

# A stomatal model of anatomical tradeoffs between photosynthesis and pathogen defense

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## 2 ABSTRACT

3 Stomatal pores control both leaf gas exchange and are an entry for many plant pathogens,  
4 setting up the potential for tradeoffs between photosynthesis and defense. To prevent colonization  
5 and limit infection, plants close their stomata after recognizing pathogens. In addition to closing  
6 stomata, anatomical shifts to lower stomatal density and/or size may also limit pathogen  
7 colonization, but such developmental changes would permanently reduce the gas exchange  
8 capacity for the life of the leaf. I developed and analyzed a spatially explicit model of pathogen  
9 colonization on the leaf as a function of stomatal size and density, anatomical traits which  
10 determine maximum rates of gas exchange. The model predicts greater stomatal size or density  
11 increases colonization, but the effect is most pronounced when stomatal cover is low. I also  
12 derived scaling relationships between stomatal size and density that preserves a given probability  
13 of colonization. These scaling relationships set up a potential conflict between maximizing defense  
14 and minimizing stomatal cover. To my knowledge, this is the first mathematical model connecting  
15 gas exchange and pathogen defense via stomatal anatomy. It makes predictions that can be  
16 tested with experiments and may explain variation in stomatal anatomy among plants. The model  
17 is generalizable to many types of pathogens, but lacks significant biological realism that may be  
18 needed for accurate predictions.

19 **Keywords:** anatomy, leaf gas exchange, model, pathogen, photosynthesis, scaling, stomata, tradeoff

## INTRODUCTION

20 Stomata evolved to regulate gas exchange in and out of the leaf (Hetherington and Woodward, 2003;  
21 Berry et al., 2010; Chater et al., 2017), but many plant pathogens take advantage of these chinks in the  
22 leaf cuticular armor to infect prospective hosts (Zeng et al., 2010; McLachlan et al., 2014; Melotto et al.,  
23 2017). The density and size of stomata set the anatomical maximum rate of stomatal conductance to CO<sub>2</sub>  
24 and water vapor (Brown and Escombe, 1900; Parlange and Waggoner, 1970; Franks and Farquhar, 2001;  
25 Franks and Beerling, 2009b; Lehmann and Or, 2015; Sack and Buckley, 2016; Harrison et al., 2019), but  
26 the pore area shrinks and expands in response to internal and external factors to regulate gas exchange  
27 dynamically (Buckley, 2019). Many plant pathogens, including viruses (Murray et al., 2016), bacteria  
28 (Melotto et al., 2006; Underwood et al., 2007), protists (Fawke et al., 2015), and fungi (Hoch et al., 1987;  
29 Zeng et al., 2010) use stomatal pores to gain entry into the leaf. Since stomatal conductance is a major  
30 limitation on photosynthesis (Farquhar and Sharkey, 1982; Jones, 1985) while pathogens reduce fitness,  
31 this sets up a potential tradeoff between increased photosynthesis and defense against pathogens. Although  
32 there have been many empirical studies on the effect that pathogens have on stomata, there is no theoretical

33 framework in which to place these findings. Lack of a theoretical framework makes it difficult to answer  
34 general questions about how selection for pathogen defense constrains maximum rates of gas exchange.

35 Stomatal anatomy is the key link between gas exchange and pathogen colonization. The density and  
36 size of stomata not only determines the theoretical maximum stomatal conductance ( $g_{s,max}$ ), but is also  
37 proportional to the operational stomatal conductance ( $g_{s,op}$ ) in many circumstances (Franks et al., 2009,  
38 2014; Dow et al., 2014a; McElwain et al., 2016; Murray et al., 2019). Therefore, I use anatomical  $g_{s,max}$  as  
39 a proxy for  $g_{s,op}$  and do not address dynamic changes in stomatal aperture (see Discussion). Harrison et al.  
40 (2019) recently reviewed the relationship between stomatal anatomy and gas exchange in detail.

41 Many pathogens rely on stomata to gain entry into the leaf, but it is unclear how anatomical traits  
42 that determine  $g_{s,max}$  (size and density) affect the ability of pathogens to colonize leaves. The impact of  
43 pathogens on host fitness is complex, but after a pathogen reaches a host, the first major step is colonization.  
44 Once infected, a pathogen can reduce fitness. Susceptible hosts can lose much of their biomass or die, but  
45 even resistant hosts must allocate resources to defense or reduce photosynthesis because of necrosis around  
46 sites of infection. Plants can limit colonization physiologically by closing stomata after they recognize  
47 pathogens, called stomatal defense (Melotto et al., 2017). Anatomy may be another layer of defense;  
48 plants can reduce pathogen colonization by developing leaves with lower stomatal density and/or size.  
49 Infection increases in leaves with higher stomatal density (McKown et al., 2014; Tateda et al., 2019;  
50 Dutton et al., 2019; Fetter et al., 2019), which suggests that both anatomical and physiological responses  
51 (stomatal closure) affect host colonization. By the same logic, if stomatal density were held constant,  
52 larger stomata should also increase colonization because they occupy more area. One key difference  
53 between physiological and anatomical defenses is that stomatal closure reduces gas exchange transiently,  
54 whereas anatomy constrains gas exchange throughout the life of the leaf. Because we do not understand the  
55 relationship between stomatal anatomy and colonization well, we cannot predict the relationship between  
56  $g_{s,max}$  and infection.

57 If stomatal size and density affect pathogen colonization, selection to limit colonization may shape  
58 stomatal size-density scaling relationships. Botanists have long recognized that stomatal size and density  
59 are inversely correlated (Weiss, 1865; Tichá, 1982; Hetherington and Woodward, 2003; Sack et al., 2003;  
60 Franks and Beerling, 2009a; Brodribb et al., 2013; de Boer et al., 2016), but the evolutionary origin of  
61 this relationship is not yet known. Here I argue that pathogens could shape selection on this relationship.  
62 Explanations for inverse size-density scaling are usually cast in terms of preserving  $g_{s,max}$  and/or total  
63 epidermal area allocated to stomata ( $f_S$ ; de Boer et al., 2016) because there are many combinations of  
64 stomatal size and density that have same  $g_{s,max}$  or same  $f_S$ :

$$g_{s,max} = bmDS^{0.5} \quad (1)$$

$$f_S = DS. \quad (2)$$

65  $b$  and  $m$  are assumed to be biophysical and morphological constants, *sensu* Sack and Buckley (2016) (see  
66 Supplementary Material). If size and density also affect pathogen colonization, then selection from foliar  
67 pathogens could significantly alter the size-density scaling relationship. The empirical size-density scaling  
68 relationship is linear on a log-log scale, determined by an intercept  $\alpha$  and slope  $\beta$ :

$$D = e^{\alpha} S^{-\beta}; \quad (3)$$

$$d = \alpha - \beta S. \quad (4)$$

69 For brevity,  $d = \log(D)$  and  $s = \log(S)$ . Rearranging Equations 1 and 2, a scaling relationship where  
70  $\beta = 0.5$  preserves  $g_{s,\max}$  while  $\beta = 1$  preserves  $f_S$ .

