environmental microbiology

Environmental Microbiology (2011) **13**(3), 792–797 doi:10.1111/j.1462-2920.2010.02382.x

Modelling sugar diffusion across plant leaf cuticles: the effect of free water on substrate availability to phyllosphere bacteria

Annemieke van der Wal[1] and Johan H. J. Leveau[1,2]*

[1]*Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Heteren, the Netherlands.*

[2]*Department of Plant Pathology, University of California, Davis, CA, USA.*

Summary

We present a continuous model for the diffusion of sugars across intact plant leaf cuticles. It is based on the flow of sugars from a source, representing the leaf apoplast, to a sink, in the shape of a hemispherical drop of water on the outside of the cuticle. Flow is a function of the difference between sugar concentrations C_{Source} and C_{Sink}, permeability P of the cuticle, volume V_{Sink} of the water drop, as well as its contact angle α with the cuticle surface. Using a bacterial bioreporter for fructose, and a two-compartment experimental set-up consisting of isolated cuticles of walnut (*Juglans regia*) carrying water droplets while floating on solutions with increasing concentrations of fructose, we determined a value of 1×10^{-6} m h^{-1} for P. Using this value, we explored different scenarios for the leaching of sugars across plant leaf cuticles to reveal in quantitative terms how diffusion takes longer when V_{Sink} increases, P decreases or α increases. Bacterial growth was modelled as a function of changes in P, α and V_{Sink} and was consistent with observations or suggestions from the literature in relation to the availability of free water on leaves. These results are discussed in the light of bacteria as ecosystem engineers, i.e. with the ability to modify the plant leaf surface environment in favour of their own survival, e.g. by increasing cuticle leakage or leaf wetness. Our model represents a first step towards a more comprehensive model which will enhance our quantitative understanding of the factors that play a role in nutrient availability to bacterial colonizers of the phyllosphere, or plant leaf surface.

Received 25 August, 2010; accepted 25 September, 2010. *For correspondence. E-mail jleveau@ucdavis.edu; Tel. (+1) 530-752-5046; Fax (+1) 530-752-5674.

Introduction

The surface of plant leaves, or phyllosphere, harbours large numbers of microorganisms. For example, it is not uncommon for plant foliage to carry 10^7 or even more bacteria per square centimetre of leaf (Lindow and Brandl, 2003; Leveau, 2006). These high densities are generally explained by the availability of nutrients which support growth of bacterial immigrants to the leaf surface (Leveau, 2004). Many different such nutrients can be washed off of leaf surfaces, the most abundant being sugars like glucose, fructose and sucrose (Tukey, 1966). On leaves of greenhouse-grown bean plants, surface sugars accumulate to an average of 2.5 μg per leaf, sufficient to support populations of around 10^7 bacteria (Mercier and Lindow, 2000). Active bacterial consumption of these leaf surface sugars was demonstrated *in vivo* by the use of bacterial bioreporters that carried a sugar-responsive promoter fused to the gene for green fluorescent protein (Leveau and Lindow, 2001).

Most leaf sugars are thought to originate as photosynthates from the plant's interior and to end up on the surface by passive diffusion in a process called leaching (Tukey, 1966). The greatest obstacle for sugars to diffuse outward from the plant apoplast is the plant leaf cuticle, which is the waxy layer at the plant–air interface. Several models predict the diffusion of sugars and other plant metabolites from the apoplast across the leaf cuticle and their consumption by epiphytic microorganisms. In the Schönherr–Baur model (Schönherr and Baur, 1996), the cuticle is made up of two compartments: one is the sorption compartment, which is closest to the apoplast (the source), and the other is the limiting skin, closest to the leaf surface (the sink). The flow F (in g h^{-1}) from source to sink is a function of the area through which metabolites diffuse (A, in m^2), the rate constant of desorption from the cuticle's limiting skin (k^*, in h^{-1}), the cuticle/water partition coefficient (K_{cw}), the thickness of the limiting skin (Δx_{ls}, in m), and the difference between the concentration in the source and sink ($C_{Source} - C_{Sink}$, in g m^{-3}) as described by the Fick equation:

