Studies on fire blight

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. H. C. van der Plas,
in het openbaar te verdedigen
op vrijdag 5 april 1991
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

STELLINGEN

1. De wilde meidoorn werd in Nederland van 1984 tot 1990 minder bedreigd door bacterievuur dan door het bloeiverbod ter bescherming tegen bacterievuur.

2. Een fruitteler zou bij het verwijderen van bacterievuur uit appel- en perebomen niet alleen op uitwendige symptomen moeten letten, maar ook op de kleur van het cambium door aansnijden van gezond lijkende takken en stammen.
   Dit proefschrift.

3. Hoge waarden van de temperatuurvereffeningscoëfficiënt van de bodem bevorderen dauwvorming op kort gras.
   Schouten, H.J. (1985). Dauwvorming; een simulatiestudie. Vakgroep Meteorologie, Landbouwuniversiteit Wageningen. 25 pp.

4. De voorspellende waarde van regressievergelijkingen is meestal hoger dan die van simulatiemodellen. Simulatiemodellen daarintegen geven veelal meer inzicht in oorzaak-gevolg-relaties.

5. De rationalist ziet een simulatiemodel als een reeks vooronderstellingen, waarbij de aanvaardbaarheid van die vooronderstellingen de validiteit vormt van het model. De positivist vindt een model valide als het nauwkeurig voorspelt, ongeacht de structuur van het model. De empirist vindt een model alleen valide als alle aannamen empirisch juist zijn bevonden. De utilist kiest één van deze drie concepten, afhankelijk van zijn doelstelling.
   Naar P.S. Teng, 1985. Ann. Rev. Phytopathol. 23: 351-379.

6. De nauwkeurigheid waarmee lidmaatschapsrelaties in de leer van de vage verzamelingen bekend dienen te zijn vormt een belangrijke hindernis bij toepassing van die leer.
   Beschrijving van de leer van de vage verzamelingen: Zadeh, L.A., 1973. Outline of a new approach to the analysis of complex systems and decision processes. IEEE Trans. Syst. Man, Cybernet. SMC-1, p. 28-44.

7. Wie probeert natuurschoon op wetenschappelijke wijze te kwantificeren ervaart het rationalistische karakter en de begrenzingen van de taal der moderne natuurwetenschap.

8. De mening van vele ouderen dat in hun jeugd de winters strenger waren dan tegenwoordig berust grotendeels op verbeterde verwarming en isolatie van woningen.

9. Bacterievuur verbrandt te veel omdat het te weinig wordt verbrand.
   Dit proefschrift.

Stellingen bij het proefschrift van Henk J. Schouten: Studies on fire blight. Wageningen, 5 april 1991.

CONTENTS

Voorwoord

Prof. J.C. Zadoks wil ik graag bedanken voor zijn begeleiding bij het schrijven van dit proefschrift. Voor de begaafde wijze waarop hij mijn manuscripten redigeerde, voor de leerzame gesprekken, voor de vrijheid die hij mij schonk bij mijn werk en voor de wetenschappelijke ondersteuning ben ik hem erkentelijk. Andere medewerkers van de vakgroep Fytopathologie, met name Theo Ruissen, Herman Frinking, Wout Hoogkamer, Corrie Geerds, Alet Leemans, Walter Rossing en Ernst van den Ende bedank ik voor gezelligheid en hulp.

Rien van Teylingen van de Plantenziektenkundige Dienst te Wageningen heeft mij geholpen snel wegwijs te worden in het wereldje van het bacterievuur. Zonder zijn medewerking en de medewerking van vele anderen van de Plantenziektenkundige Dienst zou het onderzoek naar de invloed van bloei van meidoorn op bacterievuur in peer niet mogelijk zijn geweest.

The stimulating discussion and correspondence with Eve Billing, England, are gratefully acknowledged.