71 How would adding pathogens alter these predicted scaling relationships? For simplicity, imagine two  
72 environments, one without foliar pathogens and one with lots. In the absence of foliar pathogens, we expect  
73 size-density scaling to preserve  $g_{s,\max}$ ,  $f_S$ , or some least-cost combination of them. What happens when  
74 we introduce pathogens? Assuming that stomatal size and density increase pathogen colonization, then  
75 selection will favor reduced size and/or density. This would change the intercept  $\alpha$  but not the slope. The  
76 effect of foliar pathogens on the slope depends on the relationship between size, density, and probability of  
77 colonization. If the probability of colonization is proportional to the product of *linear* stomatal size ( $S^{0.5}$ )  
78 and density ( $\propto DS^{0.5}$  as for  $g_{s,\max}$ ) then it has the same effect on the slope as  $g_{s,\max}$  because there are many  
79 combinations of  $D$  and  $S^{0.5}$  that have same probability of colonization. If the probability of colonization is  
80 proportional to the product of *areal* stomatal size ( $S$ ) and density ( $\propto DS$  as for  $f_S$ ) then it has the same  
81 effect on the slope as  $f_S$  because there are many combinations of  $D$  and  $S$  that have same probability of  
82 colonization. Alternatively, the probability of colonization may have a different scaling relationship (neither  
83 0.5 nor 1) or may be nonlinear on a log-log scale. Unlike  $g_{s,\max}$  and  $f_S$ , we do not have theory to predict a  
84 stomatal size-density relationship that preserves the probability of colonization.

85 In summary, the physical relationship between stomatal size, density, and conductance is well established  
86 (Harrison et al., 2019). The same traits likely affect the probability of pathogen colonization, but we do not  
87 have a theoretical model that makes quantitative predictions. The inverse stomatal size-density relationship  
88 has usually been explained in terms of preserving stomatal conductance and/or stomatal cover, but selection  
89 by pathogens might alter scaling. To address these gaps, the goals of this study are to 1) introduce a spatially  
90 explicit model pathogen colonization on the leaf surface; 2) use the model to predict the relationship  
91 between  $g_{s,\max}$ ,  $f_S$ , and the probability of colonization; 3) work out what these relationships predict about  
92 stomatal size-density scaling.

## MODEL

93 In this section, I introduce a spatially explicit model of pathogen colonization on a leaf surface. I explain the  
94 model structure and assumptions here; the Materials and Methods section below describes how I analyzed  
95 the model to address the goals of the study. For generality, I refer to a generic “pathogen” that lands on leaf  
96 and moves to a stomate. The model is agnostic to the type of pathogen (virus, bacterium, fungus, etc.) and  
97 the specific biological details of how it moves (biotrophy). For example, motile bacterial cells can land  
98 and move around where fungi may germinate from a cyst and grow until they form an appressorium for  
99 infection. These very different biotrophic movements on the leaf are treated identically here. I used Sympy  
100 version 1.3 (Meurer et al., 2017) for symbolic derivations.

101 **Table 1** | Glossary of mathematical symbols. The columns indicate the mathematical Symbol used in the  
102 paper, the associated symbol used in R scripts, scientific Units, and a verbal Description.

Symbol	R	Units	Description
$D, d$	$D, d$	$\text{mm}^{-2}$	stomatal density ( $d = \log D$ )
$f_s$	$f\_s$	none	stomatal cover ( $f_s = DS$ )
$g_{s,\max}$	$g\_s\max$	$\text{mol m}^{-2} \text{s}^{-1}$	anatomical maximum stomatal conductance
$g_{s,\text{op}}$	$g\_s\text{op}$	$\text{mol m}^{-2} \text{s}^{-1}$	operational stomatal conductance
$H$	$H$	$\mu\text{m}^{-1}$	death rate of pathogen on leaf surface
$R$	$R$	$\mu\text{m}$	stomatal radius ( $S = 2\pi R^2$ )
$S, s$	$S, s$	$\mu\text{m}^2$	stomatal size ( $s = \log S$ )
$\theta_i$	$\theta\_i$	radians	angles between pathogen ( $x_p, y_p$ ) and lines tangent to the circumference of stomate $i$
$U$	$U$	$\mu\text{m}$	interstomatal distance
$v_i$	$v\_i$	$\mu\text{m}$	distance between pathogen ( $x_p, y_p$ ) and stomate $i$
$x_i, y_i$	$x\_i, y\_i$	$\mu\text{m}$	position of stomate $i$
$x_p, y_p$	$x\_p, y\_p$	$\mu\text{m}$	starting position of pathogen

### 103 Spatial representation of stomata

104 Stomata develop relatively equal spacing to minimize resistance to lateral diffusion (Morison et al., 2005),  
 105 allow space between stomata (Dow et al., 2014b), and prevent stomatal interference (Lehmann and Or,  
 106 2015). Here I assume that stomata are arrayed in an equilateral triangular grid with a density  $D$  and size  
 107 (area)  $S$ . This assumption ignores veins, trichomes, and within-leaf variation in stomatal density. Stomata  
 108 are therefore arrayed in an evenly spaced grid (Figure 1a). The interstomatal distance  $U$ , measured as the  
 109 distance from the center of one stomata to the next, is the maximal diagonal of the hexagon in  $\mu\text{m}$  that  
 110 forms an equal area boundary between neighboring stomata. The area of a hexagon is  $A_{\text{hexagon}} = \frac{\sqrt{3}}{2}U^2$ .  
 111 By definition the stomatal density is the inverse of this area, such that  $D = A_{\text{hexagon}}^{-1} = \frac{2}{\sqrt{3}}U^{-2}$ . Therefore,  
 112 interstomatal distance can be derived from the stomatal density as:

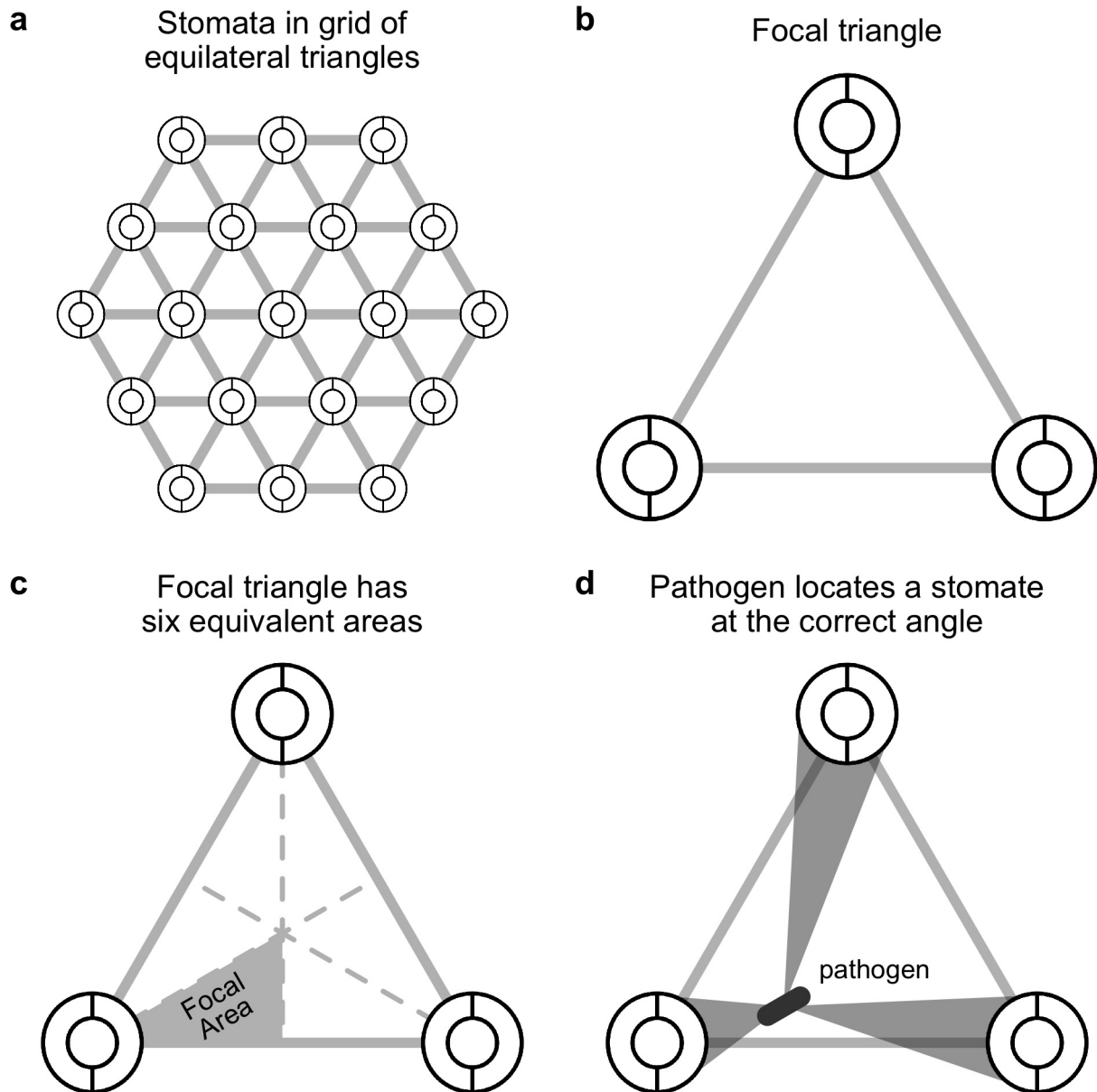
$$D = \frac{2}{\sqrt{3}}U^{-2}$$

$$U = \left( \frac{2}{\sqrt{3}}D^{-1} \right)^{0.5}$$

114 For example, if the density is  $D = 10^2 \text{ mm}^{-2} = 10^{-4} \mu\text{m}^{-2}$ , then  $U$  is 107.5  $\mu\text{m}$ . Parkhurst (1994)  
 115 described this result previously. I also make the simplifying assumption that stomata are perfectly circular  
 116 with radius  $R$ . This may be approximately true for fully open stomata with kidney-shaped guard cells.  
 117 Although I assume stomata are circular here, in calculating  $g_{s,\max}$ , I assume typical allometric relationships  
 118 between length, width, and pore area (see Supplementary Material).

### 119 Spatial representation of pathogen search

120 Now imagine that a pathogen lands at a uniform random position within the focal region and must arrive  
 121 at a stomate to colonize. If it lands on a stomate, then it infects the leaf with probability 1; if it lands  
 122 between stomata, then it infects the leaf with probability  $p_{\text{locate}}$ . This is the probability that it locates a  
 123 stomate, which I will derive below. The probabilities of landing on or between a stomate are  $f_s$  and  $1 - f_s$ ,  
 124 respectively. Hence, the total probability of colonization is:



**Figure 1. A spatially explicit model of stomatal anatomy and pathogen colonization.** a. Stomata are assumed to be in a homogenous equilateral triangular grid, which means that we can extrapolate from b. a focal triangle to the entire leaf. The circles represent idealized stomata; the grey lines between them are for visualization. c. By symmetry, a single focal region within the focal triangle can be modeled and extrapolated to the rest of the area. d. The model assumes that a pathogen, depicted as a grey rod, lands somewhere on the leaf surface and will successfully locate a stomate if it moves at the correct angle, depicted by the grey polygons.

$$p_{\text{colonize}} = f_s + (1 - f_s)p_{\text{locate}}. \quad (5)$$

125 I assume that the pathogen cannot sense where stomata are and orients at random, thereafter traveling in  
 126 that direction. If it successfully locates a stomate, it colonizes the leaf, but otherwise does not infect. If  
 127 there is a high density of stomata and/or large stomata, the probability of locating a stomate increases. By

128 assuming that stomata form an equilateral triangular grid (see above), we can extrapolate what happens in  
 129 a focal triangle (Figure 1b) by symmetry. Further, since an equilateral triangle can be broken up into six  
 130 identical units (Figure 1c), we can simply calculate  $p_{\text{locate}}$  in this focal area. This implicitly assumes that  
 131 the probability of colonizing stomata outside the focal area is 0 because they are too far away.

