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Notes on the role of water potential in the pathogenesis of fire blight, caused by Erwinia amylovora

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Abstract

The rate of multiplication of fire blight causing bacterium *Erwinia amylovora* (Burrill) Winslow et al. depends on the availability of water. Water availability can be quantified by means of the parameter 'water potential'. The relationship between water potential and relative multiplication rate of *E. amylovora* was derived from experiments of L. Shaw (1935). This relationship appears to be applicable to *E. amylovora* in plant tissues and in nectar of flowers.

Multiplication and expansion of *E. amylovora* in a restricted space, e.g. an intercellular hole, creates a pressure, which may cause schizogenic cavities in soft tissue. Strong tissue, however, may be able to resist this multiplication pressure of the bacteria, so that symptom progression can be prevented. A hypothesis is formulated on how the multiplication pressure may be quantified by means of the parameter water potential. Expansion of bacterial ooze may also be due to absorbtion of water without increase of dry weight (e.g. a daily cycle of shrinkage and expansion). This expansion may give rise to a swelling pressure, which again may be quantified by means of the parameter water potential.

Additional keywords: pressure, bacterial disease, pear.

Introduction

The (bio)chemical aspects of host-pathogen relations receive ample attention in the scientific literature. The physical aspects of host-pathogen relations receive little attention, at least with respect to bacterial pathogens.

In the case of fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) Winslow et al., circumstantial evidence points to the importance of water availability (Billing, 1976; Van der Zwet and Keil, 1979; Schulz and Schröder, 1978), which can be expressed as 'water potential' (symbol: ψ). The present paper reviews this evidence. In addition, attention is paid to the mechanical pressure of the bacterial mass on the surrounding plant tissue during pathogenesis. Perhaps, this pressure of the bacterial mass can be quantified by means of the parameter water potential. The method of quantification is summarized by two additional hypotheses. Application of the concept 'water potential' organizes and clarifies scattered information, stimulates quantification of qualitatively described relationships, and may lead to new experiments.

Shaw's experiment

Shaw (1935) demonstrated the influence of water availability on multiplication of *E. amylovora* (Fig. 1). He did not use the parameter 'water potential', but the now obsolete parameter 'equivalent relative humidity'. The physical parameters water potential and equivalent relative humidity are closely related (Slatyer, 1967; Papendick and Mulla, 1986) so that they easily can be interchanged.

The parameter 'numbers of bacteria per ml of a 24-hour-old culture' was used by Shaw to define bacterial multiplication. This parameter can be transformed approximately into the more preferable parameter 'relative multiplication rate'. If the bacteria divide at a constant rate, they multiply exponentially:

$$\frac{dN_t}{dt} = r N_t \qquad (1)$$

where: N_t = number of bacteria per volume broth (bacterial density) at time t (n ml^{-1})
t = time (hours)
r = relative multiplication rate ($hour^{-1}$)

Equation 1 can be integrated and solved for r:

$$r = \frac{1}{t} (\ln N_t - \ln N_0) \qquad (2)$$

In Shaw's experiments t (= 24 hours) and N_{24} are known. In his experiments $\ln(N_0)$ is negligible in comparison to $\ln(N_{24})$, so that Equation 2 can be rewritten as

$$r \approx \frac{1}{24} \ln N_{24} \qquad (3)$$

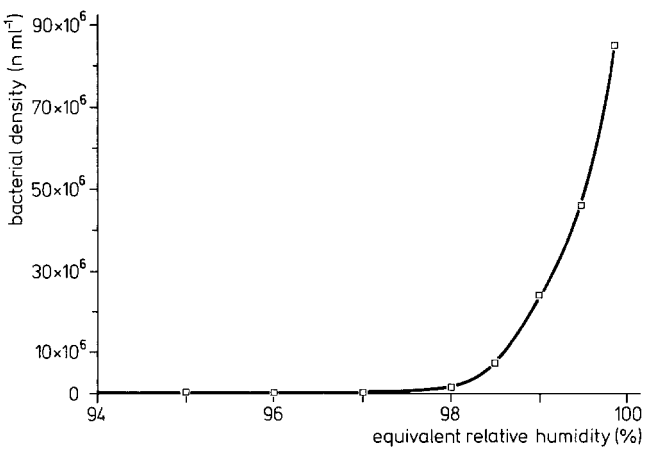

Fig. 1. Approximate numbers of cells of *Erwinia amylovora* per ml in a 24-hour-old culture in beef-extract-peptone medium, of which the different relative humidity equivalents were produced by varying the concentrations of diluted sugars (after Shaw, 1935).