$$F = A \cdot P \cdot (C_{Source} - C_{Sink}) \quad (1)$$

in which permeability P (m h^{-1}) equals

$$P = k* \cdot K_{cw} \cdot \Delta x_{ls}. \quad (2)$$

On the basis of this formula and a number of assumptions, Schönherr and Baur rejected the hypothesis that carbohydrates diffuse across intact cuticles in rates sufficient to sustain growth of epiphytic microorganisms. A reconstruction of their argument follows. For a bacterium made up of 1.5×10^{-13} g carbon to double in 24 h, a flux F/A is required of 6.3×10^{-3} g carbon $h^{-1}\,m^{-2}$, if one assumes that the area A through which metabolites diffuse equals the area that is covered by the bacterium, i.e. approximately 1 μm^2, or 10^{-12} m^2. Further assuming that P ranges from 2.8×10^{-9} to 9.0×10^{-8} m h^{-1} (calculated from $\Delta x_{ls} = 10^{-7}$ m and $k^* \times K_{cw}$ for carbohydrates between 0.028 and 0.9 h^{-1}), and that the bacterium immediately consumes any of the carbohydrates that reach it, so that $C_{Sink} = 0$, this would mean that C_{Source} would have to be at least 6.9×10^4 g carbon m^{-3}. This corresponds to 1.7×10^5 g glucose equivalents m^{-3}, or 0.94 M. This value is several orders of magnitude higher than the current estimates for the combined concentrations of glucose, fructose and sucrose in the apoplast (0.1–5 mM; Lohaus *et al.*, 1995). Thus, Schönherr and Baur concluded that diffusion of sugars through intact cuticles is not sufficiently fast to support bacterial growth on the leaf surface.

There are two assumptions in the Schönherr–Baur model that are contestable. The first is the assumed P value for carbohydrates. This value does not have an experimental basis but rather is an estimate based on extrapolation from P values of more hydrophobic compounds (Schönherr and Baur, 1996). Studies that provide experimental estimates for the permeability of sugars across plant leaf cuticles are very few: the only one we are aware of is on *Prunus laurocerasus* or cherry laurel (Stammitti *et al.*, 1995), and provides much higher estimates (i.e. $1.9–8.4 \times 10^{-6}$ m h^{-1}) than Schönherr and Baur. Here, using a novel method for measuring fructose diffusion, we will present a similarly higher estimate P for fructose diffusion across isolated leaf cuticles of *Juglans regia* (common walnut). Another assumption of the model is that the area of diffusion equals the area on the cuticle that is covered by the bacterium. This ignores the fact that bacteria on leaf surfaces are oftentimes present in free water, e.g. in drops of water (Brewer *et al.*, 1991). Our hypothesis is that these bodies of water can act as very efficient sinks for carbohydrates that diffuse from the apoplast, mainly because they cover a larger surface than does a single bacterium. We formulate here a model to simulate, explain and predict the effect of water drops on sugar diffusion across plant cuticles. This is a first step towards a more comprehensive quantitative model which will enhance our understanding of the factors that play a role in nutrient availability to bacterial colonizers of plant leaf surfaces.

Results and discussion

Estimating P *for cuticle diffusion of fructose across isolated cuticles using bacterial bioreporters*