132 Imagine that the pathogen lands in position  $(x_p, y_p)$  within the triangle. The centroid of the triangle  
 133 is at position  $(x_c, y_c)$  and a reference stomate is at position  $(0, 0)$  (Figure 2a). Therefore  $x_c = U/2$  and  
 134  $y_c = \sqrt{3}U/6$ . The other stomata are at positions  $(U/2, \sqrt{3}U/2)$  and  $(U, 0)$  (Figure 2).  $x_p$  and  $y_p$  are defined  
 135 as the horizontal and vertical distances, respectively, from the pathogen to the reference stomate at position  
 136  $(0, 0)$ .

137 Given that the pathogen starts at position  $(x_p, y_p)$ , what's the probability of contacting one of the stomata  
 138 at the vertices of the focal triangle? I assume the probability of contacting a stomate is equal to the  
 139 proportion of angular directions that lead to a stomate (Figure 1d). I solved this by finding the angles  
 140  $(\theta_1, \theta_2, \theta_3)$  between lines that are tangent to the outside of the three stomata and pass through  $(x_p, y_p)$   
 141 (Figure 2a). If stomate  $i$  is centered at  $(x_i, y_i)$ , the two slopes of tangency as function of pathogen position  
 142 are:

$$t_{i,1}(x_p, y_p) = \frac{-Re_{i,2}(x_p, y_p) + e_{i,3}(x_p, y_p)}{e_{i,1}(x_p, y_p)} \quad (6)$$

$$t_{i,2}(x_p, y_p) = \frac{Re_{i,2}(x_p, y_p) + e_{i,3}(x_p, y_p)}{e_{i,1}(x_p, y_p)} \quad (7)$$

143 where

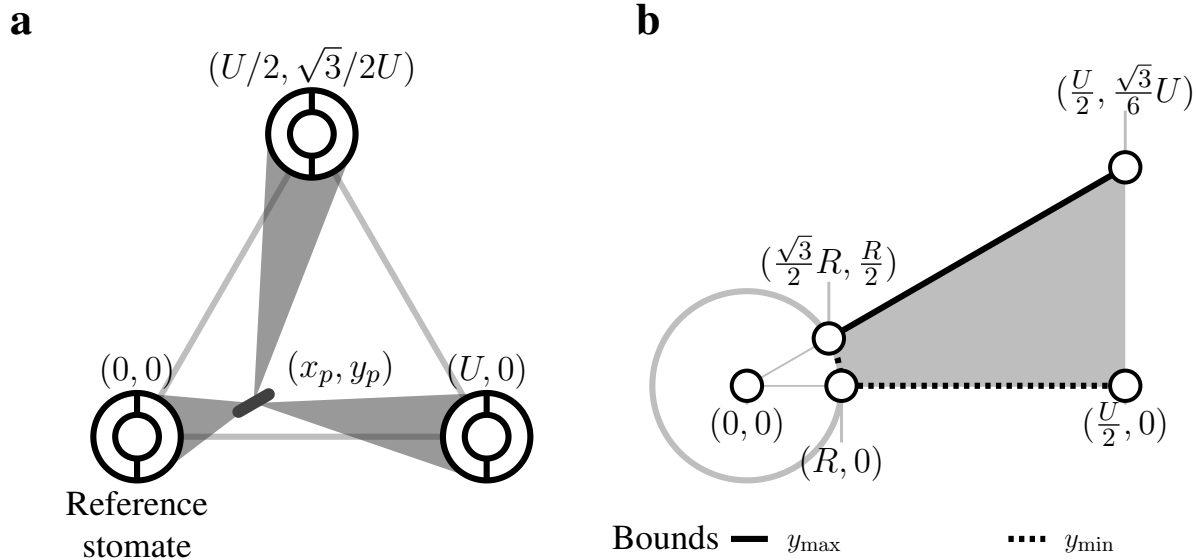
$$e_{i,1}(x_p, y_p) = (R^2 - x_i^2 + 2x_i x_p - x_p^2), \quad (8)$$

$$e_{i,2}(x_p, y_p) = \sqrt{-e_{i,1} + (y_i - y_p)^2}, \quad (9)$$

$$e_{i,3}(x_p, y_p) = -x_i y_i + x_i y_p + x_p y_i - x_p y_p. \quad (10)$$

144 Note that  $i \in \{1, 2, 3\}$ , indexing the three stomata in the focal triangle. The angle in radians between  
 145  $t_{i,1}(x_p, y_p)$  and  $t_{i,2}(x_p, y_p)$  is:

$$\theta_i(x_p, y_p) = \arctan\left(\frac{t_{i,1}(x_p, y_p) - t_{i,2}(x_p, y_p)}{1 + (t_{i,1}(x_p, y_p)t_{i,2}(x_p, y_p))}\right) \quad (11)$$



**Figure 2. Spatial representation of stomata and pathogen.** **a.** The pathogen starts at a uniform random position within the focal region denoted  $(x_p, y_p)$ . Within the focal triangle, the reference stomata is at position  $(0, 0)$  by definition, and other stomatal positions are determined by the interstomatal distance  $U$ . **b.** Within the focal region, a pathogen can land within the stomate (white circle with grey outline and radius  $R$ ) or in the grey area. The outer borders of this area are shown and depend on  $R$  and  $U$ . For a given position  $x$ , there is a minimum  $y$ -value ( $y_{min}$ , dashed line) and maximum  $y$ -value ( $y_{max}$ , solid line).

146 I further assumed that the longer distance a pathogen must travel, the less likely it would be to locate  
 147 a stomate. For example, if stomata are at very low density, then a pathogen may die before it reaches a  
 148 stomate because of UV, dessication, or another factor. I included this effect by assuming the probability of  
 149 reaching a stomate declines exponentially at rate  $H$  with the Euclidean distance  $v_i(x_p, y_p)$  between the  
 150 pathogen location and the edge of stomata  $i$ , which is distance  $R$  from its center at  $x_i, y_i$ :

$$v_i(x_p, y_p) = \sqrt{(x_i - x_p)^2 + (y_i - y_p)^2} - R. \quad (12)$$

151 The probability of locating a stomate as a function of  $x_p$  and  $y_p$  ( $f_{locate}(x_p, y_p)$ ) is the sum of the angles  
 152 divided by  $2\pi$ , discounted by their distance from the stomate:

$$f_{locate}(x_p, y_p) = \frac{1}{2\pi} \sum_{i=1}^3 e^{-Hv_i(x_p, y_p)} \theta_i(x_p, y_p) \quad (13)$$

153 When  $H = 0$ ,  $p_{locate}$  is the fraction of angles that lead from  $(x_p, y_p)$  to a stomate. When  $H > 0$ ,  $p_{locate}$  is  
 154 proportional to this fraction, but less than it depending on stomatal density, size, and starting location of  
 155 the pathogen.