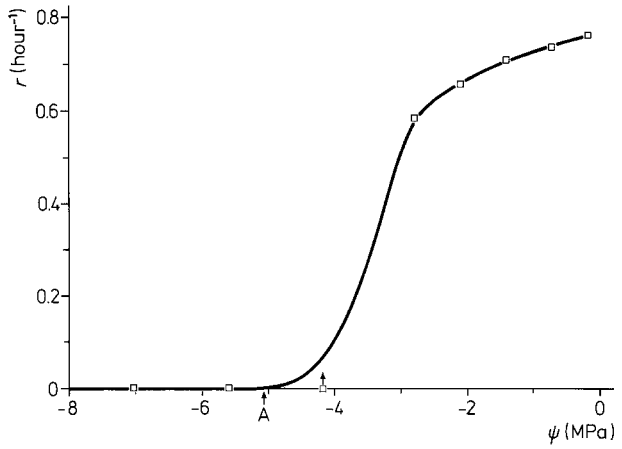

Fig. 2. Relationship between relative multiplication rate (r) of *Erwinia amylovora in vitro* and water potential (ψ) (derived from data by Shaw, 1935).

which gives the relationship between 'numbers of bacteria per ml in a 24-hour-old culture' and 'relative multiplication rate'. By means of this equation and the relationship between water potential and 'equivalent relative humidity', Fig. 1 was transformed to Fig. 2. As it is risky to assume immediate exponential growth after seeding, without a lag phase, it is possible that the values of r are not accurate. Therefore more refined experiments are undertaken.

Water potential of pear shoots

The value of ψ of a pear shoot in an orchard usually fluctuates between 0 and -3 MPa (Klepper, 1968; personal observations) (1 megapascal = 10^6 Pa = 10 bar). So, according to Fig. 2, lack of water availability may be an important factor limiting multiplication of *E. amylovora*. When the water potential of the diseased plant part is known, Fig. 2 indicates to what extent the water availability at that moment is limiting the multiplication of *E. amylovora*. The water potential of a plant can be measured, e.g. by means of a pressure chamber (Boyer, 1967; Millar and Hansen, 1975), or it can be estimated by means of a simulation program (e.g. Powell and Thorpe, 1977) when sufficient other relevant data are known.

Water potential of nectar

Fig. 2 can be applied to multiplication of bacteria in plant parts, e.g. a pear shoot. The graph can also be applied to calculations on fire blight infection. For blossom infection, high numbers of *E. amylovora* bacteria are needed (Ivanoff and Keitt, 1941). Before blossom infection can occur under natural circumstances without wounds, *E. amylovora* must multiply epiphytically on the flower. Multiplication is possible in the flower's nectar, when the sugar concentration of the nectar is not too high (Ivanoff and Keitt, 1941). Thomas and Ark (1934) suggested the need to quantify the nectar's concentration by the parameter 'osmotic potential'. For nectar in a flower, the osmotic potential equals

the water potential, so that by means of Fig. 2 the osmotic potential of the nectar can be related to the relative multiplication rate of *E. amylovora* in that nectar. After multiplication, when a threshold number of bacteria per flower is reached, infection may occur (Miller and Schroth, 1972; Thomson et al., 1975).

Measurements of the osmotic potential of the nectar (e.g. with an osmometer or a Spanner psychrometer) and use of Fig. 2 indicate when multiplication of *E. amylovora* is possible with respect to water availability. Such measurements indicate if dilution of the nectar by dew, rain or irrigation is required for multiplication, and whether, in this respect, there are differences in susceptibility between plant species and cultivars.