We designed a simple two-compartment source/sink setup, consisting of 15 mm isolated cuticle discs from leaves of *J. regia* floating in a Petri dish on a fructose solution (the source) and each carrying a single drop of water (the sink). Cuticles were isolated as described elsewhere (Knoll and Schreiber, 2008) from the adaxial surface (avoiding the primary vein) of *J. regia* leaves which lack hairs, stomata or glands that would otherwise leave holes in the isolated cuticle. Individual cuticle discs were floated surface-side up on 15 ml of a fructose solution (the 'source') in a sterile Petri dish. Fructose concentrations in the source were 0, 0.01, 0.1, 1, 11 or 111 mM (i.e. 2, 20, 200, 2000 or 20 000 g m^{-3}). A 20 µl drop of sterile, double-distilled water (referred to as the 'sink') was placed in the centre of the cuticle, and left for 24 h at 30°C under conditions of high humidity. At that time, 2 µl was taken from both source and sink and added to 18 µl of a cell suspension of the GFP-based fructose bioreporter *Erwinia herbicola* 299R (pP_{fruB}-*gfp*[AAV]) (Leveau and Lindow, 2001). This suspension was taken directly from an exponentially growing culture on M9 minimal medium plus 0.4% galactose, 0.2% Casamino acids and 2 mM $MgSO_4$, and supplemented with 50 µg of kanamycin per millilitre for maintenance of plasmid pP_{fruB}-*gfp*[AAV]. After 3.5 h at 30°C, cell suspensions were examined by fluorescence microscopy and image cytometry to quantify the average GFP content per cell, as described previously (Leveau and Lindow, 2001). We plotted the average GFP content per cell as a function of fructose concentration in the source for both source and sink samples (Fig. 1). As expected, the bioreporter cells reacted to the source solutions in a dose-dependent manner, i.e. higher GFP content with increasing fructose concentrations (Fig. 1A). We estimated a value of 0.18 mM or 32 g m^{-3} for $[fructose]_{1/2max}$, i.e. the fructose concentration at which the bioreporter signal was half of its maximum value. When presented with the sink solutions, the response curve was similar in shape, but clearly shifted to the right. The apparent $[fructose]_{1/2max}$ was 8.8 mM, or 1.6×10^3 g m^{-3}, indicating that the bacteria experienced in each sink a fructose concentration that was 50 times lower than that in its corresponding source. From this 50-fold difference, it is possible to approximate an apparent value P for the diffusion of fructose across isolated *J. regia* cuticles, as follows. As $C_{Source} - C_{Sink} \approx C_{Source}$, we can rewrite Eq. 1 as $F = A \times P \times C_{Source}$. F also equals the product of concentration C_{Sink} ($= 1/50 \times C_{Source} = 0.02 \times C_{Source}$) and volume V_{Sink}, divided by time t. Substituting t with 24 h, V_{Sink} with 2.0×10^{-8} m^3 (20 µl), A with 1.6×10^{-5} m^2 (= 16 mm^2, calculated from measured 4.5 mm diameter of circular drop),

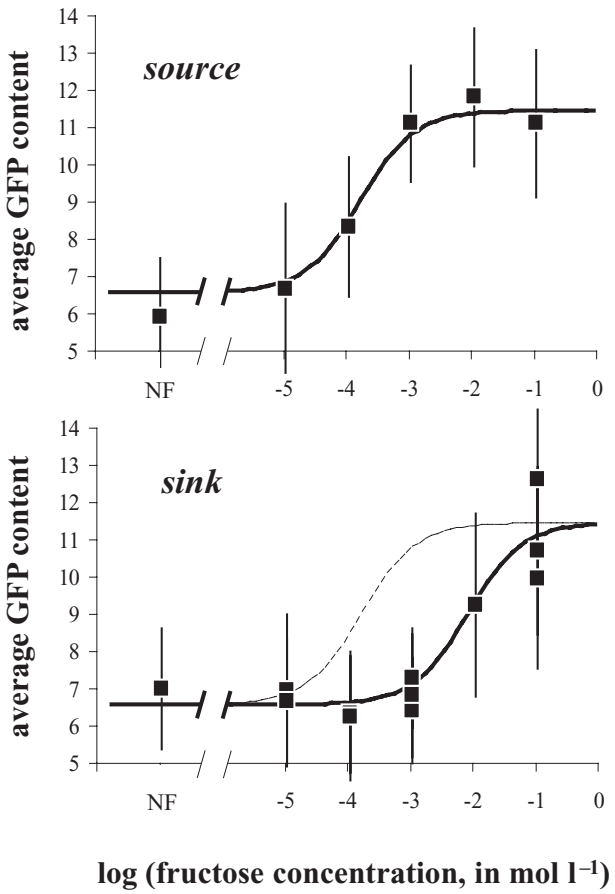

Fig. 1. Differential detection of fructose in source (top) and sink (bottom) of a two-compartment diffusion set-up (see text for details). Average GFP contents of *E. herbicola* (pP$_{fruB}$-*gfp*[AAV]) cells exposed to either source or sink were plotted as a function of the logarithm of the fructose concentration (mol l^{-1}) in the source. NF stands for 'no fructose'. Error bars indicate the standard deviation in the GFP content of bioreporter cells. Solid lines are best-fit curves for the source and sink data; the stippled line in the sink graph is the best-fit curve for the source data and serves as a reference.