156 To obtain the average  $p_{locate}$ , we must integrate  $f_{locate}(x_p, y_p)$  over all possible starting positions  $(x_p, y_p)$   
 157 within the focal area. The focal area is a 30-60-90 triangle with vertices at the center of the reference  
 158 stomate  $(0, 0)$ , the midpoint of baseline  $(U/2, 0)$ , and the centroid of the focal triangle  $(U/2, \sqrt{3}/6U)$   
 159 (Figure 1c). Colonization occurs with probability 1 if the pathogen lands in the reference stomate, so we

160 need to integrate the probability of colonization if it lands elsewhere. This region extends from the edge of  
161 the stomate, at  $\sqrt{3}/2R$  to  $U/2$  (Figure 2b). At any  $x$ , we integrate from the bottom of the focal area ( $y_{\min}$ )  
162 to the top ( $y_{\max}$ ):

$$y_{\min} = f(x) = \begin{cases} \sqrt{R^2 - x^2}, & \text{if } \frac{\sqrt{3}}{2}R < x < R \\ 0, & \text{if } R \leq x \leq \frac{U}{2} \end{cases} \quad (14)$$

$$y_{\max} = f(x) = \frac{\sqrt{3}}{3}x \quad (15)$$

163 The integral is:

$$p_{\text{locate}} = \frac{1}{a_{\text{focal}}} \int_{\frac{\sqrt{3}}{2}R}^{U/2} \int_{y_{\min}}^{y_{\max}} f_{\text{locate}}(x, y) dx dy \quad (16)$$

164  $a_{\text{focal}}$  is the area of the focal region depicted in grey in Figure 2b:

$$a_{\text{focal}} = \frac{U^2}{8\sqrt{3}} - \frac{\pi R^2}{12}$$

## MATERIALS AND METHODS

165 The Model calculates a probability of host colonization (Equation 5) as a function stomatal density, size,  
166 and position of a pathogen on the leaf. I solved  $p_{\text{colonize}}$  using the `integral2()` function in the **pracma**  
167 package version 2.2.5 (Borchers, 2019) for numerical integration. I used R version 3.6.1 (R Core Team,  
168 2019) for all analyses and wrote the paper in **rmarkdown** version 1.17 (Xie et al., 2018; Allaire et al., 2019).  
169 Source code is deposited on GitHub (<https://github.com/cdmuir/stomata-tradeoff>) and  
170 will be archived on Zenodo upon publication.

### 171 What is the relationship between stomatal size, density, and colonization?

172 I calculated  $p_{\text{colonize}}$  over a biologically plausible grid of stomatal size and density for hypostomatous  
173 species based on de Boer et al. (2016). Stomatal density ranges from  $10^1 - 10^{3.5} \text{ mm}^{-2}$ ; stomatal size  
174 ranges from  $10^1 - 10^{3.5} \mu\text{m}^2$ . I only considered combinations of size and density where  $f_S$  was less than  
175  $1/3$ . For simplicity, I have not extended the current analysis to amphistomatous leaves. I crossed stomatal  
176 traits with three levels of  $H \in \{0, 0.01, 0.1\}$ . When  $H = 0$ , a pathogen persists indefinitely on the leaf  
177 surface.  $H = 0.01$  and  $H = 0.1$  correspond to low and high death rates, respectively. These values are not  
178 necessarily realistic, but illustrate qualitatively how a hostile environment on the leaf surface alters model  
179 predictions.

### 180 How do pathogens alter optimal stomatal size-density scaling?

181 The stomatal size-density scaling relationship can be explained in terms of preserving a constant  $g_{s,\text{max}}$   
182 that is proportional to  $DS^{0.5}$  when  $bm$  is constant (Equation 1). In other words, there are infinitely many  
183 combinations of  $D$  and  $S^{0.5}$  with the same  $g_{s,\text{max}}$ . If  $g_{s,\text{max}}$  is held constant at  $C_g$ , then the resulting  
184 size-density scaling relationship on a log-log scale is:



$$d = c_g - 0.5s$$

185 where lowercase variables are log-transformed equivalents of their uppercase counterparts. The scaling  
186 exponent  $\beta_g = 0.5$  preserves  $C_g$ .

187 Next, imagine there is similarly a scaling exponent  $\beta_p$  that preserves  $p_{\text{colonize}}$  for the product  $DS^{\beta_p}$ .  
188 If  $\beta_p = 0.5$ , then  $p_{\text{colonize}}$  is always proportional to  $g_{s,\text{max}}$ . If  $\beta_p > 0.5$ , small, densely packed stomata  
189 would be better defended (lower  $p_{\text{colonize}}$ ) compared to larger, sparsely spaced stomata with the same  
190  $g_{s,\text{max}}$ . If  $\beta_p < 0.5$ , small, densely packed stomata would be less defended (higher  $p_{\text{colonize}}$ ) compared to  
191 larger, sparsely spaced stomata with the same  $g_{s,\text{max}}$ . I refer to the three outcomes ( $\beta_p = 0.5$ ,  $\beta_p < 0.5$ ,  
192 and  $\beta_p > 0.5$ ) as iso-, hypo-, and hyper-conductance, respectively. I was unable to solve analytically  
193 for  $\beta_p$ , so I numerically calculated isoclines of  $p_{\text{colonize}}$  over the grid of  $D$  and  $S$  values described in  
194 the preceding subsection. I numerically calculated the scaling relationships at a constant  $p_{\text{colonize}} \in$   
195  $\{0.025, 0.05, 0.1, 0.2, 0.4\}$  for  $H \in \{0, 0.01, 0.1\}$ .

## RESULTS

196 I analyzed an idealized, spatially explicit Model of how a pathogen lands on a leaf and finds a stomate to  
197 colonize the leaf using a random search. To my knowledge, this is the first model that makes quantitative  
198 predictions about the relationship between stomatal anatomy, the probability of colonization, and their  
199 impact on stomatal size-density scaling.