Piet Kostense maakte de grafieken die in dit proefschrift zijn opgenomen. Uit het "Fonds Landbouw Export Bureau 1916/1918" werd het drukken van dit proefschrift grotendeels bekostigd, waarvoor ik de beheerders van dit fonds erkentelijk ben.

Veruit de meeste dank ben ik verschuldigd aan mijn God, die mij inzicht wilde schenken. Hij motiveerde me en droeg zorg over het verloop van het onderzoek.

Henk Schouten

CHAPTER 0

Context and structure of this thesis; a work account

The research assignment

When I started my Ph.D. research at the Department of Phytopathology of the Wageningen Agricultural University, I was charged with the development of a warning system for chemical treatment of fire blight in apple and pear orchards. Timing of sprays against fire blight (*Erwinia amylovora*) should be optimized.

Study of the literature

During the first months of the study, I read scientific and practice oriented literature, gradually focusing on existing warning systems against fire blight. Especially in the United States and in England much effort was spent on timing chemical treatments against fire blight (e.g. Billing, 1980; Thomson *et al.*, 1982; van der Zwet *et al.*, 1988). A warning system developed by Billing (1976, 1978) in South-eastern England, looked most appropriate for the Netherlands. Several research workers and Ph.D. students (Baumm, 1985; Bazzi *et al.*, 1984; Brulez and Zeller, 1981; Dinesen *et al.*, 1984; Jacquart-Romon *et al.*, 1984; Lauwers *et al.*, 1984; Paulin *et al.*, 1983; Sobiczewski, P., 1984; Timmermans *et al.*, 1984; Zeller, 1984) had validated Billing's system in Western Europe by means of retrospective comparisons between real fire blight outbreaks and 'predicted' outbreaks. Billing and Meyneke (1981) and Meyneke and van Teylingen (1984) validated in this way Billing's system for dutch circumstances. Rather than repeating this kind of validation, I chose to study Billing's system in a more fundamental way. I checked the internal consistency of her system, verifying whether her system had an inherently logical structure. Results of this analysis

were published in two papers (Schouten, 1987a, 1987b). Billing herself validated the proposed corrections for fire blight prediction, using field data, and concluded to adopt and to recommend most corrections (Billing, 1990).

Transformations of temperature and rainfall have played important roles in Billing's system. Temperature has been transformed into potential doublings (= relative multiplication rate / ln(2)) of *E. amylovora* (Billing, 1974, 1976; Schouten, 1987b), and rainfall into a daily rain score R (if rain = 0 mm day^{-1}, then R = 0 day^{-1}; if 0 < rain < 2.5 mm day^{-1}, then R = 0.5 day^{-1}; if rain ≥ 2.5 mm day^{-1}, then R = 1 day^{-1}). The rainfall transformation into R-values, although very useful in practical warning systems, did not satisfy me from a scientific point of view. The R-values, combined with potential doubling-values, appeared to be well correlated with development rates of fire blight (Billing, 1976; Schouten, 1987a), but they gave no clear insight into the underlying effects of water on fire blight. A variable which refers to the environment of a host plant (e.g. rainfall, relative humidity, soil water content) might contribute less to understanding than a variable for the environment of the bacterium itself. The variable 'water content of the diseased host tissue' would be better but still not fully satisfactory, because it would not account for binding of water to ions, dissolved molecules, and macromolecules. Needed was a measure for the availability of water to the bacterium. I chose water potential of the environment of *E. amylovora* as the basic variable. Water potential is a well defined entity in physics, and is also used in plant physiology. It is a measure for the energy level (J m^{-3} = Pa) of the water considered, compared to that of free pure water under atmospheric pressure, and therewith it is also a measure for the availability of water. Re-interpretation of literature led to a paper on the role of water potential in the pathogenesis of fire blight (Schouten, 1988).

