Neth. J. Pl. Path. 97 (1991) 139-141

# Simulation of pressure caused by multiplication and swelling of Erwinia amylovora in intercellular space of host tissue

HENK J. SCHOUTEN[1]

Department of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, the Netherlands

Accepted 22 November 1990

**Abstract**

When *Erwinia amylovora* grows in an intercellular space of a host, and fills this space, further multiplication or swelling may create a pressure, and may cause tearing of host tissue. Theoretically, this bacterial pressure equals the actual water potential of the host tissue minus the water potential at which the bacterial biomass would completely fill the intercellular space, but without exerting pressure.

Simulation runs indicate that, when the pressure increases, the extracellular slime of *E. amylovora* shrinks by releasing water, thus allowing further production of bacterial dry matter. The slime remains around the bacterial cells as a dense substance, low in water content, having a strong capacity to swell when the pressure induces tearing apart of the host tissue. Simulation runs show that the pressure attains its highest values at evening and night.

Some fire blight symptoms that illustrate the evidence of bacterial pressure are discussed.

*Additional keywords:* fire blight, extracellular slime, extracellular polysaccharide, relative growth rate, water potential.

## Introduction

The histopathology of fire blight, incited by *Erwinia amylovora* (Burrill) Winslow et al., has been studied extensively by various workers, e.g. Eden-Green (1972), Hockenhull (1974), Huang (1974), Huang and Goodman (1976), and Wilson et al. (1987). Several histopathological aspects of the disease, however, have remained unclear. One of these is the damage caused by the bacterium to host tissue. Eden-Green (1972) suggested that *E. amylovora* and other bacteria which grow in intercellular space of host tissue, create a mechanical pressure. When bacterial biomass grows and fills the available intercellular space, further multiplication, in a restricted volume, would create a pressure, and continued division would cause expansion of the bacterial mass in those directions offering least resistance. Bacterial masses migrate either longitudinally, as internal 'strands', or radially, finally emerging as exudate.

The 'multiplication pressure' might be a good explanation for the formation of

[1]Present address: Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, the Netherlands.

often reported long longitudinally-oriented spaces, full of bacteria, in soft tissue. Extrusion of pasty bacterial biomass through natural openings of the epidermis, so that strands are formed (Keil and Van der Zwet, 1972; Eden-Green and Billing, 1972) points to existence of a mechanical pressure. The bacterial pressure may play an important role in expansion of the disease through a plant (Schouten, 1989). The idea, however, has remained largely unchallenged, as it is difficult to verify.

Bacterial pressure may originate not only from multiplication, but additionally from swelling of the bacterial mass (Schouten, 1988). The extracellular slime of *E. amylovora*, mainly consisting of extracellular polysaccharide (EPS), has a strong capacity to swell at increasing water potential (Schouten, 1989). The maximum multiplication pressure and maximum swelling pressure exerted by the bacterial mass on the surrounding plant cells has been quantified using straightforward physics theory (Schouten, 1988).

In this paper the development of pressure by cells of *E. amylovora* and by its extracellular slime is simulated by means of a computer model. This provides with insight into the dynamics of the bacterial pressure and into te role of the extracellular bacterial slime.

## Methods

### *Assumptions in the simulation model*

Several assumptions were made to simulate the growth rate of *E. amylovora*.

*Temperature.* Temperature affects the relative multiplication rate, $r$, according to Schouten (1987b), Equation (6). This relationship temperature - $r$ is derived from in vitro experiments (Billing, 1974). For calculation purposes, the effect of temperature is standardized in Equation (1) and (2) to factor $f^T$:

$$r = r_{max} \times f_T \tag{1}$$

and

$$f_T = \sin (4.2 \times 10^{-4} \times T^{2.46}) \tag{2}$$

with $0 \leq f_T \leq 1$

where: $r$ = relative multiplication rate (hour$^{-1}$), the number of daughter bacteria per mother bacterium per hour; $r_{max}$ = maximum value of $r$ (= 0.57 hour$^{-1}$; Schouten, 1987b); $f_T$ = standardized $r$, expressing the reducing effect of temperature on $r$; $T$ = temperature (°C).