Multiplication pressure

In the course of pathogenesis, *E. amylovora* appears in the intercellular spaces of plant tissues. The adjacent plant cells look healthy for some time. Even when the intercellular spaces are full of bacteria and an exudate oozes out of the shoot, most plant cells may still function well (Bachmann, 1913; Eden Green, 1972). Toward the end of pathogenesis, *E. amylovora* causes dysfunction and death of plant cells. The cells are crushed and large schizogenic cavities appear in the plant tissue. Eden Green (1972) hypothesized that multiplication of the bacteria in a restricted volume would create a pressure, which would cause the schizogenic holes. Continued division would induce expansion of the bacterial mass in those directions offering least resistance. Bacterial masses would migrate either longitudinally, as internal intercellular 'strands', or radially, finally emerging as exudate. We will call this pressure of the multiplying bacteria 'multiplication pressure'. Until now, the multiplication pressure had never been quantified. Nevertheless quantification is possible, namely by application of the parameter water potential. The way of quantification is explained below.

The bacteria already present in an intercellular space, multiply at a certain rate as given by Fig. 2, until the intercellular hole is filled. The bacteria still continue to multiply, so that a pressure arises between the plant cells and the intercellular bacterial ooze. Because of this multiplication pressure, the bacteria will be less capable to expand by absorbing water, so that the absorbability of water for the bacteria decreases. The availability of water keeps the same (the water potential does not change), but the bacteria are less able to absorb the available water, because of the multiplication pressure. The ease with which *E. amylovora* can absorb water is no longer expressed by the parameter water potential, but by the water potential minus the multiplication pressure. So long as the multiplication pressure is smaller than the absolute value of the water potential, the bacteria are able to absorb some water and multiply. Note that the parameter water potential has the dimension 'energy per volume' ($J\ m^{-3}$) (Papendick and Mulla, 1986), which is equivalent to the dimension of the parameter multiplication pressure ($Pa \equiv N\ m^{-2} = J\ m^{-3}$). When a certain multiplication pressure exists, the relative rate of multiplication can still be derived from Fig. 2. On the X-axis ψ is substituted by $(\psi - \psi_{pressure})$, where $\psi_{pressure}$ represents the pressure of the bacterial mass on its surrounding plant tissue, which equals here the multiplication pressure. The value of $\psi_{pressure}$ equals 0 when the intercellular space is not filled, but when the space is being filled $\psi_{pressure}$ becomes positive. This positive $\psi_{pressure}$ reduces the absorbability of water for the bacteria, so that the relative multiplication rate decreases. The increase of the multiplication pressure slows down. Two possibilities ensue.

The first possibility is that the bacterial multiplication ceases. The plant tissue is able to resist the pressure of the bacterial ooze, and the progress of the disease through the plant stops. The value of r now equals 0 and ($\psi - \psi_{pressure}$) equals A (see Fig. 2), so that

$$\text{multiplication pressure} = \psi_{pressure} = \psi - A \qquad (4)$$

The value of A is more or less fixed (determined by the bacterium), but ψ varies according to the weather. When the plant is water-saturated ($\psi = 0$), the multiplication pressure will have its maximum value (multiplication pressure = $-A$ MPa). When the plant is 'dry' ($\psi < 0$), the multiplication pressure will be lower.

The second possibility is that the plant tissue cannot resist the bacterial pressure, and that the bacterial mass tears the tissue apart. Schizogenic cavities appear and the bacterial ooze forces its way through the plant. This happens when the plant tissues are too weak and soft to resist the pressure ($\psi - A$). According the above argument, schizogenic holes will appear in the soft tissues, especially in humid weather. There is considerable evidence that orchards planted on wet soils are more susceptible to fire blight than those on well-drained soils (e.g. Van der Zwet and Keil, 1979).

Swelling pressure

Expansion of bacterial ooze may be due to multiplication, or to water uptake without increase of dry weight (e.g. a daily cycle of shrinkage and expansion). The hygroscopic polysaccharide capsules of *E. amylovora* and the bacterial cells themselves will deliver and absorb water when ψ fluctuates. Assume that the intercellular hole is completely filled with ooze, that there is no bacterial pressure ($\psi_{pressure} = 0$), and that the water potential of the plant tissue and the bacterial ooze equals ψ_1 (Fig. 3). Further assume that the multiplication pressure is negligible (no increase of dry weight). Suddenly the water potential changes from ψ_1 to ψ_2 (for instance because of a shower). The bacterial mass will then tend to absorb water and expand (see Fig. 3). Because the intercellular space was already filled, a pressure arises:

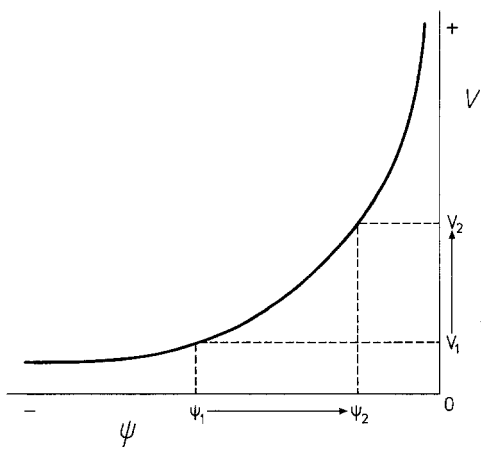

Fig. 3. An explanatory diagram of the hypothetical relationship between water potential (ψ in MPa) and the volume of fresh ooze of *Erwinia amylovora* per unit dry weight of that ooze (V in ml g^{-1}). The figure is not based on experimental data.

$$\psi_{\text{pressure}} = \psi_1 - \psi_2 \qquad (5)$$

The greater the difference between ψ_1 and ψ_2, the greater the 'swelling pressure' of the bacterial mass will be. The plant can resist this pressure only if its tissue is elastic or strong.

Quantitative hypotheses and some ideas for experiments

The last two sections give rise to two quantitative and more specific hypotheses:
1. Continued multiplication of *E. amylovora* can lead to a bacterial pressure in plant tissues. The magnitude of this pressure, in this paper called multiplication pressure, can be derived from Equation 4 and Fig. 2.
2. Swelling of the bacterial mass because of absorbtion of water only, without multiplication of the bacteria, can lead to a bacterial pressure in plant tissues too. This bacterial pressure, here called swelling pressure, can be quantified by means of Equation 5.

For verification of these hypotheses, the values of two variables need to be measured, the water potential and the softness of the plant tissues. The water potential can be measured e.g. by means of a pressure chamber or a Spanner psychrometer (Wiebe et al., 1971). Softness of tissues may be measured by means of a penetrometer (Kaufmann, 1964). A penetrometer gives a value for the ease with which a needle can force its way into a tissue. Such measurements may quantify differences in softness or resistance between parts of one tree and difference in resistance between plant species, cultivars and effects of cultural practices. Perhaps, softness measurement in combination with Fig. 2 and 3 can give insight into whether the disease in a tree will progress or stop. Notice in this respect the difference between determinate cancers, where disease progress ceases, and indeterminate cancers, where the necrosis progresses (Beer and Norelli, 1977; Shigo, 1984).

The evidence, admittedly circumstantial, is that moistness and succulence of host tissue are closely related with susceptibility to fire blight (Van der Zwet and Keil, 1979). The underlying physical relationship discussed in this paper may increase our understanding of the pathogenesis of *E. amylovora*. Experiments are in course to test the hypotheses described above.

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Samenvatting

Aantekeningen over de rol van waterpotentiaal in de pathogenese van bacterievuur, veroorzaakt door Erwinia amylovora

De vermenigvuldigingssnelheid van de bacterie die bacterievuur veroorzaakt (*Erwinia amylovora* (Burrill) Winslow et al.,), hangt af van de beschikbaarheid van water. De beschikbaarheid van water kan worden gekwantificeerd met de parameter 'waterpotentiaal'. De relatie tussen waterpotentiaal en relatieve vermenigvuldigingssnelheid van *E. amylovora* werd afgeleid uit experimenten van L. Shaw (1935, Cornell University Agricultural Experiment Station, Ithaca. Memoir 181). Deze relatie kan zowel worden toegepast op de pathogenese in planteweefsel als op de epifytische ontwikkeling in nektar.

Vermenigvuldiging van *E. amylovora* in een beperkte ruimte, bijvoorbeeld in een intercellulaire holte, creëert een druk, die tot scheuren van zacht weefsel kan leiden. Sterk weefsel kan de vermenigvuldigingsdruk van de bacteriën vermoedelijk wel weerstaan, zodat uitbreiding wordt verhinderd. In een hypothese wordt beschreven hoe de vermenigvuldigingsdruk zou kunnen worden gekwantificeerd met behulp van de parameter 'waterpotentiaal'. Wateropname door bacterieslijm zonder toename van het drooggewicht (bijvoorbeeld een dagelijkse gang van krimpen en zwellen) kan ook leiden tot een druk. Deze druk kan eveneens worden berekend met de parameter 'waterpotentiaal'.

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