------------------------------|--------------------------------|--------------------------|--------------|
| Reaction constant 1 | $k_1$ | 0.039 | 1/s | Jolly [32] |
| Reaction constant 2 | $k_2$ | 23 | 1/s | Jolly [32] |
| Acid dissociation constant for H$_2$CO$_3$ | $K_{hyd}$ | 0.25 | mol/m$^3$ | Jolly [32] |
| pH | | | | |
| pH | | | | |
| 7.0, Water | | | | |
| 6.5, Leaf | | | | |

| **Light absorption by chloroplasts** | | | | |
|--------------------------------------|--------------------------------|--------------------------|--------------|
| Specific absorption, Blue light | $a_{chl, blu}^*$ | 2600 | m$^2$/mol | Assumed from Vogelmann and Evans [20] |
| Specific absorption, Green light | $a_{chl, grn}^*$ | 1500 | m$^2$/mol | Assumed from Vogelmann and Evans [20] |
| Specific absorption, Red light | $a_{chl, red}^*$ | 2000 | m$^2$/mol | Assumed from Mitchell and Kiefer [34] and Vogelmann and Evans [20] |
| Specific absorption, White light | $a_{chl, whit}^*$ | 2100 | m$^2$/mol | Assumed |
| **Nutrient uptake by bacteria** |  |  |  |
|--------------------------------|-----------------------------|-------------------|---|
| Yield of glucose on bacteria  | $Y_{\text{gluc/bac}}$      | $9.4 \times 10^{-15}$ | mol/cell | Assumed from Kayser et al. [35] |
| Yield of oxygen on bacteria   | $Y_{\text{o2/bac}}$        | $2.9 \times 10^{-14}$ | mol/cell | Assumed from Shiloach and Fass [36] |

| **AI-2 reaction** |  |  |  |
|-------------------|-----------------------------|-------------------|---|
| Synthesis rate constant $k_{1,AI2}$ | $1 \times 10^{-24}$, no glucose | mol/cell · s | Li et al. [37], Xu et al. [38] and Wang et al. [39] |
|                   | $1 \times 10^{-23}$, with glucose | mol/cell · s |  |

| **Bacterial chemotaxis** |  |  |  |
|--------------------------|-----------------------------|-------------------|---|
| Chemotactic sensitivity coefficient, $E. coli$ $\chi_0$ | $10 \times 10^{-9}$ | m$^2$/s | Ford et al. [40] |
| Receptor-ligand dissociation constant, glucose $K_{d,\text{gluc}}$ | 0.1 | mol/m$^3$ | Ford and Lauffenburger [41] |
| Receptor-ligand dissociation constant, oxygen $K_{d,o2}$ | 0.013 | mol/m$^3$ | Delgado-Nixon et al. [42] |
| Receptor-ligand dissociation constant, AI-2 $K_{d,AI2}$ | 0.007 | mol/m$^3$ | Assumed from Jani et al. [12] |

| **Bacteria growth** |  |  |  |
|---------------------|-----------------------------|-------------------|---|
| Maximum growth rate constant, at 25 °C $\zeta_{\text{max,gr}}$ | $1.00 \times 10^{-4}$ | 1/s | Assumed from Kovarova et al. [43] |
| Monod half saturation constant, glucose $K_{\text{gluc}}$ | $184.8 \times 10^{-6}$ | mol/m$^3$ | Kovarova et al. [43] |
| Monod half saturation constant, Oxygen | $K_{o_2}$ | $121 \times 10^{-6}$ | mol/m$^3$ | Stolper et al. [44] |
|--------------------------------------|----------|----------------------|-----------|---------------------|

**Experimental procedure**

**Bacterial strains and inoculum preparation**

A loop of frozen (at -80 °C) culture of *E. coli* cells (wild type ampicillin-resistant K-12 MG1655 carrying pUC19 plasmid, or incessantly motile (but not chemotactic) kanamycin-resistant K-12 BW25113 ($\Delta$ CheZ)) were grown in sterile LB broth (a 25 g/l of LB powder dissolved in Milli-Q water), supplemented with 100 $\mu$g/ml ampicillin or 30 $\mu$g/ml kanamycin, in a shaker incubator at 37 °C and 220 rpm. This was followed by a second overnight incubation in fresh LB broth. The bacterial culture were then harvested by two successive centrifugation steps (Sorvall legend RT+centrifuge, Thermo Scientific, USA) at 2700 g for 10 min to efficiently remove the LB broth. The cell pellets were resuspended in sterile 0.85% NaCl (saline) solution and the concentration of final bacterial populations was adjusted to $\sim 10^8$ CFU/ml by using spectrophotometry analysis (Agilent Technologies, Inc., Mattapoiset, MA).