Reconsideration of the assignment

Using my first three papers on fire blight (Schouten, 1987a, 1987b, 1988) as a starting point, I applied dynamic simulation to the effect of temperature and water on the blossom infection process by *E. amylovora*. Prediction of 'infection days' would be very useful for the timing of preventive sprays. However, looking into the fruit growing practices it became clear to me that the antibiotic streptomycin,

the only bactericide registered for treatment of fire blight during the growing season in pear and apple orchards, has been hardly used in the Netherlands. The major reasons are: Streptomycin 1) hardly has a curative effect; 2) has a rather short preventive effect; 3) is expensive; 4) may not be used eight weeks before harvest; 5) A fruit grower is not allowed to buy Streptomycin unless he receives permission of the Plant Protection Service. Complete withdrawal of Streptomycin from the dutch market is pending (Anonymous, 1990a). Moreover, it is unlikely that other bactericides against fire blight in fruit orchards will become available.

In the dutch fruit growing practice, fire blight is predominantly controlled in two ways: 1) The government issued a regulation on the control of fire blight in all host plants (Anonymous, 1984; Meyneke, 1984). The Plant Protection Service checks the observance of this regulation. 2) Fruit growers use to inspect their orchards to remove and burne diseased tree parts.

Other topics

In view of this reconsideration, I departed from my original assignment 'Development of a warning system for chemical treatment against fire blight in apple and pear orchards', and focused on two other, more relevant topics. 1) The governmental regulation with respect to fire blight, and 2) the development of fire blight within trees after infection.

The regulation on fire blight says among other things that flowering of hawthorn (*Crataegus monogyna* and *C. laevigata*) is prohibited in large parts of the Netherlands. The flowers of hawthorn are the most important point of entry for the bacterium (Wilson *et al.*, 1987). Prevention of flowering of hawthorn lowers the risk of hawthorn becoming a source of infection for orchards and nurseries (Billing, 1981). Flowering can be prevented by cutting down the hawthorn bushes at least once per three years or by clipping the hawthorn hedges annually. From the point of view of nature and landscape conservation the blooming prohibition has aroused much opposition. Unfortunately, little was known about the effect of flowering prevention of hawthorn on fire blight in nurseries and orchards. The lack of knowledge about this subject, and its relevance to dutch society challenged me to do research in this field. In close collaboration with the Plant Protection Service, the effect of flowering prevention

of hawthorn on fire blight in pear orchards was studied (Schouten and van Teylingen, 1990a and 1990b; Schouten, 1991a). The most important conclusions from this field research were 1) the flowering prohibition hardly affected fire blight in pear orchards; 2) usually, fruit growers removed obvious fire blight symptoms from diseased pear trees by pruning and sawing, but in many cases the bacterium remained present in these trees, because sanitation was not rigorous enough. Tens of newspapers and professional journals payed attention to these results, and during several lawsuits on flowering hawthorns they have been used. At the moment (autumn, 1990), changes in the governmental regulation are proposed and vehemently opposed (Anonymous, 1990b).

The bottle-neck of fire blight control in dutch pear orchards is the removal of diseased tree parts. Therefore, I studied the development of fire blight within the tree (Schouten, 1989, 1990), using the earlier paper on water potential (Schouten, 1988). The quantified effects of temperature and water potential on multiplication of and pressure development by *E. amylovora* within host tissue allowed integration of previous results into simulation models (Schouten, 1991b-d).

Structure of this thesis

This dissertation contains two parts:

Part 1: Effects of temperature and water potential on multiplication of *E. amylovora* within the host plant and on development of pressure exerted by the bacterium on surrounding plant cells (Chapters 1 - 7).

Part 2: Effectiveness of flowering prevention of hawthorn on fire blight in pear orchards (Chapter 8).