### 200 Nonlinear relationships between colonization, stomatal cover, and conductance

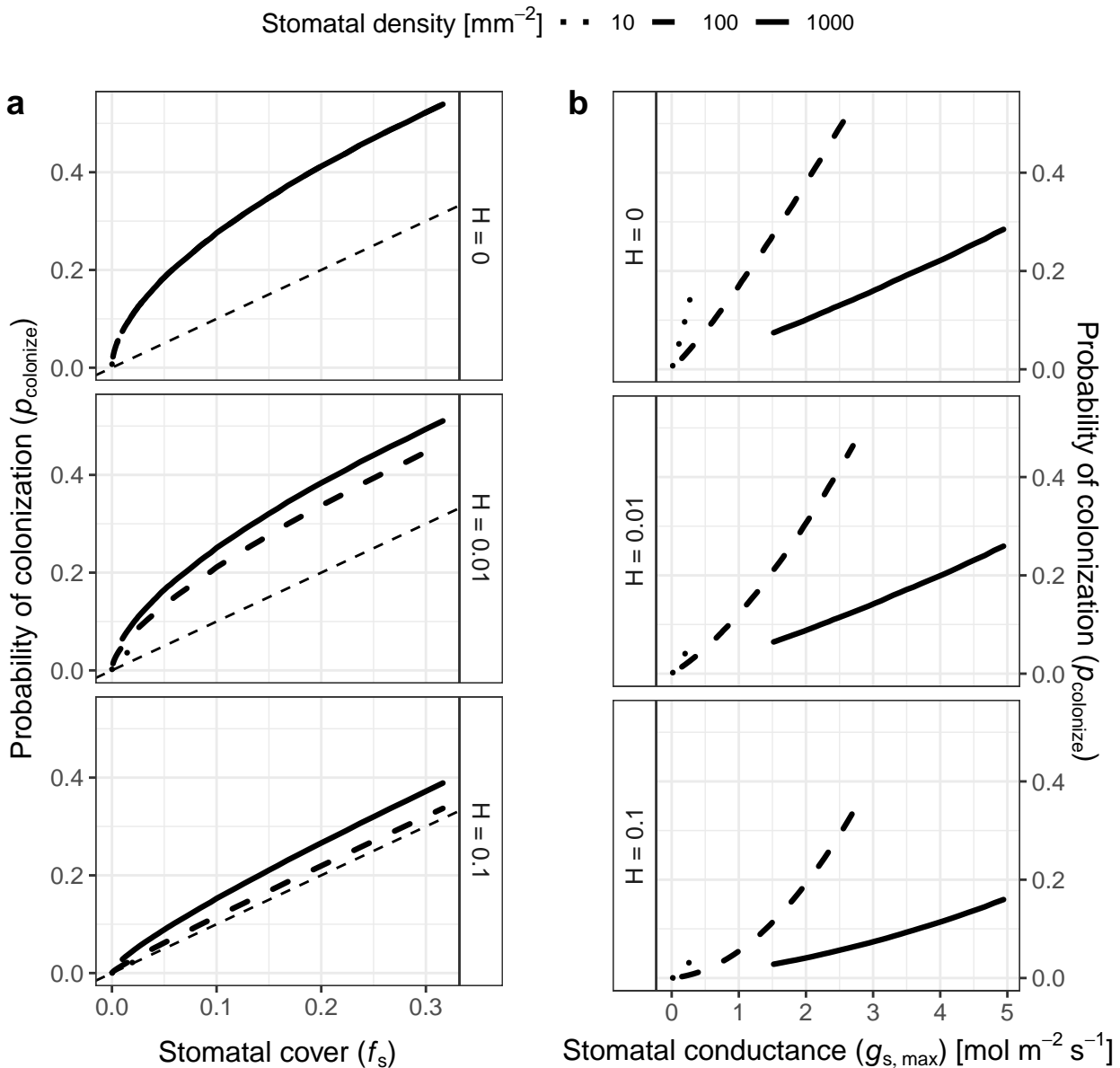
201 The probability of colonization ( $p_{\text{colonize}}$ ) is not simply a one-to-one relationship between the fraction of  
202 epidermal area allocated to stomata ( $f_S$ ). At low  $f_S$ ,  $p_{\text{colonize}}$  increases faster rapidly relative to  $f_S$  at first  
203 Figure 3a). At higher  $f_S$ , the  $p_{\text{colonize}}$  increases linearly with  $f_S$ . When  $H = 0$ , any combination of stomatal  
204 size ( $S$ ) and density ( $D$ ) with the same  $f_S$  have the same effect on  $p_{\text{colonize}}$ . When  $H > 0$ , pathogens are  
205 less likely to land close enough to a stomate to infect before dying, so  $p_{\text{colonize}}$  is closer to  $f_S$  (Figure 3a).  
206 Furthermore,  $p_{\text{colonize}}$  depends on  $D$  and  $S$ , not just  $f_S$ . For the same  $f_S$ , leaves with greater  $D$  have higher  
207  $p_{\text{colonize}}$  (Figure 3a). Holding  $f_S$  constant, leaves with lower  $D$  and higher  $S$  will have a greater distance  
208 ( $v_i$ ) between a pathogen and its stomata. When  $H > 0$ , this extra distance leads more pathogens to die  
209 before they can find a stomate. In contrast to  $f_S$ ,  $p_{\text{colonize}}$  increases at a greater than linear rate with  $g_{s,\text{max}}$ .  
210 Greater  $D$  (smaller  $S$ ) is associated with lower  $p_{\text{colonize}}$  when  $g_{s,\text{max}}$  is held constant (Figure 3b). This  
211 happens because  $p_{\text{colonize}}$  increases approximately linearly with  $S$  whereas  $g_{s,\text{max}}$  is proportional to  $S^{0.5}$ .

### 212 Hyper-conductance size-density scaling

213 The scaling relationship between  $S$  and  $D$  that preserves  $p_{\text{colonize}}$  is always greater 0.5 (hyper-  
214 conductance), but usually less than 1. When  $H = 0$ , the scaling relationship is essentially 1 (Figure  
215 4), which means that an increase  $f_S$  leads to a proportional increase in  $p_{\text{colonize}}$ . Because the scaling  
216 relationship is greater than 0.5, leaves with greater stomatal density will have lower  $p_{\text{colonize}}$  than leaves  
217 lower stomatal density but the same  $g_{s,\text{max}}$ . In other words, increasing  $D$  and lowering  $S$  allows plants to  
218 reduce  $p_{\text{colonize}}$  while maintaining  $g_{s,\text{max}}$ . The scaling relationship is slightly less than 1, but still greater  
219 than 0.5, when  $H > 0$  (Figure 4). In this area of parameter space, lower stomatal density can reduce  $f_S$   
220 while  $p_{\text{colonize}}$  is constant, but this will still result in lower  $g_{s,\text{max}}$ .