**Leaf inoculation and light exposure**

Experimental procedure to determine total amount of infiltration is shown in Fig. E. Fresh baby spinach leaves were purchased at a local retail store. Samples of 5 g containing about 8 leaves were used. The leaves were placed in sterile petri dishes with cover lids and were kept at room temperature for about 45 min under the illumination condition that was under study. This was done to make sure that the leaf microstructures were in equilibrium with the illumination condition. Then the samples were spot inoculated with 500 $\mu$l of *E. coli* cell suspension to reach an initial population of $\sim 3 \times 10^7$ CFU/g. The inoculum was then gently spread on the leaf surface using a sterile loop to uniformly cover the surface. The lid of petri dishes were placed to avoid evaporation.
of the inoculum at the leaf surface. The samples were exposed to white/blue/red/green light with
intensity of 100 $\mu$mol/m$^2$.s, or kept in the dark. All exposures were performed at room temperature
for 2 h. The light intensity was measured by a digital luxmeter (PM6612, Peak Meter R⃝, China)
with ±3% accuracy. The illumination chamber was shielded thoroughly to make sure that the
samples would only receive the expected wavelengths.

**Bacterial infiltration assay**

After exposure treatment, the surface of the leaves was washed by sterile 0.85% NaCl (saline)
solution and left to dry. Then, they were sprayed with 70% ethanol in two successive steps to
remove any surface bacteria. The surface sterilization was examined (Fig. F) by gently pushing
the surface of some treated leaves on LB agar plates and incubate them. The surface sanitation
was considered effective when more than 99.99999% of inoculated bacteria at the surface were
inactivated (about 8-log CFU/ml reduction).

The surface sterilized leaves of each sample were crushed in a sterile bowl and was added with
45 ml sterile 0.85% NaCl (saline) solution. The homogenized sample was serially diluted in sterile
0.85% NaCl (saline) solution and surface plated onto LB agar containing 100 $\mu$g/ml ampicillin
or 30 $\mu$g/ml kanamycin. To make sure that the natural microbiota on the leaf surface were not
growing in the growth medium containing ampicillin or kanamycin, control samples without inoc-
ulation were also homogenized and plated. The inoculated petri dishes were incubated at 30°C
for 24 h and the colonies were enumerated to find the bacterial count. No growth was observed in
any of the uninoculated plates (Fig. G) implying that the natural microbiota were not ampicillin or
kanamycin resistant and would not be counted as infiltrated bacteria.

**Microscopy imaging of stomatal aperture**

The microscopy imaging experiments were done using an epi-fluorescent microscope (DM5500,
Leica Microsystems, Exton, PA, USA) with 20x or 63x water immersion objectives. Before
microscopy, three leaves were either exposed to white/blue/red/green light with an intensity of
Figure E: Experimental procedure to determine total amount of infiltration.
### Bacteria: ampicillin-resistant *E. coli* K-12 MG1655

| Test  | Illumination condition | Number of colonies detected | Dilution factor | Leaf side | Number of bacteria at the leaf surface after surface sanitation (CFU) |
|-------|------------------------|----------------------------|-----------------|-----------|---------------------------------------------------------------------|
| **Test 1** | White 100 (μmol/m².s) | 3                          | 1/1             | Adaxial   | 3                                                                   |
| **Test 2** | Green 100 (μmol/m².s) | 1                          | 1/1             | Adaxial   | 1                                                                   |
| **Test 3** | Green 100 (μmol/m².s) | 0                          | 1/1             | Adaxial   | 0                                                                   |

**Figure F:** Results of the leaf surface sanitation treatments. The inoculated surface of the leaves were washed by sterile 0.85% NaCl (saline), after 2 h illumination, and let to dry. Then, they were sprayed with 70% ethanol in two successive steps to remove any surface bacteria.
Figure G: Control tests to make sure the natural microbiota on the leaf surface were not growing in the growth medium containing ampicillin or kanamycin.
100 µmol/m²·s, or were kept in the dark for 45 min. After illumination, samples (5 mm × 5 mm) from three arbitrary locations of each leaf were cut and immediately used for microscopy. For each experimental condition, data of stomatal aperture were gathered from more than 100 stomata. The measurements were done using ImageJ software. The results of measurements of the stomatal aperture when using various light wavelengths as well as dark condition, are shown in Fig. H.
Figure H: Measured stomatal aperture of spinach leaves under different light colors of 100 \( \mu \text{mol/m}^2 \cdot \text{s} \), and dark condition for 45 min. Each data presents an average of 100 measurements of the stomatal aperture. The error bars show the standard deviations. Representative confocal microscopy images related to dark condition (smallest aperture) and blue light illumination condition (widest aperture) are shown as insets. Red arrows in the inset images show the location of the stomata.