C_{Source} can be eliminated to reveal that $P = 0.02 \times 2.0 \times 10^{-8}/(24 \times 1.6 \times 10^{-5}) = 1.0 \times 10^{-6}$ m h^{-1}. This value is 10 times higher than the highest theoretical estimate for *P* by Schönherr and Baur (1996), and close to but lower than the most conservative value obtained experimentally with *P. laurocerasus* cuticles (Stammitti *et al.*, 1995). To the best of our knowledge, it is the first experimental *P* value reported for sugar diffusion across cuticles of *J. regia*. The lower value for *J. regia* compared with *P. laurocerasus* is inconsistent with previous observations (Kirsch *et al.*, 1997) that *P. laurocerasus* is less permeable than *J. regia*, at least for non-sugar hydrocarbons. In one unpublished study, Krimm (2005) arrived at sugar permeabilities of $0.4–2.4 \times 10^{-7}$ m h^{-1} for isolated *P. laurocerasus* cuticles, which would place our estimate for *J. regia* in better agreement with the observation by Kirsch and colleagues (1997). These discrepancies between studies, on top of the relative scarcity of available estimates for *P*, signal a need for additional, independent studies into the diffusion of sugars across plant leaf cuticles. The bioreporter approach described here presents a viable experimental alternative for obtaining those data from different plant species.

A quantitative model for sugar diffusion across plant leaf cuticles

To formulate a quantitative model for the diffusion of sugars from the apoplast across the cuticle to a drop of water, we visualized (Fig. 2) a hemispherical sink with volume V_{Sink} and contact angle α that is separated by a barrier with permeability *P* from a source with a constant sugar concentration C_{Source}. The interface between the sink and barrier covers an area *A* that is a function of V_{Sink} and α as follows:

$$A = \pi \cdot \sin^2 \alpha \cdot \left(\frac{3 \cdot V_{Sink}}{\pi \cdot (2 - 3 \cdot \cos \alpha + \cos^3 \alpha)} \right)^{2/3} \quad (3)$$

On the basis of Eq. 1, changes in sink concentration C_{Sink} over time can be described as

$$\frac{\partial C_{Sink}}{\partial t} = \frac{A \cdot P}{V_{Sink}} \cdot (C_{Source} - C_{Sink}). \quad (4)$$

Equation 4 can be solved (assuming that $C_{Sink} = 0$ at $t = 0$) to reveal that

$$C_{Sink} = C_{Source} \cdot \left(1 - e^{-\frac{A \cdot P}{V_{Sink}} t} \right) \quad (5)$$

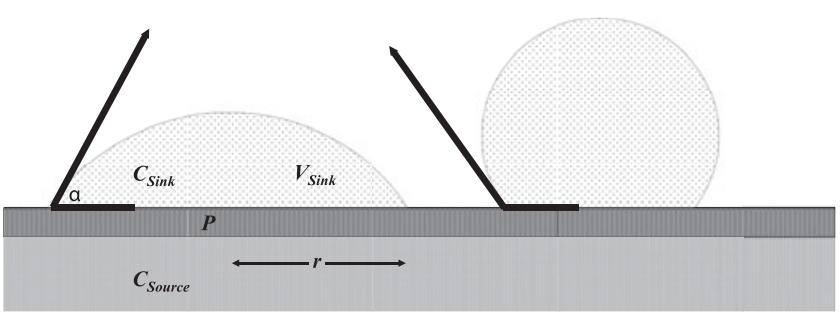

Fig. 2. Schematic representation of the sugar diffusion model presented in this article. Shown are two water drops on the surface of a simulated cuticle with permeability *P*, which separates the drops (sink with sugar concentration C_{Sink} and volume V_{Sink}) from the source with sugar concentration C_{Source}. A smaller contact angle α (left-hand drop) results in a flatter drop and a larger area *A* (larger radius *r*) covered by water.