Part 1 starts with an exploratory chapter, which discusses correlations between temperature and rainfall on the one hand and the development rate of fire blight under field conditions on the other hand (Chapter 1). Chapters 2 and 3 go into more detail. The effects of temperature T (Chapter 2), and water potential ψ (Chapter 3) on the relative multiplication rate r of *E. amylovora* are described, using data from laboratory experiments carried out earlier by other researchers. The relationship ψ-r allowed quantification of a supposed pressure by *E. amylovora* on surrounding host tissue (Chapter 3). Pressure caused by

multiplication of *E. amylovora* ('multiplication pressure') and pressure caused by swelling of bacterial biomass ('swelling pressure') were distinguished. Chapter 4 shows that the swelling pressure has to be ascribed mainly to the extracellular slime of *E. amylovora*. In Chapter 5, the effects of T and ψ on the growth, swelling, and shrinking of the bacterial biomass within the intercellular spaces of the host plant are investigated by dynamic simulation. The simulation model of Chapter 5 is extended in Chapter 6, so that the effect of weather and soil on T and ψ in the host plant, and therewith on r of the bacterium in the intercellular spaces can be studied. The sensitivity of r to weather factors is studied in more detail in Chapter 7, using statistical methods and a simulation model from Chapter 6.

Chapter 8 describes the research on the effectiveness of the regulatory flowering prevention of hawthorn. The general discussion, Chapter 9, contains among other things, practical implications of Part 1 for removal of fire blight from diseased trees.

Table 1 gives an short overview of the Chapters 1-8.

Table 1. Structure of this thesis. T = temperature; ψ = water potential; r = relative multiplication rate of *Erwinia amylovora*.

Chapter	Method	Source	Relationship
PART 1.			
1	statistics	field data from literature	T, rain → incubation period
2	simulation	laboratory data from literature	$T \rightarrow r$
3	physics	laboratory data from literature	$\psi \rightarrow r$, bacterial pressure on host tissue; re-interpretation of literature
4	experiments	Chapter 3; laboratory experiments	extracellular bacterial slime, ψ → bacterial pressure
5	simulation	Chapters 1, 2, 3, 4; literature	extracellular bacterial slime, ψ → r and pressure
6	simulation	Chapter 5; literature	weather, soil → T, ψ → r within host plant
7	simulation and statistics	Chapter 5	weather → r within host plant; methods for sensitivity analysis
PART 2.			
8	surveys; statistics	field observations and inquiries	flowering hawthorn → fire blight in pear

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PART 1

CHAPTER 1

Analysis of field data on temperature, rainfall, and development rate of fire blight

Abstract

Analysis of field data from Eve Billing, England, on the duration of the incubation period of fire blight revealed that temperature and rainfall were positively and interactively correlated with the development rate of fire blight. Values of standard regression coefficients suggest that temperature had more impact on the variation in the development rate than rainfall.

Introduction

In England, Billing (1976) studied effects of weather on the duration of the incubation period of fire blight, caused by *Erwinia amylovora* (Burrill) Winslow *et al.* She started from the assumption that the duration of the incubation period, I, is determined by temperature only. She transformed temperature into 'potential doublings' of *E. amylovora*, using the optimum curve of the bacterium in a shaking culture (Billing, 1974). Knowing the temperature course during an incubation period and the optimum curve, the number of potential doublings of the bacterium over the incubation period can be calculated. The number of potential doublings is represented in this chapter by ΣPD. Billing assumed ΣPD

to be constant, irrespective of the temperature course during the incubation period, other weather factors, host species, soil characteristics, and so on.From field observations on five rosaceous species, however, it appeared that ΣPD was not constant at all (Table 1). Therefore, she involved other weather factors, and found a reasonable fit when not only ΣPD but also a rain score, R, was inserted into a regression model. The incubation period was expected to be completed when

$$\Sigma PD \geq \frac{36}{\bar{R}} - 6 \qquad (1)$$

where: ΣPD = sum of potential doublings of *E. amylovora* from infection (-);
$\bar{R}$ = average R from infection (day^{-1});
R = rain score per day, determined as follows:
if rain = 0 then R = 0 day^{-1};
if 0 < rain < 2.5 mm day^{-1} then R = 0.5 day^{-1};
if rain ≥ 2.5 mm day^{-1} then R = 1 day^{-1}.