Results

Measured microbial infiltration into spinach leaves

The results of the measurement of wild-type bacterial infiltration into adaxial side of spinach leaves are shown in Fig. I for dark condition, and in Fig. J for white light, Fig. K for blue light, Fig. L for green light, and Fig. M for red light, with light intensity of 100 \( \mu \text{mol/m}^2 \cdot \text{s} \). The corresponding results for infiltration of CheZ mutant bacteria under white light of the same intensity are shown in Fig. N. Also, the corresponding results for infiltration of wild-type bacteria under white light of the same intensity into the abaxial side of the spinach leaves are shown in Fig. O. Evidence presented here are to support data shown in Fig. 4c-4e and Fig. 5a.
| Test   | Illumination condition | Number of colonies detected | Dilution factor | Leaf side | Leaf sample weight (g) | Number of bacterial infiltration into the leaves (CFU/g) | Bacterial infiltration (log CFU/g) |
|--------|------------------------|----------------------------|----------------|-----------|------------------------|-------------------------------------------------------|----------------------------------|
| Test 1 | Dark                   | 12                         | 1/500          | Adaxial   | 5.06                   | 2400                                                  | 3.38                             |
| Test 2 | Dark                   | 3                          | 1/500          | Adaxial   | 5.05                   | 600                                                   | 2.78                             |
| Test 3 | Dark                   | 9                          | 1/500          | Adaxial   | 5.01                   | 1800                                                  | 3.26                             |
| Mean   |                        |                            |                |           |                        | 1600                                                  | 3.13                             |
| Standard deviation |                        |                            |                |           |                        | 748.3                                                 | 0.26                             |

Figure I: Results of the colony growth of ampicillin-resistant *E. coli* K-12 MG1655 on LB-agar medium containing 100 µg/ml ampicillin. The inoculated leaves were kept in dark condition for 2 h.
### Bacteria: ampicillin-resistant *E. coli* K-12 MG1655

| Test  | Illumination condition | Number of colonies detected | Dilution factor | Leaf side | Leaf sample weight (g) | Number of bacterial infiltration into the leaves (CFU/g) | Bacterial infiltration (log CFU/g) |
|-------|------------------------|-----------------------------|----------------|-----------|------------------------|--------------------------------------------------------|----------------------------------|
| Test 1 | White 100 (µmol/m².s)  | 14                          | 1/1000         | Adaxial   | 5.015                  | 5600                                                   | 3.75                             |
| Test 2 | White 100 (µmol/m².s)  | 39                          | 1/1000         | Adaxial   | 5.011                  | 15600                                                  | 4.19                             |
| Test 3 | White 100 (µmol/m².s)  | 54                          | 1/1000         | Adaxial   | 5.013                  | 21600                                                  | 4.33                             |
| Mean   |                         |                             |                |           |                        | 14266.7                                                | 4.09                             |
| Standard deviation |                   |                             |                |           |                        | 6599.7                                                 | 0.25                             |

**Figure J:** Results of the colony growth of ampicillin-resistant *E. coli* K-12 MG1655 on LB-agar medium containing 100 µg/ml ampicillin. The inoculated leaves were exposed to white light, from adaxial side, with an intensity of 100 µmol/m² · s for 2 h.
### Test 1