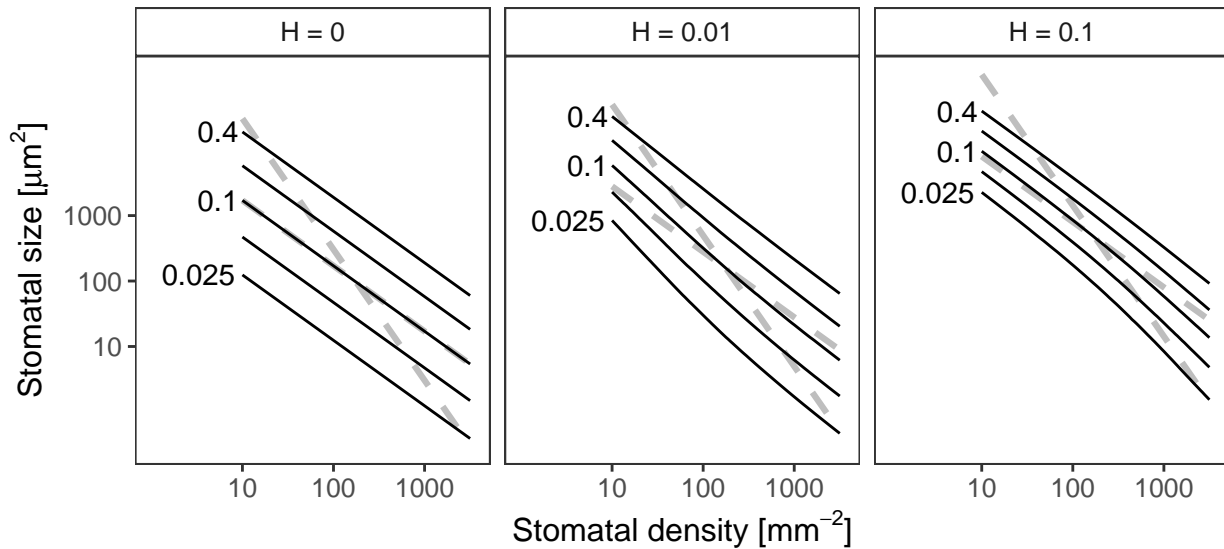
## DISCUSSION

221 Stomatal density and size set the upper limit on gas exchange in leaves (Harrison et al., 2019) and is often  
222 closely related to operational stomatal conductance in nature (Murray et al., 2019). Despite the fact that



**Figure 3. The probability of colonization increases with both stomatal cover and conductance a.** The probability of colonization ( $p_{\text{colonize}}$ ,  $y$ -axis) initially increases rapidly with stomatal cover ( $f_s$ ), then slows down to a linear relationship. Overall,  $p_{\text{colonize}}$  is lower when pathogens can die on the leaf surface ( $H > 0$ ). The relationship between  $f_s$  and  $p_{\text{colonize}}$  is the same regardless of stomatal density when  $H = 0$  (upper facet). When  $H > 0$ , higher density (solid lines) increase  $p_{\text{colonize}}$  (lower facets). **b.**  $p_{\text{colonize}}$  increases exponentially with  $g_{s, \text{max}}$  at all stomatal densities, but  $p_{\text{colonize}}$  is much lower at higher densities for a given  $g_{s, \text{max}}$ . The relationship between  $g_{s, \text{max}}$  and  $p_{\text{colonize}}$  is similar for all values of  $H$ .

223 many ecologically and economically significant plant pathogens infect through stomata, the relationship  
 224 between stomatal anatomy and susceptibility to foliar pathogens is less clear than it is for gas exchange.  
 225 To develop testable predictions, we need mathematical models that can clarify the potential for tradeoffs  
 226 between stomatal conductance, stomatal cover, and disease resistance. I used a spatially explicit model of  
 227 a pathogen searching for a stomate to colonize a host. From this Model, I derived predictions about the  
 228 relationship between stomatal anatomy and disease resistance for the first time. The model predicts that the



**Figure 4.** Log-log scaling relationships between stomatal density ( $D$ ,  $x$ -axis) and size ( $S$ ,  $y$ -axis) that preserve the probability of colonization ( $p_{\text{colonize}}$ ). In each panel, solid lines indicate values of  $D$  and  $S$  where  $p_{\text{colonize}}$  is 0.025 (lowest line), 0.05, 0.1, 0.2, or 0.4 (highest line). For reference, dashed grey lines show scaling relationships that preserve  $f_s$  ( $\beta = 1$ , slope =  $-1/\beta = -1$ ) and  $g_{s,\text{max}}$  ( $\beta = 0.5$ , slope =  $-1/\beta = -2$ ) drawn through the centroid of the plotting region. When the death rate on the leaf surface is low ( $H = 0$ ), the scaling exponent is very close to  $\beta = 1$ . When  $H > 0$ ,  $0.5 < \beta < 1$  and is slightly nonlinear on a log-log scale.

229 probability of colonization is not always proportional to the surface area of leaf covered by stomata ( $f_s$ ), as  
 230 one might intuitively predict. If the leaf surface is a hostile environment and pathogens have a limited time  
 231 to search, lower stomatal density decreases the probability of colonization even if  $f_s$  is constant. However,  
 232  $g_{s,\text{max}}$  decreases proportionally more than the probability of colonization. The model reveals the potential  
 233 for conflicting demands of maximizing disease resistance, minimizing stomatal cover, and maintaining  
 234 stomatal conductance. Including the effect of anatomy on disease resistance therefore has the potential to  
 235 change our understanding of how stomatal size-density scaling evolves in land plants.