In this chapter, Billing's data (Table 1) are utilized for further analysis of effects of temperature and rainfall on the incubation period of fire blight. The results of the analysis are used in simulation studies (Chapter 7, 8, and 9). This chapter is a revised version of Schouten (1987).

Material and Methods

Billing's data (Table 1) were subjected to regression analysis, using the inverse of the incubation period ($1/I$) as dependent variable. ($1/I$) is equivalent to the average development rate of fire blight over the considered incubation period. The average PD-value and the average R-value over the incubation period ($\overline{PD}$ and $\bar{R}$, respectively) were chosen as the explanatory variables. Several regression models were tested for fit and their biological meanings were interpreted.

The importance of $\overline{PD}$ compared to that of $\bar{R}$ in determining the development rate of fire blight, was studied on basis of the standard regression coefficients of

Table 1. Data on incubation periods (*I*) of fire blight (from Billing, 1976). The data refer to different locations in Kent and Suffolk, England, and to different years (1959-1970). ΣPD = sum of potential doublings of *Erwinia amylovora* from infection; ΣR = sum of daily rain scores (see text) from infection.

host	*I* (days)	ΣPD (-)	ΣR (-)
stranvaesia	6	48	4.5
quince	8	69	4.5
hawthorn	10	68	5
pear	11	80	3.5
apple	13	103	4
pear	15	94	6
pear	16	110	5
pear	16	57	9.5
pear	16	99	8
apple, hawthorn	16	46	11
pear	17	72	9
apple, hawthorn	17	129	4.5
hawthorn	20	37	12
pear	29	49	14
pear	37	59	17.5

$\overline{PD}$ and $\overline{R}$. The standard regression coefficient (*src*) is defined as (Snedecor and Cochran, 1980)

$$src = \beta * \frac{\sigma_x}{\sigma_y} \qquad (2)$$

where: ß = regression coefficient of y on x (slope);
σ_x, σ_y = standard deviations of x and y.

Results

Table 2 shows that the r^2-value of the regression equation was rather low when the temperature derivative $\overline{PD}$ was used as the only explanatory variable. This agrees with the finding of Billing that ΣPD was not constant over different incubation periods. The r^2-value increased when $\bar{R}$ was added as second explanatory variable (Equation (5)). The partial regression coefficients of $\bar{R}$ and $\overline{PD}$ differed both significantly from 0, which suggests that not only $\overline{PD}$ but also $\bar{R}$ affects the development rate. Up to this point, the analysis has not revealed extra information to be derived from Billing's data.

Table 2. Regression equations explaining the development rate of fire blight ($1/I$). The regression coefficients of Equation (3), (5), and (6) are significant, but not of Equation (4) ($P < 0.05$).

regression equation	r^2	Equation
$1/I = 0.019 + 0.010 * \overline{PD}$	0.51	(3)
$1/I = 0.032 + 0.086 * \bar{R}$	0.12	(4)
$1/I = -0.077 + 0.013 * \overline{PD} + 0.16 * \bar{R}$	0.89	(5)
$1/I = 0.013 + 0.024 * (\overline{PD} * \bar{R})$	0.91	(6)

To find a regression equation with on the one hand as few explanatory variables as possible and on the other hand an optimal fit, multiple regression analysis with stepwise variable selection was applied. The set of explanatory variables consisted of $\overline{PD}$, $\bar{R}$, and $\overline{PD} * \bar{R}$, whereas F-to-enter and F-to-remove equalled 4.0. The forward as well as the backward selection procedure yielded a final regression equation which contained only one explanatory term: the interaction term $\overline{PD} * \bar{R}$. The other two terms $\overline{PD}$ and $\bar{R}$ were not included. The r^2-value of the final equation is high (Table 2; Equation (6)). The result indicates