It is assumed that the relative growth rate of the bacterial biomass (cells and extracellular slime) equals $r$ of the bacterial cells. Also, $r_{max}$ in vivo is assumed to equal $r_{max}$ in vitro.

*Water potential.* The water potential, $\psi$, affects $r$ as described in Schouten (1988), Fig. 1. This relationship is derived from in vitro and in vivo experiments by Shaw (1935).

$$r = r_{max} \times f_T \times f_\psi \quad (3)$$

with $0 \leq f_\psi \leq 1$

Equation (3) implies the assumption that the effect of temperature and water potential on $r$ are multiplicative and not additive (as in $r = a + b \times f_T + c \times f_\psi$). This assumption is based on a regression analysis by Schouten (1987a), showing that temperature and rainfall affected the development rate of fire blight multiplicatively.

*Pressure.* When biomass of *E. amylovora* fills an intercellular space of a host and tends to grow or swell but cannot escape, it will exert a pressure on the surrounding plant cells (Schouten, 1988 and 1989). This pressure can be calculated. Assume that an intercellular space has a constant volume $vol_{space}$, and that the dry weight of the bacterial biomass in this space equals M. Fig. 1 shows the volume of the bacterial biomass per gram dry weight ($V_{potential}$) when the bacterial biomass exerts no pressure at the actual water potential, $\psi_{actual}$. The product of $V_{potential}$ and dry weight equals the total volume of the bacterial biomass, $vol_{potential}$. If $vol_{potential} < vol_{space}$, the intercellular space is not completely filled, so that there is no bacterial pressure, but if $vol_{potential} > vol_{space}$, then the bacterial pressure $> 0$ MPa. The volume available per gram dry weight equals $vol_{space}/M = V_{space}$. The bacterial biomass tends to have a volume $V_{potential}$ per gram dry weight, but if $V_{potential} > V_{space}$, the biomass is compressed and has the volume $V_{space}$ per gram dry weight. The pressure needed to prevent swelling of the biomass from $V_{space}$ to $V_{potential}$ by absorbing water equals $\psi_{actual} - \psi'$, where $\psi'$ represents the water potential at which the biomass completely fills the intercellular space, but without exerting pressure (Fig. 1).

$$\text{pressure} = \psi_{actual} - \psi' \quad (4)$$

Because the available space is limited, the bacterial biomass is not allowed to expand by absorbing water, so that a water shortage is induced. The suction power (per unit of area) of the bacterial biomass for water does no longer equal $\psi_{actual}$, but $\psi_{actual}$ – pressure (Schouten, 1988), and Equation (3) becomes:

$$r = r_{max} \times f_T \times f_{(\psi\text{-pressure})} \quad (5)$$

omitting the subscript 'actual' in $\psi_{actual}$.

When the bacterial biomass exerts a pressure on the surrounding host cells, the host tissue may be torn apart, especially when the tissue is soft, thus allowing spread and further growth of the bacteria. The softness of the host tissue probably is a function of the growth rate during its formation (Fisher et al., 1959). Because no quantitative information is available on the pressure needed to tear apart the tissue of shoots of fruit-trees, the tearing process itself has not been incorporated in the growth and pressure model. Other factors such as nutrition of and assimilation by the trees probably affect $r$ of *E. amylovora* (Parker et al., 1961) directly (nutrition for the bacteria) and indirectly (softness of the host tissue). These effects were not simulated either. In the simulations, all environmental factors were assumed to be constant, except the variables mentioned.