236 The model predicts that in most cases, increasing stomatal cover should lead to a proportional increase  
 237 in susceptibility, which is the implicit assumption of some empirical studies (e.g. McKown et al. (2014);  
 238 Tateda et al. (2019); Dutton et al. (2019); Fetter et al. (2019)). It also makes new, testable predictions that  
 239 are less intuitive. At very low  $f_s$ , there is a rapid increase in susceptibility (Figure 3a). If there are no  
 240 stomata, the probability of colonization is 0, so the first few stomata dramatically increase the probability.  
 241 This is unlikely to be significant for abaxial (lower) leaf surfaces, which usually have most of the stomata  
 242 (Salisbury, 1928; Metcalfe and Chalk, 1950; Mott et al., 1984; Peat and Fitter, 1994; Jordan et al., 2014;  
 243 Muir, 2015; Bucher et al., 2017; Drake et al., 2019). However, many adaxial (upper) leaf surfaces have zero  
 244 or very few stomata. Using adaxial leaf surfaces, it should be possible to test if small changes in stomatal  
 245 size or density have a larger effect on disease susceptibility when  $f_s$  is low. The nonlinear increase in  
 246  $p_{\text{colonize}}$  is less apparent when  $H > 0$  (Figure 3a). A more hostile microenvironment (e.g. drier, higher UV)  
 247 should therefore reduce the effect of increased size or density as low  $f_s$ . If true, the diminishing marginal  
 248 effect of  $f_s$  on colonization could explain why stomatal ratio on the upper and lower surface is bimodal  
 249 (Muir, 2015). The initial cost of adaxial (upper) stomata is high, but if the benefits outweigh the costs,  
 250 then equal stomatal densities on each surface maximize  $\text{CO}_2$  supply for photosynthesis (Parkhurst, 1978;  
 251 Gutschick, 1984; Parkhurst and Mott, 1990).

252 An effect of stomatal size and density on susceptibility to foliar pathogens could change our understanding  
253 of stomatal size-density scaling. Since allocating leaf epidermis to stomata may be costly (Franks and  
254 Farquhar, 2007; Assmann and Zeiger, 1987; Dow et al., 2014b; Lehmann and Or, 2015; Baresch et al.,  
255 2019), selection should favor leaves that achieve a desired  $g_{s,max}$  while minimizing  $f_S$  (de Boer et al., 2016).  
256 Because of their different scaling exponents (Equation 1, 2), smaller, densely packed stomata can achieve  
257 the same  $g_{s,max}$  at minimum  $f_S$ . However, many leaves have larger, sparsely packed stomata. Incorporating  
258 susceptibility to disease may explain why. If pathogens have a limited time to find stomata before dying  
259 ( $H > 0$ ), then the scaling exponent between size and density that keeps  $p_{colonize}$  constant is between 0.5  
260 and 1, the scaling exponents for  $g_{s,max}$  and  $f_S$ , respectively (Figure 4). Greater density of smaller stomata  
261 can increase  $g_{s,max}$  while keeping  $p_{colonize}$  constant, but this will increase  $f_S$ . Conversely,  $f_S$  could decrease  
262 while keeping  $p_{colonize}$  constant, but this will decrease  $g_{s,max}$ . This sets up the potential for conflict between  
263 competing goals. The optimal stomatal size and density will therefore depend on the precise costs and  
264 benefits of infection, stomatal conductance, and stomatal cover. This may explain why many leaves have  
265 large, sparsely packed stomata despite the fact that they could achieve the same  $g_{s,max}$  and lower  $f_S$  with  
266 smaller, more densely packed stomata.

267 The model examines the probability of colonization for a single pathogen. The calculated probabilities of  
268 colonization should not be interpreted as exact predictions, but rather as depicting qualitative relationships  
269 between stomatal anatomy and infection severity. The model is most applicable to diseases where the host  
270 has some resistance. The energetic cost and lost photosynthetic capacity (closed stomata, necrosis, etc.) of  
271 dealing with a pathogen is assumed to be proportional to the amount of infection. The actual fitness cost  
272 will be modulated by the number of pathogens landing on the leaf and the cost of infection. In environments  
273 with fewer or less virulent pathogens, the fitness cost of infection will be less than in environments with  
274 more abundant, virulent pathogens. The model is less relevant to very susceptible host plants that can be  
275 severely damaged or killed by a small number of colonizations that spread unchecked throughout the host  
276 tissue.

277 The purpose of this model is to provide a general foundation to examine the relationship between stomatal  
278 size, density, and defense against foliar pathogens. In its generality, it overlooks interesting natural history  
279 and biologically important features of specific plants and their pathogens. For example, some pathogens  
280 actively seek out and find stomata (Kiefer et al., 2002), whereas I assumed that pathogens randomly orient  
281 themselves on the leaf. Including sensing should increase the probability of colonization. I also assumed  
282 that the plant does not respond by, for example, closing stomata when it senses a pathogen. However, plants  
283 can sense and close stomata when pathogens land on the leaf, but pathogens can pry stomata back open  
284 (Melotto et al., 2006). Future work on specific plant-pathogen interactions could build on this model by  
285 adding more biological realism to provide more precise predictions.

## CONCLUSION

286 The model makes two non-intuitive predictions. First, the effect of increased stomatal density or size  
287 on susceptibility to foliar pathogens is greatest when stomatal cover is very low. Second, maximizing  
288 disease resistance sets up a potential conflict between minimizing stomatal cover and maximizing stomatal  
289 conductance. The first prediction may be relatively straightforward to test experimentally with adaxial  
290 (upper) stomata that occur at low and moderate densities within the same or closely related species (Muir  
291 et al., 2014; McKown et al., 2014; Fetter et al., 2019). The second prediction about size-density scaling is  
292 more complex because we would need to know the relationships between colonization, stomatal cover,  
293 stomatal conductance, and fitness in natural conditions. Testing these predictions in a variety of species

294 would help determine whether pathogens have played an important role shaping stomatal anatomy in land  
295 plants.

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## CONFLICT OF INTEREST STATEMENT

297 The author declares that the research was conducted in the absence of any commercial or financial  
298 relationships that could be construed as a potential conflict of interest.

## SUPPLEMENTARY MATERIAL

299 I calculated  $g_{s,max}$  (Equation 1) to water vapor at a reference leaf temperature ( $T_{leaf} = 25^\circ \text{C}$ ) following  
300 Sack and Buckley (2016). They defined a biophysical and morphological constant as:

$$b = D_{wv}/v$$
$$m = \frac{\pi c^2}{j^{0.5}(4hj + \pi c)}$$

301  $b$  is the diffusion coefficient of water vapor in air ( $D_{wv}$ ) divided by the kinematic viscosity of dry air ( $v$ ).  
302  $D_{wv} = 2.49 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$  and  $v = 2.24 \times 10^{-2} \text{ m}^3 \text{ mol}^{-1}$  at  $25^\circ$  (Monteith and Unsworth, 2013). For  
303 kidney-shaped guard cells,  $c = h = j = 0.5$ .

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