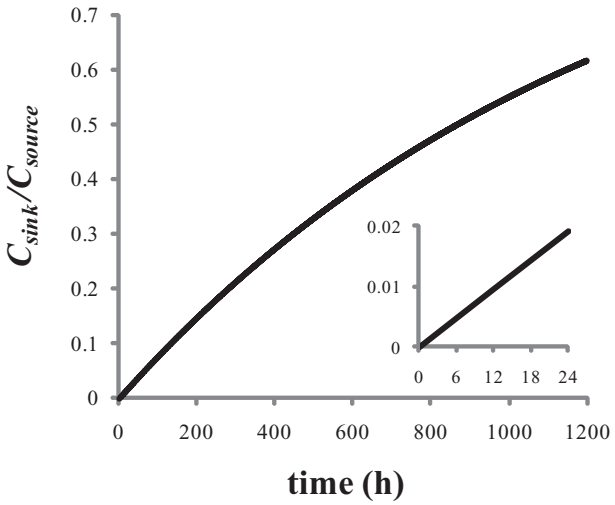

Fig. 3. Simulated diffusion of sugar across a cuticle, expressed as C_{Sink}/C_{Source} as a function of time. Parameters used were $A = 1.6 \times 10^{-5}$ m^2, $V_{Sink} = 2.0 \times 10^{-8}$ m^3 and $P = 1.0 \times 10^{-6}$ m h^{-1}. The inset shows in detail the first 24 h of the simulation.

or

$$\frac{C_{Sink}}{C_{Source}} = 1 - e^{-\frac{A \cdot P}{V_{Sink}} t}. \quad (6)$$

In Fig. 3, we plotted C_{Sink}/C_{Source} as a function of time, assuming the same values for A, V_{Sink} and P as in the experiment, i.e. $A = 1.6 \times 10^{-5}$ m^2, $V_{Sink} = 2.0 \times 10^{-8}$ m^3 and $P = 1.0 \times 10^{-6}$ m h^{-1}. It shows a typical asymptotic curve. The inset reveals that during the first 24 h C_{Sink} increases linear with time to a value C_{Sink}/C_{Source} of about 0.02, which is in agreement with the bioreporter data in Fig. 1. With time, the C_{Sink}/C_{Source} curve levels off to 1, indicating that C_{Sink} approaches C_{Source}. As a measure for the rate of diffusion we can define $t_{1/2}$ as the time it takes for C_{Sink} to reach half the value of C_{Source}, i.e. $C_{Sink}/C_{Source} = 0.5$. Substituted in Eq. 5, this gives

$$t_{1/2} = \frac{V_{Sink} \cdot \ln 2}{A \cdot P}. \quad (7)$$

By combination of Eqs 3 and 7, A can be eliminated to reveal that

$$t_{1/2} = \frac{{V_{Sink}}^{1/3} \cdot \ln 2}{\pi^{1/3} \cdot \sin^2 \alpha \cdot \left(\frac{3}{2 - 3 \cdot \cos \alpha + \cos^3 \alpha} \right)^{2/3} \cdot P}. \quad (8)$$

This formula states in quantitative terms that diffusion takes longer when V_{Sink} increases, P decreases or α increases. A change in P will result in a reverse proportional change in diffusion time, e.g. multiplying P with a factor of 1000 will result in a 1000 times lower value for $t_{1/2}$. Similarly, an eightfold increase in V_{Sink} requires double the time for a drop to fill. With an increase in α from 28° to 90°, diffusion slows down by a factor 3, same as for an increase in α from 90° to 135°.