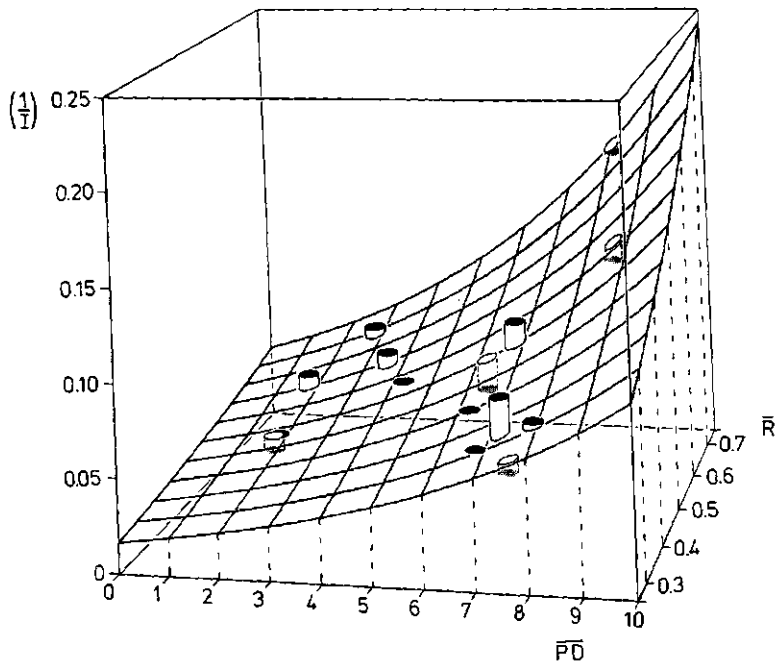

Fig. 1. Relation between the dependent variable 'average development rate of *Erwinia amylovora*' ($1/I$ in day^{-1}), and the explanatory variables 'average potential doublings' ($\overline{PD}$ in day^{-1}) and 'average rain score' ($\overline{R}$ in day^{-1}; see text). The points indicate the observed development rates (Table 1), whereas the plane is drawn according to Equation (3) (Table 2).

that $\overline{PD}$ and $\overline{R}$ affect the development rate not additively, but interactively. An increase of $\overline{PD}$ had more effect on the development rate at high $\overline{R}$-values than at low $\overline{R}$-values (Fig. 1).

The high r^2-value of Equation (3) does not necessarily imply that temperature affects the development rate of fire blight only by the way of the multiplication rate ($\overline{PD}$) of *E. amylovora*. The growth rate of the host plant, strongly affected by temperature (Barlow, 1975), is also important for fire blight development (van der Zwet and Keil, 1979). The effect of temperature changes might be underestimated when only the effect of temperature on the multiplication rate of

Erwinia amylovora is taken into account, using the *PD*-transformation. If the curve which relates the relative multiplication rate to temperature is similar to the curve which relates the growth rate of the host plant to temperature, transformation from temperature into $(PD)^2$ may be better than transformation into *PD* for linearisation of the relationship between temperature and development rate of fire blight. The fit of $(\overline{PD})^2$, which admittedly does not equal $\overline{PD^2}$, was evaluated by means of multiple regression analysis with stepwise variable selection. The set of explanatory variables consisted of $\overline{PD}$, $\bar{R}$, $\overline{PD}*\bar{R}$, $(\overline{PD})^2$, and $(\overline{PD})^2*\bar{R}$. The forward selection procedure gave as the final model Equation (6), but the backward selection procedure yielded:

$$\frac{1}{I} = 0.034 + 0.0025*(\overline{PD})^2*\bar{R} \qquad (7)$$

with $r^2 = 0.90$. This equation has approximately the same explanatory value as Equation (6).