*Fluctuations of temperature and water potential.* In case of fluctuating temperature,

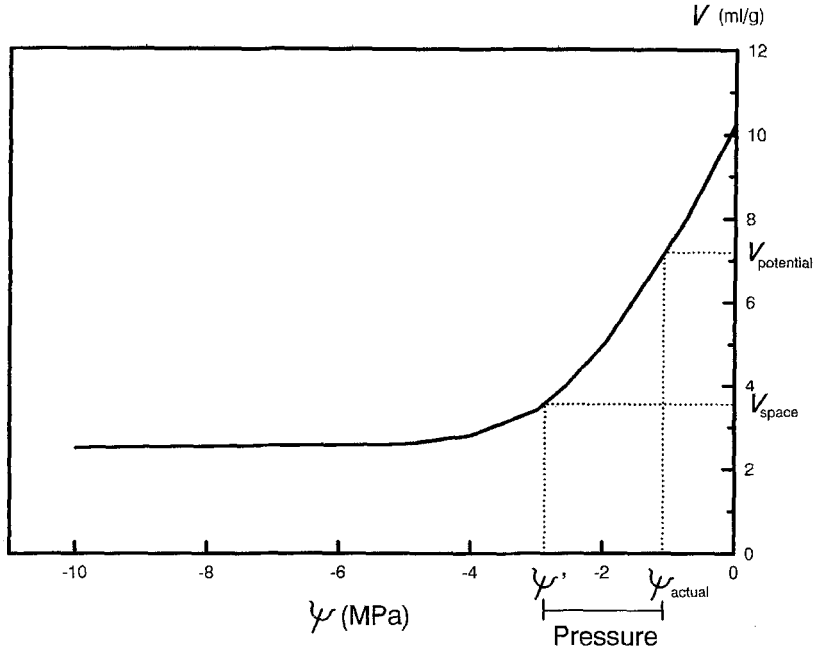



Fig. 1. The volume ($V$) per gram dry weight of fresh biomass of *Erwinia amylovora* in dependence of water potential, $\psi$ (see text). Derived from Schouten (1989). $V_{space}$ = volume of the intercellular space per gram bacterial dry weight; $V_{potential}$ = the volume the bacterial mass tends to have per gram bacterial dry weigth at the actual water potential $\psi$; $\psi'$ = water potential below which there would be no bacterial pressure because of dehydration of the bacterial biomass.

the daily temperature course was simulated according to Schouten (1987b), Equations (2) to (5). Water potentials and water flows in fruit-trees were dynamically simulated by analogy with an electric network, containing resistors and capacitors, according to Powell and Thorpe (1977). This module on water potential of the plant required as inputs (1) the daily transpiration of the tree, and (2) the water potential of the soil. The latter was assumed to equal the water potential of the shoots at night, when there is no transpiration. The output variable of this plant module was water potential of (the intercellular spaces of) the shoots of fruit-trees.

The relationships between the main variables in the growth and pressure model are indicated in Fig. 2.

*Examples of simulation runs*

In Example 1, a form of *E. amylovora* is considered which has lost its ability to produce extracellular polysaccharide (EPS). EPS has been often implicated as virulence factor (Ayers et al., 1979). As a consequence of EPS-deficiency, the considered bacterial biomass contains no extra-cellular slime. In this example temperature and water potential in the intercellular space are constant (12 °C and −0.1 MPa respectively) and the volume of the intercellular space equals 1 ml constantly. The initial