<image of petri dish with bacterial colonies>

### Test 2

<image of petri dish with bacterial colonies>

### Test 3

<image of petri dish with bacterial colonies>

### Test 4

<image of petri dish with bacterial colonies>

#### a.

**Bacteria: ampicillin-resistant *E. coli* K-12 MG1655**

| Test   | Illumination condition | Number of colonies detected | Dilution factor | Leaf side | Leaf sample weight (g) | Number of bacterial infiltration into the leaves (CFU/g) | Bacterial infiltration (log CFU/g) |
|--------|------------------------|----------------------------|-----------------|-----------|------------------------|-------------------------------------------------|----------------------------------|
| Test 1 | Blue 100 (µmol/m².s)   | 1978                       | 1/500           | Adaxial   | 5.012                  | 395600                                         | 5.60                             |
| Test 2 | Blue 100 (µmol/m².s)   | 887                        | 1/500           | Adaxial   | 5.010                  | 177400                                         | 5.25                             |
| Test 3 | Blue 100 (µmol/m².s)   | 861                        | 1/500           | Adaxial   | 5.021                  | 172200                                         | 5.23                             |
| Test 4 | Blue 100 (µmol/m².s)   | 170                        | 1/500           | Adaxial   | 5.011                  | 34000                                          | 4.53                             |
| Mean   |                         |                            |                 |           |                        | 194800                                         | 5.15                             |
| Standard deviation |                |                            |                 |           |                        | 129413                                         | 0.39                             |

#### b.

Figure K: Results of the colony growth of ampicillin-resistant *E. coli* K-12 MG1655 on LB-agar medium containing 100 µg/ml ampicillin. The inoculated leaves were exposed to blue light, from adaxial side, with an intensity of 100 µmol/m² · s for 2 h.
| Test 1 | Test 2 | Test 3 |
|--------|--------|--------|
| **Illumination condition** | Green 100 (μmol/m².s) | Green 100 (μmol/m².s) | Green 100 (μmol/m².s) |
| **Number of colonies detected** | 13 | 11 | 20 |
| **Dilution factor** | 1/500 | 1/500 | 1/500 |
| **Leaf side** | Adaxial | Adaxial | Adaxial |
| **Leaf sample weight (g)** | 5.051 | 5.018 | 5.008 |
| **Number of bacterial infiltration into the leaves (CFU/g)** | 2600 | 2200 | 4000 |
| **Bacterial infiltration (log CFU/g)** | 3.42 | 3.34 | 3.60 |

**Mean**

| Test 1 | Test 2 | Test 3 |
|--------|--------|--------|
| **Number of colonies detected** | | | |
| **Dilution factor** | | | |
| **Leaf side** | | | |
| **Leaf sample weight (g)** | | | |
| **Number of bacterial infiltration into the leaves (CFU/g)** | | | |
| **Bacterial infiltration (log CFU/g)** | | | |

| **Mean** | **Standard deviation** |
|----------|------------------------|
| 2933.3 | 771.7 |
| 3.45 | 0.11 |

**Bacteria: ampicillin-resistant *E. coli* K-12 MG1655**

Figure L: Results of the colony growth of ampicillin-resistant *E. coli* K-12 MG1655 on LB-agar medium containing 100 µg/ml ampicillin. The inoculated leaves were exposed to green light, from adaxial side, with an intensity of 100 µmol/m² · s for 2 h.
### a.

**Bacteria: ampicillin-resistant *E. coli* K-12 MG1655**

| Test | Illumination condition | Number of colonies detected | Dilution factor | Leaf side | Leaf sample weight (g) | Number of bacterial infiltration into the leaves (CFU/g) | Bacterial infiltration (log CFU/g) |
|------|------------------------|----------------------------|-----------------|-----------|------------------------|--------------------------------------------------------|-----------------------------------|
| Test 1 | Red 100 (µmol/m².s) | 60 | 1/500 | Adaxial | 5.010 | 12000 | 4.08 |
| Test 2 | Red 100 (µmol/m².s) | 27 | 1/500 | Adaxial | 5.021 | 5400 | 3.73 |
| Test 3 | Red 100 (µmol/m².s) | 32 | 1/500 | Adaxial | 5.001 | 6400 | 3.81 |
| Mean | | | | | 7933.3 | | 3.87 |
| Standard deviation | | | | | 2904.4 | | 0.15 |

### b.

Figure M: Results of the colony growth of ampicillin-resistant *E. coli* K-12 MG1655 on LB-agar medium containing 100 µg/ml ampicillin. The inoculated leaves were exposed to red light, from adaxial side, with an intensity of 100 µmol/m² · s for 2 h.
| Test   | Illumination condition | Number of colonies detected | Dilution factor | Leaf side | Leaf sample weight (g) | Number of bacterial infiltration into the leaves (CFU/g) | Bacterial infiltration (log CFU/g) |
|--------|------------------------|-----------------------------|----------------|-----------|-----------------------|--------------------------------------------------------|-----------------------------------|
| Test 1 | White 100 (μmol/m².s)  | 6                           | 1/500          | Adaxial   | 5.051                 | 1200                                                   | 3.08                              |
| Test 2 | White 100 (μmol/m².s)  | 8                           | 1/500          | Adaxial   | 5.024                 | 1600                                                   | 3.20                              |
| Test 3 | White 100 (μmol/m².s)  | 1                           | 1/500          | Adaxial   | 5.021                 | 200                                                    | 2.30                              |