From Equation (6) (Table 2) the relative importance of $\overline{PD}$ compared to that of $\bar{R}$ in determining $1/I$ cannot be estimated. Therefore, the natural log transformation was used to separate $\overline{PD}$ from $\bar{R}$, maintaining their interactive effect on $1/I$:

$$\ln(\frac{1}{I}) = \alpha + \beta * \ln(\overline{PD}*\bar{R}) \qquad (8)$$

which can be rewritten to

$$\ln(\frac{1}{I}) = \alpha + \beta * \ln(\overline{PD}) + \beta * \ln(\bar{R}) \qquad (9)$$

If the size of the effect of $\ln(\overline{PD})$ is distinguished from that of $\ln(\bar{R})$, this equation changes into

$$\ln(\frac{1}{I}) = \alpha + \beta_1 * \ln(\overline{PD}) + \beta_2 * \ln(\overline{R}) \quad (10)$$

The regression coefficients were estimated,

$$\ln(\frac{1}{I}) = -3.26 + 0.75 * \ln(\overline{PD}) + 0.78 * \ln(\overline{R}) \quad (11)$$

and appeared to be significant ($P < 0.001$; $r^2 = 0.87$). For calculation of the standard regression coefficients (*src*), standard deviations were estimated:

$$s_{\ln(1/I)} = 0.45$$

$$s_{\ln(\overline{PD})} = 0.61$$

$$s_{\ln(\overline{R})} = 0.31$$

so that, according to Equation (2),

$$src_{\ln(\overline{PD})} = 0.75 * \frac{0.61}{0.45} = 1.0$$

$$src_{\ln(\overline{R})} = 0.78 * \frac{0.31}{0.45} = 0.54$$

The *src*-values indicate that the impact of $\ln(\overline{PD})$ on variation in $\ln(1/I)$ was larger than that of $\ln(\overline{R})$. Apparently, $\overline{PD}$ was more important in estimating the development rate of fire blight than $\overline{R}$.

Discussion

A common feature of weather factors is their mutual dependence. The rain score R, for example, is correlated with a.o. potential transpiration rate and, after a delay, with water potential of the soil (Schouten, 1991). The regression equations in this chapter give no clear insight into cause - effect pathways with respect to $\overline{PD}$, $\overline{R}$, and $1/I$, but provide insight into correlations.

Samenvatting

Analyse van veldgegevens over temperatuur, regen en ontwikkelingssnelheid van bacterievuur

Uit analyse van veldgegevens van E. Billing, Engeland, betreffende de duur van de incubatieperiode van bacterievuur bleek dat temperatuur en regen positief en interactief gecorreleerd waren met de ontwikkelingssnelheid van bacterievuur. Waarden van standaard regressiecoefficiënten duiden erop dat temperatuur meer invloed had op variatie in de ontwikkelingssnelheid dan regen.

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CHAPTER 2

Neth. J. Pl. Path. 93 (1987) 55-60

A revision of Billing's potential doublings table for fire blight prediction

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Accepted 14 November 1986

Abstract

In her fire blight prediction systems, E. Billing (1978, in: P.R. Scott & A. Bainbridge (Eds), Plant disease epidemiology, p. 159-166) has used the parameter 'potential doublings per day' (PD) of the fire blight causing bacterium, *Erwinia amylovora*. Reconsideration of her calculations of PD revealed, however, that the PD values in Billing's table were underestimated. This leads to overestimation of the duration of incubation periods. A corrected PD table is presented. Sensitivity analyses indicated that day-of-the-year and latitude have little effect on the values in the PD table.

Additional keywords: prediction system, incubation period, *Erwinia amylovora*.

Introduction

In the fire blight prediction systems of E. Billing (1978), the parameter 'potential doublings per day' (PD) of the bacterium *Erwinia amylovora* (Burril) Winslow et al., the causal organism of fire blight in pears and other Rosaceae, is crucial. Billing (1974) investigated the relationship between temperature and potential doublings in vitro (pd) of *E. amylovora* (Fig. 1). She translated the potential doublings in vitro to potential doublings over one day (PD) according to Equation 1, using naturally occurring daily temperature courses.

$$PD = \frac{1}{24} * \sum_{t=1}^{24} (pd_{T_t}) \qquad (1)$$

where: PD = estimated potential doublings per day (day^{-1}); t = solar time (hours); T_t = temperature at hour t (°C); pd_{T_t} = potential doublings per day in vitro at temperature T_t (day^{-1}).