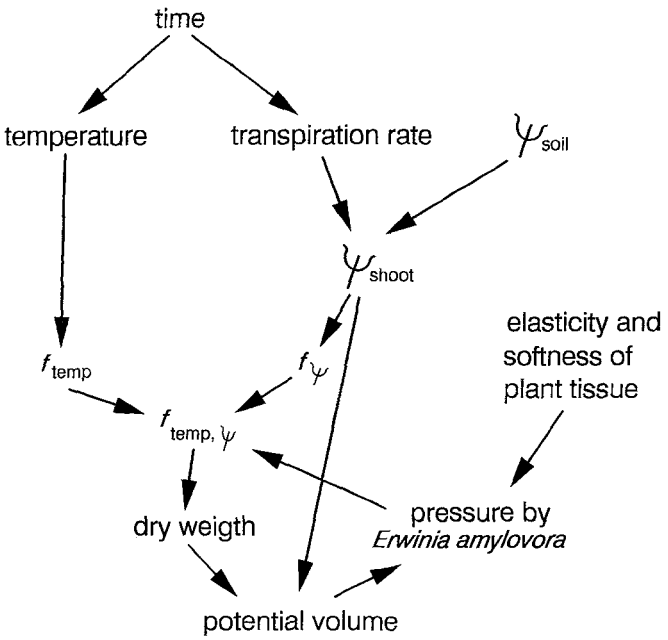



Fig. 2. Relational diagram of the major variables in the simulation model.

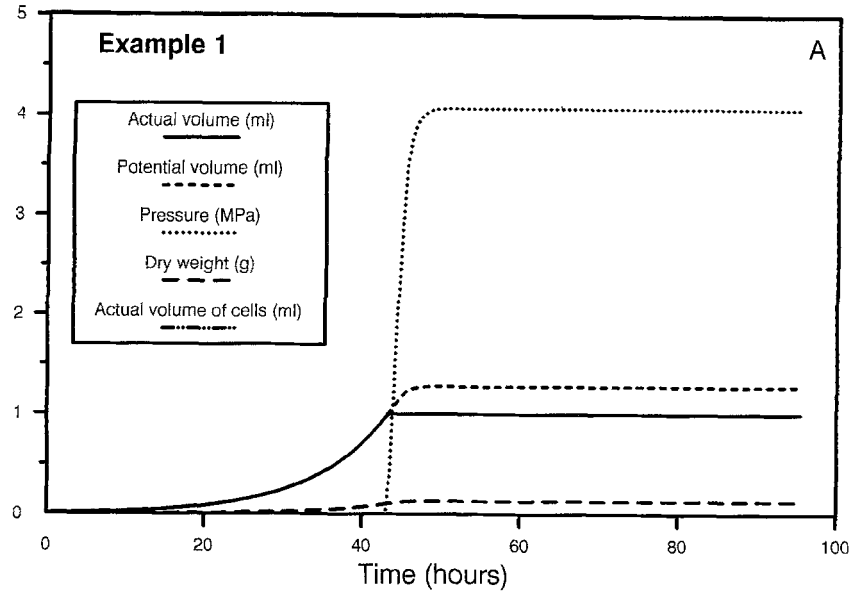
dry weight of the bacterial biomass equals 1 mg. The pressure and growth of the bacteria was simulated, using Equations 4 and 5. For determination of $\psi$', Fig. 1 was replaced by a similar graph for bacterial cells only, without extracellular slime (Schouten, 1989, Fig. 1).

In Example 2, *E. amylovora* with extra-cellular slime is considered. Temperature, water potential, intercellular volume and initial dry weight of the bacterial cells have the same values as in Example 1. The proportion of dry weight of the bacterial cells to that of the extracellular slime is assumed to be 1 to 4 continuously, in accordance with experiments of Keil and Van der Zwet (1972) and Eden-Green and Knee (1974).

Example 3 is similar to Example 2, but temperature and water potential vary. The minimum temperature equals 5 °C, the maximum temperature 15 °C, and the day length 12 h. Giving the soil a constant water potential of −0.1 MPa and the daily transpiration the value 2 l water per tree, the water potential of intercellular space in shoots of fruit-trees was simulated.

Example 4 resembles Example 3, but elasticity of the intercellular space is added. In Example 3, as in Examples 1 and 2, the intercellular space has a constant volume (1 ml), but in Example 4 the volume of the intercellular space varies because of elasticity. The volumetric elasticity of the intercellular space is assumed to equal 10 MPa, which means that a pressure increase of 1 MPa by the bacterial biomass in the intercellular space would enlarge that space by 1/10 = 10 %. The volume of the intercellular space without bacterial pressure equals 1 ml, like in the other examples.