Predictions of the cuticle diffusion model, also in relation to bacterial growth

Using the model, we explored several scenarios for the leaching of sugars across isolated plant leaf cuticles by changing values for P, α and V_{Sink} in ways for which experimental evidence exists in the literature. As a point of departure, simulation A in Fig. 4 shows the predicted accumulation of sugar from an 18 g m^{-3} source into a 20 µl sink with a contact angle of 83° across a cuticle with permeability $P = 10^{-6}$ m h^{-1}. This scenario is the same as the one used to construct Fig. 3. Under these conditions, approximately 20 ng of sugars will have diffused into the sink after 72 h. In Fig. 4, simulation B shows that increasing P by half results in a 50% rise in the amount of sugar that leaches out. Such a change in P is not uncommon during the lifetime of a leaf (Schreiber, 1994), and was also observed for cuticles of *P. laurocerasus* and *Hedera helix* (ivy) after inoculation of the cuticle with bacteria (Schreiber *et al*., 2005). Bacterial products such as extracellular enzymes that degrade the cutin polymer (Riederer and Schönherr, 1990) have been proposed to explain this effect. Another mechanism by which bacteria may enhance diffusion of sugars is through stimulation of leaf wetting, e.g. by secretion of surfactants. It was shown previously that an increase in the bacterial coverage of the leaf surface from 1% to 25% resulted in a decrease in the contact angle from 83° to 43° (Knoll and Schreiber, 2000). Simulation C in Fig. 4 shows the effect of such a reduction: due to the increased contact area with the leaf surface almost double the amount of sugar diffuses into the sink. A third prediction of the model is that many small droplets of water on leaf surfaces leach more sugars across the cuticle than few larger droplets. Simulation D in

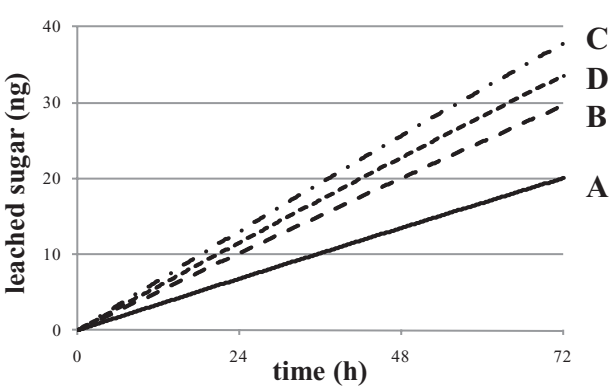

Fig. 4. Simulation of sugar diffusion over a 72 h time period. Settings were as follows: (A) $P = 1 \times 10^{-6}$ m h^{-1}, $C_{Source} = 18$ g m^{-3}, $V_{Sink} = 2 \times 10^{-8}$ m^3, $\alpha = 83°$, $A = 1.59 \times 10^{-5}$ m^2 and $\delta t = 0.2$ h; (B) same as A, except $P = 1.5 \times 10^{-6}$ m h^{-1}; (C) same as A, except $\alpha = 43°$ and $A = 3.08 \times 10^{-5}$ m^2; (D) same as A, except that the amount of diffused sugar shown is the total leached into five 4 µl drops instead of one 20 µl drop, so $V_{Sink} = 4 \times 10^{-9}$ m^3 and $A = 5.44 \times 10^{-6}$ m^2.

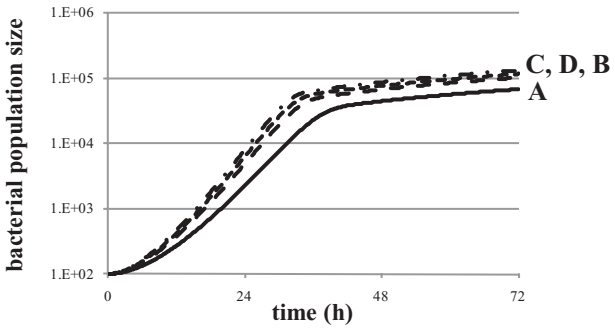

Fig. 5. Simulated bacterial growth in response to sugar diffusion across a cuticle. Shown are the numbers of bacteria as a function of time under conditions that are identical as those shown in Fig. 4, except that at $t = 0$, the sink was seeded with 100 bacteria per 20 μl (20 bacteria per 4 μl for simulation D).

Fig. 4 shows that the amount of sugar that leaks into five individual droplets of 4 μl combined is significantly larger than what leaks into a single drop of 20 μl (Fig. 4, line A). This difference is due to the larger combined surface area for the smaller drops. In nature, water ends up on leaves during precipitation events such as rain, dewfall, ground fog and cloud mist (Brewer *et al.*, 1991). It was noted (Tukey, 1966) that rain that falls as a light drizzle removes more nutrients from foliage than rain that comes down in coarser drops, which is consistent with our model prediction.