Mean: 1000 2.86
Standard deviation: 588.8 0.40

Figure N: Results of the colony growth of kanamycin-resistant *E. coli K-12 BW25113 (∆CheZ)* on LB-agar medium containing 30 μg/ml kanamycin. The inoculated leaves were exposed to white light, from adaxial side, with an intensity of 100 μmol/m² · s for 2 h.
### Test 1

| Test 1 | White 100 (µmol/m²·s) | 114 | 1/500 | Abaxial | 5.025 | 22800 | 4.36 |
|--------|-------------------|-----|-------|---------|-------|-------|------|

### Test 2

| Test 2 | White 100 (µmol/m²·s) | 153 | 1/500 | Abaxial | 5.051 | 30600 | 4.49 |
|--------|-------------------|-----|-------|---------|-------|-------|------|

### Test 3

| Test 3 | White 100 (µmol/m²·s) | 511 | 1/500 | Abaxial | 5.017 | 102200 | 5.01 |
|--------|-------------------|-----|-------|---------|-------|-------|------|

### Mean

|       |                   |     |       |         |       |       |      |
|-------|-------------------|-----|-------|---------|-------|-------|------|
|       | Illumination      |     |       |         |       |       |      |
|       | condition         |     |       |         |       |       |      |
|       | Number of         |     |       |         |       |       |      |
|       | colonies          |     |       |         |       |       |      |
|       | detected          |     |       |         |       |       |      |
|       | Dilution          |     |       |         |       |       |      |
|       | factor            |     |       |         |       |       |      |
|       | Leaf side         |     |       |         |       |       |      |
|       | Leaf sample       |     |       |         |       |       |      |
|       | weight (g)        |     |       |         |       |       |      |
|       | Number of         |     |       |         |       |       |      |
|       | bacterial         |     |       |         |       |       |      |
|       | infiltration      |     |       |         |       |       |      |
|       | into the leaves   |     |       |         |       |       |      |
|       | (CFU/g)           |     |       |         |       |       |      |
|       | Bacterial         |     |       |         |       |       |      |
|       | infiltration      |     |       |         |       |       |      |
|       | (log CFU/g)       |     |       |         |       |       |      |

### Standard deviation

|               |                   |     |       |         |       |       |      |
|---------------|-------------------|-----|-------|---------|-------|-------|------|
|               | Illumination      |     |       |         |       |       |      |
|               | condition         |     |       |         |       |       |      |
|               | Number of         |     |       |         |       |       |      |
|               | colonies          |     |       |         |       |       |      |
|               | detected          |     |       |         |       |       |      |
|               | Dilution          |     |       |         |       |       |      |
|               | factor            |     |       |         |       |       |      |
|               | Leaf side         |     |       |         |       |       |      |
|               | Leaf sample       |     |       |         |       |       |      |
|               | weight (g)        |     |       |         |       |       |      |
|               | Number of         |     |       |         |       |       |      |
|               | bacterial         |     |       |         |       |       |      |
|               | infiltration      |     |       |         |       |       |      |
|               | into the leaves   |     |       |         |       |       |      |
|               | (CFU/g)           |     |       |         |       |       |      |
|               | Bacterial         |     |       |         |       |       |      |
|               | infiltration      |     |       |         |       |       |      |
|               | (log CFU/g)       |     |       |         |       |       |      |

### Figure O: Results of the colony growth of ampicillin-resistant *E. coli* K-12 MG1655 on LB-agar medium containing 100 µg/ml ampicillin. The inoculated leaves were exposed to white light, from abaxial side, with an intensity of 100 µmol/m² · s for 2 h.
Model predictions for bacterial flux inside stomatal cavity

Predicted bacterial flux (toward the leaf interior) within stomatal cavity after 1 h of illumination with white light intensity of 100 \( \mu \text{mol/m}^2 \cdot \text{s} \) are shown in Fig. P.
Figure P: Predicted bacterial flux (toward the leaf interior) within stomatal cavity after 1 h of illumination with white light intensity of 100 $\mu$mol/m$^2 \cdot$ s.
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