The simulation models are available at the author as text files and as executable files. The models are written in Pascal and contain explanation and variable names in Dutch.



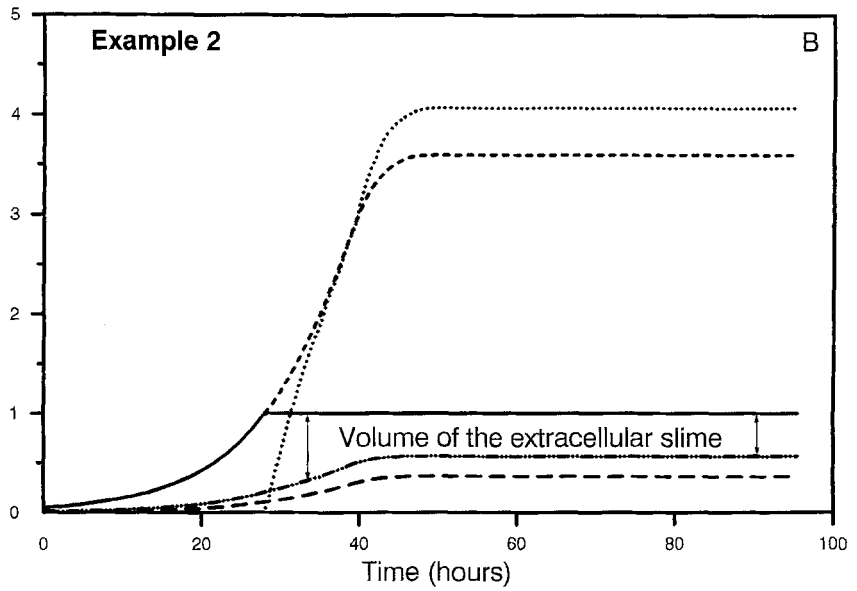
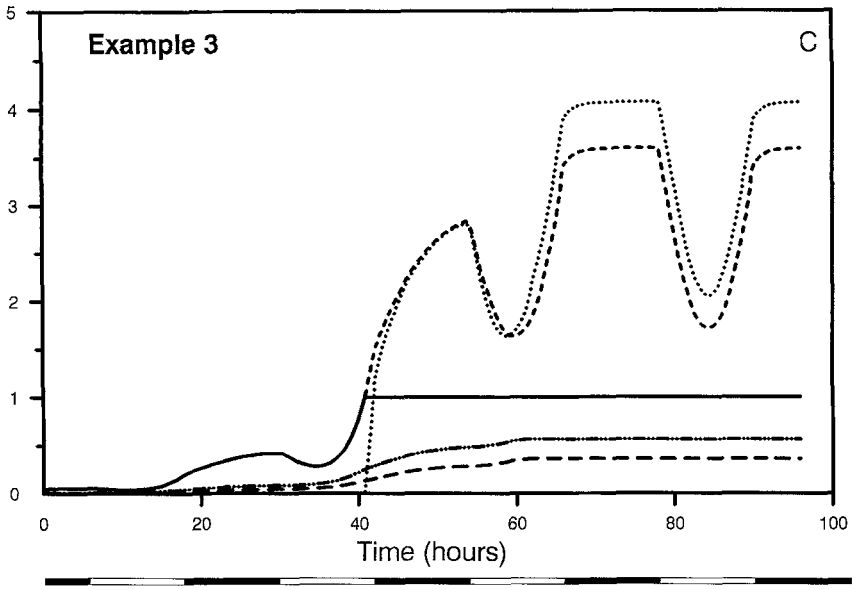
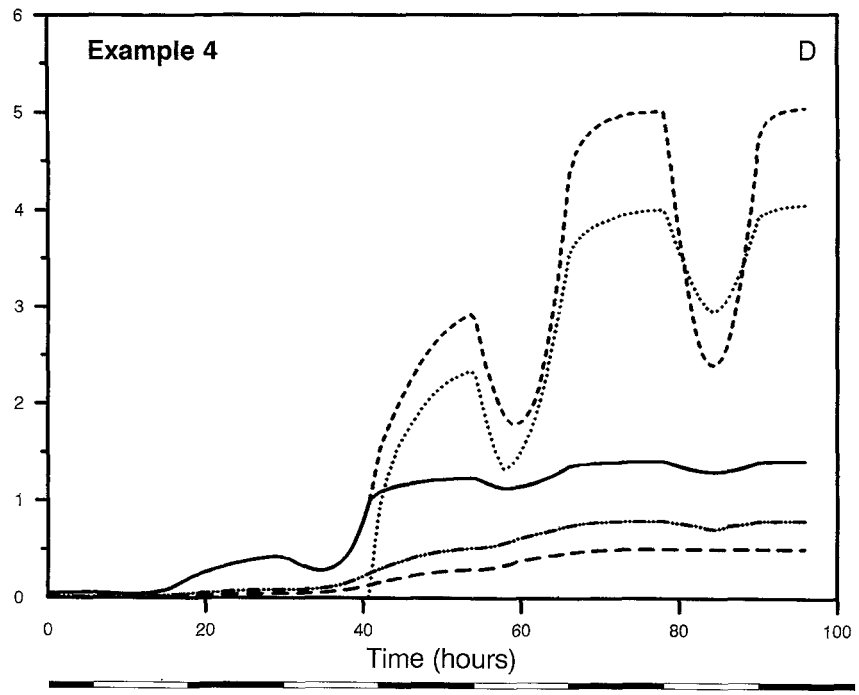