Figure 5 shows how the conditions modelled in Fig. 4 would affect the growth of bacteria on the cuticle surface as a function of available sugars. For these simulations A–D, the same parameters were used as in simulations A–D in Fig. 4, respectively, except that in each case the sink was seeded with 100 bacterial cells that were assumed to be able to utilize the sugar to multiply, following a Monod model of growth with $\mu_{max} = 0.4\ h^{-1}$ and $K_S = 0.3\ g\ m^{-3}$ (Table 1). It is clear from these simulations that an increase in P (Fig. 5, line B), a decrease in contact angle (Fig. 5, line C) and a decrease in drop volume (Fig. 5, line D) all had a positive effect on bacterial population sizes. This effect was mainly due to the higher rates at which bacteria were able to multiply in the simulated environment under conditions B, C and D (0.23, 0.25, 0.24 h^{-1}) compared with A (0.20 h^{-1}). The shape of the curves in Fig. 5, i.e. initial rapid growth followed by a level-off in the doubling rate, is typical of bacterial colonization of plant leaves under experimental (Brandl and Lindow, 1998) and field (Hirano and Upper, 1996) conditions under which increases in bacterial population sizes are limited by growth of initial immigrants rather than the arrival of new immigrants.

Summary

We present experimental data that back the hypothesis for the leaching of sugars from intact plant leaf cuticles in amounts that support growth of bacterial epiphytes. We demonstrate through modelling the positive role of free water on the dynamics of diffusion and consequently on bacterial growth. The model is compatible with existing experimental data on the ability of bacteria to act as ecosystem engineers and to alter the permeability of the leaf cuticle or to increase leaf wetting, thereby amplifying the flow of nutrients from the leaf's interior. The model presented here continues to serve as the baseline for a more comprehensive model that takes into account additional complexities of the phyllosphere environment which determine nutrient flow to bacterial colonizers on the leaf surface. One of these is the notion that at the micrometre scale, the cuticle landscape is not a homogeneous environment, but consists of different structures including trichomes and stomates, which are thought to differ in sugar permeability (Schönherr and Baur, 1996; Schreiber *et al.*, 2005) and in their ability to retain water (Brewer *et al.*, 1991). Thus, a single leaf at the scale of individual bacteria may represent a wide range of sugar availabilities and opportunities to survive and thrive.

Acknowledgements

We thank Mitja Remus-Emsermann and Lukas Schreiber for practical and theoretical discussions about cuticle diffusion

Table 1. Description, units and values for the parameters used in the model.

Parameter	Description	Unit	Values
C_{Sink}	Solute concentration in sink	$g\ m^{-3}$	Variable
C_{Source}	Solute concentration in source	$g\ m^{-3}$	18–900 (Lohaus *et al.*, 1995)
P	Solute permeance	$m\ h^{-1}$	1×10^{-6} (this article); 2.8×10^{-9} to 9.0×10^{-8} (Schönherr and Baur, 1996)
V_{Sink}	Sink volume	m^3	μl range
α	Contact angle leaf surface	rad	0.09–3.05 (i.e. 5–175°)
$t_{1/2}$	Time to reach $C_{Sink}/C_{Source} = 0.5$	h	Variable
A	Sink surface area	m^2	Function of V_{Sink} and α (Eq. 3)
μ_{max}	Maximum rate of growth of bacteria in the phyllosphere	h^{-1}	0.4 (based on *E. herbicola* 299R data; Leveau and Lindow, 2001)
K_S	Substrate affinity constant	$g\ m^{-3}$	0.3 (i.e. 1.7 μM, Leveau and Lindow, 2001)
Y	Grams of sugar needed for a bacterium to replicate	g	3×10^{-13} (Leveau and Lindow, 2001)

and Steve Lindow and Maria Marco for valuable feedback on draft versions of the manuscript. Funding was provided by the Netherlands Organisation of Scientific Research (NWO) in the form of a personal VIDI grant to J.H.J.L. This is NIOO-KNAW publication No. 4907.

References

Brandl, M.T., and Lindow, S.E. (1998) Contribution of indole 3-acetic acid production to the epiphytic fitness of *Erwinia herbicola*. *Appl Environ Microbiol* **64:** 3256–3263.