Fig. 3. Simulated volume and dry weight of *Erwinia amylovora* in an intercellular space of a host, and simulated pressure exerted by the bacterial biomass on surrounding plant tissue (see text). The volume of the bacterial biomass without compression is called 'potential volume'. The initial bacterial dry weight equals 1 mg.

A. Example 1. *E. amylovora* without extracellular slime, at constant temperature and constant water potential (12 °C and −0.1 MPa, respectively). The volume of the intercellular space equals 1 ml.

B. Example 2. As A., but with extracellular bacterial slime.




C. Example 3. As B., but temperature and water potential fluctuate as shown in Fig. 4. The horizontal bar indicates the day-night rhythm (white blocks represent daytime and black blocks nighttime).

D. Example 4. As C., but the intercellular space is assumed to be elastic in this example.

## Results

The output of the simulation run of Example 1 is shown in Fig. 3A. In this example (temperature, water potential and volume of the intercellular space are constant; no extracellular slime) the volume and dry weight of the biomass increase exponentially, until the intercellular space is filled (at time $t$ = 43 h). When the available space is full, the bacteria still continue to multiply and a pressure on the surrounding host tissue arises. The intercellular space has a constant volume of 1 ml in this example, so that expansion of the bacterial biomass is impossible. Water cannot be absorbed and becomes limiting to further production of bacterial biomass: $f_{\psi\text{-pressure}}$ decreases (Equation (5)). Because of the pressure, the bacterial cells shrink a little (the volumetric elasticity, $\epsilon$, of the bacterial cells equals 0.19 MPa according to Schouten, 1989), allowing some multiplication and dry matter production. Finally, the dry matter production ceases and pressure reaches its maximum value. The pressure then equals $\psi_{actual} - A$, where A represents the lowest value of water potential at which the bacterium is still capable to multiply and produce dry matter without pressure. Because A = −4.2 MPa (Schouten, 1988) and $\psi_{actual}$ = -0.1 MPa, the maximum pressure equals 4.1 MPa. The 'potential volume' (= $vol_{potential}$; the volume of the bacterial biomass without compression) is shown in Fig. 3A. The difference between potential and actual volume is the capacity to swell by absorbing water.