Brewer, C.A., Smith, W.K., and Vogelmann, T.C. (1991) Functional interaction between leaf trichomes, leaf wettability and the optical properties of water droplets. *Plant Cell Environ* **14:** 955–962.

Hirano, S.S., and Upper, C.D. (1996) Raindrop momentum triggers growth of leaf-associated populations of *Pseudomonas syringae* on field-grown snap bean plants. *Appl Environ Microbiol* **62:** 2560–2566.

Kirsch, T., Kaffarnik, F., Riederer, M., and Schreiber, L. (1997) Cuticular permeability of the three tree species *Prunus laurocerasus L., Ginkgo biloba L.* and *Juglans regia L.*: comparative investigation of the transport properties of intact leaves, isolated cuticles and reconstituted cuticular waxes. *J Exp Bot* **48:** 1035–1045.

Knoll, D., and Schreiber, L. (2000) Plant–microbe interactions: wetting of ivy (*Hedera helix* L.) leaf surfaces in relation to colonization by epiphytic microorganisms. *Microb Ecol* **40:** 33–42.

Knoll, D., and Schreiber, L. (2008) Methods for analysing the interactions between epiphyllic micro-organisms and leaf cuticles. In *Plant Surface Microbiology*. Varma, A., Abbott, L., Werner, D., and Hampp, R. (eds). Berlin, Germany: Springer-Verlag, pp. 471–487.

Krimm, U. (2005) Untersuchungen zur Interaktion epiphyller Bakterien mit Blattoberflächen und Veränderungen in der Phyllosphäre während der Vegetationsperiode. Thesis. Bonn, Germany: Bonn University.

Leveau, J.H.J. (2004) Leaf surface sugars. In *Encyclopedia of Plant and Crop Science*. Goodman, R.M. (ed.). New York, NY, USA: Marcel Dekker, pp. 642–645.

Leveau, J.H.J. (2006) Microbial communities in the phyllosphere. In *Biology of the Plant Cuticle*. Riederer, M., and Mueller, C. (eds). Oxford, UK: Blackwell Publishing, pp. 334–367.

Leveau, J.H.J., and Lindow, S.E. (2001) Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere. *Proc Natl Acad Sci USA* **98:** 3446–3453.

Lindow, S.E., and Brandl, M.T. (2003) Microbiology of the phyllosphere. *Appl Environ Microbiol* **69:** 1875–1883.

Lohaus, G., Winter, H., Riens, B., and Heldt, H.W. (1995) Further-studies of the phloem loading process in leaves of barley and spinach – the comparison of metabolite concentrations in the apoplastic compartment with those in the cytosolic compartment and in the sieve tubes. *Bot Acta* **108:** 270–275.

Mercier, J., and Lindow, S.E. (2000) Role of leaf surface sugars in colonization of plants by bacterial epiphytes. *Appl Environ Microbiol* **66:** 369–374.

Riederer, M., and Schönherr, J. (1990) Effects of surfactants on water permeability of isolated plant cuticles and on the composition of their cuticular waxes. *Pestic Sci* **29:** 85–94.

Schönherr, J., and Baur, P. (1996) Cuticle permeability studies – a model for estimating leaching of plant metabolites to leaf surfaces. In *Aerial Plant Surface Microbiology*. Morris, C.E., Nicot, P.C., and Nguyen-The, C. (eds). New York, USA: Plenum Press, pp. 1–23.

Schreiber, L. (1994) Comparative investigations of cuticular permeability of conifer needles from healthy and damaged trees. *New Phytol* **128:** 251–261.

Schreiber, L., Krimm, U., Knoll, D., Sayed, M., Auling, G., and Kroppenstedt, R.M. (2005) Plant–microbe interactions: identification of epiphytic bacteria and their ability to alter leaf surface permeability. *New Phytol* **166:** 589–594.

Stammitti, L., Garrec, J.P., and Derridj, S. (1995) Permeability of isolated cuticles of *Prunus laurocerasus* to soluble carbohydrate. *Plant Physiol Biochem* **33:** 319–326.

Tukey, H.B., Jr (1966) Leaching of metabolites from aboveground plant parts and its implications. *Bull Torrey Bot Club* **93:** 385–401.