In Example 2, *E. amylovora* is considered with extracellular slime (Fig. 3B). Initially, the bacterial mass grows exponentially as in Example 1, but the intercellular space is filled sooner because of the extracellular bacterial slime. Consequently, the pressure arises earlier ($t$ = 28 h). In the previous example, the dry matter production stopped soon after the intercellular space was filled, but in Example 2 the dry matter production holds on for a longer period, even though the available space is full. The extracellular slime shrinks easily by releasing water when pressure increases (Schouten, 1989), thus allowing further dry matter production. Although the dry weight of the extracellular slime increases considerably after $t$ = 28 h (dry weight of the extracellular slime equals 80 % of the total dry weight continuously), the volume of this slime decreases, as indicated in Fig. 3B. Finally, when the pressure reaches its maximum value, the extracellular slime is a dense substance around the bacterial cells, with a low water content and with a strong capacity to swell by absorbing water when the pressure induced tearing apart of the host tissue. At maximum pressure and $\psi_{actual} = -0.1$ MPa, the bacterial biomass tends to swell to a volume which equals 1.3 ml in Example 1 (bacterial cells only) and 3.6 ml in Example 2 (bacterial cells and extracellular slime), while the actual volume of the biomass equals 1.0 ml in both examples. The maximum pressure equals $\psi_{actual} - A$, like in Example 1.

In Example 3 temperature and water potential vary as shown in Fig. 4. In contrast to previous example, the bacterial mass does not grow exponentially until the intercellular space is filled (Fig. 3C), because of the fluctuations in temperature and water potential. When the volume of the bacterial biomass attains 1 ml, a pressure originates, which does not increase gradually as in the previous examples, but fluctuates. Initially, the pressure increases because of dry matter production at a temperature of about 13 °C. During the night, temperature gradually goes down to 5 °C (Fig. 4), so that the production of dry matter and the increase of pressure slow down. After sunrise, the transpiration rate of the tree increases, and the water potential of the inter-

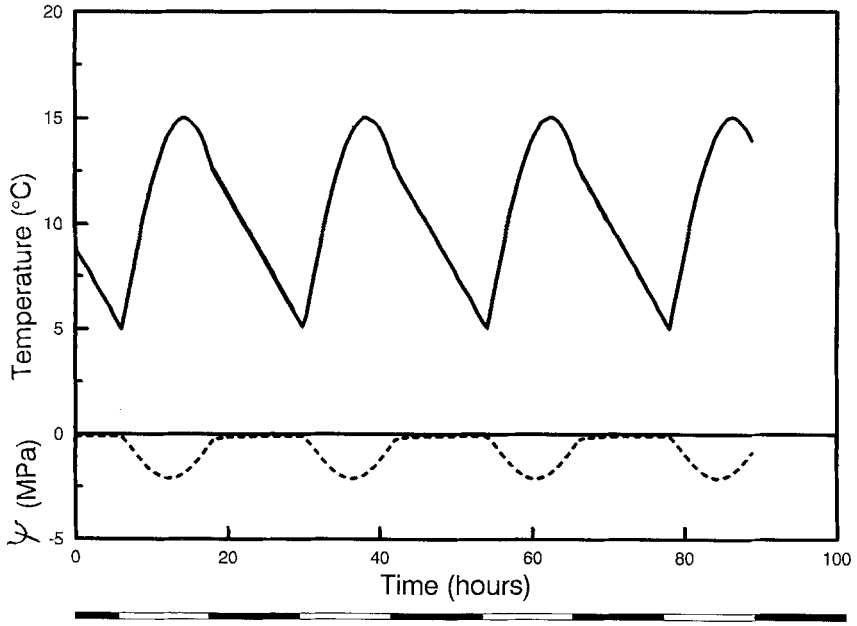



Fig. 4. Simulated courses of temperature and water potential $\psi$ for a shoot of a fruit-tree. These courses were used as inputs to the simulation runs in Examples 3 and 4, Figures 3C, D. The horizontal bar indicates the day-night rhythm.

cellular space of the shoot decreases, so that the tendency of the extracellular slime to swell decreases too. Therefore, the pressure decreases during the morning, although bacterial dry matter is still produced. In the afternoon, the water potential in the shoot rises again, inducing swelling pressure. The potential volume has roughly the same course as the pressure, after filling the available space. According to this example, formation of large cavities by tearing apart host cells and filling the cavities by swelling slime is expected during evening, night or early morning before sunrise, when the potential volume is increasing. If the cavities connect with rays and lenticels the plants will ooze. Oozing might occur also at daytime, according to other runs of the model, if temperature is high (between 20 and 30 °C), and if the water potential of the shoots is not low, because then rapid bacterial dry matter production compensates the decreased swelling capacity per gram dry weight. At nighttime, however, when the tree is (nearly) water saturated (high water potential), the pressure and potential volume probably will have their highest values.

The simulation output of Example 4 (Fig. 3D) differs slightly from that of Example 3. In Example 4 the pressure approaches its maximum value somewhat later, because it takes extra time to fill the enlarged, stretched space.

## Discussion

In order to validate this model on development of pressure caused by multiplication and swelling of *E. amylovora*, the volume and dry weight of bacterial biomass in host tissue and the bacterial pressure on surrounding plant cells should be measured.

Measurements of pressure in intercellular space, however, seem technically not feasible, so that validation of the model is not possible in that way.

Another, but less direct and therefore less convincing validation method is formulating consequences of the theory and testing whether these consequences agree with experimental results. One consequence of the supposed pressure development is that bacterial biomass should migrate faster in soft, easily torn tissue than in tough tissue. This agrees well with general experience: Succulent tissues of pear, apple, pyracantha, hawthorn, or any other host plant usually are very susceptible to blight, in contrast to harder and stronger tissues, in which the disease progress often comes to a stop (Van der Zwet and Keil, 1979). In succulent, 'loose' parenchyma tissues bacterial mass expands rapidly (Hockenhul, 1974; Huang, 1974).

Sometimes, thin diseased shoots of hawthorn (*Crataegus* spp.) and *Cotoneaster* spp. have blisters which are full of bacterial mass. The blisters can be recognized with unaided eye as bumps of the shoot. When pressing on the bumps with one's nail, damaging the upper layer of the cortex, bacterial masses are squeezed out of the shoot (personal observations). The blisters provide evidence of bacterial pressure, which tears soft tissue.

Probably, the theory applies better to the initial stages of the disease process than to the final stages when plant cells have degraded. Leakage of the host cells, which occurs in later stages of the pathogenesis, inhibits pressure development. Aerial strands, indicating pressure, usually emerge during the initial stages, when plant parts are still green. Aerial strands correspond to the long internal, longitudinally-oriented 'strands' in apparently healthy tissue (Hockenhull, 1974, Fig. 20; Wilson et al., 1987, Fig. 3C).

**Acknowledgements**

The critical reading of the manuscript by Professor J.C. Zadoks is appreciated.

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# Book review

D.W. Parry, 1990. Plant pathology in agriculture. Cambridge University Press, Cambridge, GB. 385 pp. Price paperback in GB 17.50 GBP.

In fresh inviting shades of green, a new book on plant pathology has appeared, aiming at a wide readership in agriculture. The book consists of two parts. Part I (158 pages) explains the principles of plant pathology. Pathogens considered are fungi, bacteria, mycoplasma-like organisms and viruses. Part II (189 pages) focuses on practice, presenting a catalogue of the main diseases of major temperate field crops. Clearly a choice had to be made. Of the pathogens, nematodes (and viroids) are excluded and of the crops, horticultural and tropical crops are missing.