To estimate the average daily temperature courses at different combinations of T_{min} and T_{max}, Billing examined thermogrammes from 15 growing seasons in southern England. Her results are given in Table 1.

Where the temperature is constant during the whole 24 hours period, PD should equal pd. In other words, the PD values of Table 1 on the diagonal line $T_{min} = T_{max}$ should equal the pd values of Fig. 1. However, they do not. At 13 °C, for instance, pd = 5 day^{-1}, while in Table 1 PD = 1.5 day^{-1}. Apparently Billing made a small, although notable error. The present paper presents a corrected PD table. In addition,

Table 1. Billing's estimation of potential doublings per day (PD) in relation to daily minimum and daily maximum temperature (T_{min} and T_{max}) (Billing, 1980).

T_{max} (°C)	T_{min} (°C)				
	< 10	10-11	12-14	15-17	18-20
< 10	0.0				
10-11	0.0	0.5			
12-14	0.5	1.0	1.5		
15-17	1.5	2.0	2.5	4.5	
18-20	3.5	4.5	5.0	7.0	10.5
21-23	7.0	8.0	9.0	10.5	12.0
24-30	9.0	10.5	11.0	11.5	12.5

attention will be given to the effects of month and latitude on the values in the PD table.

Methods

The first step to reconstruct the PD table was to fit a curve to Billing's experimental data on the potential doublings in vitro, pd. The second step was to simulate an average daily temperature course, with input variables: daily minimum temperature (T_{min}), daily maximum temperature (T_{max}) and daylength. T_{min} was assumed to be reached at sunrise. As illustrated in Fig. 2, the temperature at day-time was approximated with a sinus-function with amplitude ($T_{max} - T_{min}$):

$$T_t = (T_{max} - T_{min}) * \sin(d_t) + T_{min} \quad (2)$$

$$d_t = \tfrac{1}{2}\pi * \frac{(t - t_{sunrise})}{\tfrac{1}{2} * \text{daylength} + A} \quad (3)$$

$$t_{sunrise} = 12 - \tfrac{1}{2} * \text{daylength} \quad (4)$$

where A = average time (hours) between 12:00 solar time and the moment that T reaches T_{max} (A = 2.3).

For night temperatures a rectilinear interpolation was used (see Fig. 1):

$$T = T_{sunset} + (T_{min} - T_{sunset}) * \frac{B}{(24 - \text{daylength})} \quad (5)$$

where B = time since sunset (hours).

The temperature at sunset, T_{sunset}, in Equation 5 was approximated with Equations 2, 3 and 4. Daylength was calculated according to Goudriaan (1982), as a function of day-of-the-year and latitude.

The PD values for various combinations of T_{min} and T_{max} were calculated with Equation 1. The effects of day-of-the-year and latitude on the values in the PD table were examined by way of the variable daylength.

Table 2. Estimated potential doublings per day (PD) on June 1, at 50° North latitude, in relation to daily minimum and maximum temperatures (T_{min} and T_{max}).

T_{max} (°C)	T_{min} (°C)																		
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
0	0.0																		
2	0.0																		
4	0.1	0.2	0.3																
6	0.3	0.4	0.5	0.7															
8	0.6	0.7	0.9	1.1	1.4														
10	1.0	1.1	1.4	1.6	2.0	2.4													
12	1.6	1.7	2.0	2.3	2.7	3.2	3.8												
14	2.3	2.5	2.8	3.2	3.6	4.2	4.8	5.5											
16	3.1	3.4	3.7	4.2	4.7	5.3	5.9	6.7	7.6										
18	4.1	4.5	4.8	5.3	5.9	6.5	7.2	8.0	9.0	9.9									
20	5.3	5.6	6.1	6.6	7.2	7.8	8.6	9.5	10.4	11.4	12.5								
22	6.5	6.9	7.3	7.9	8.5	9.3	10.1	11.0	11.9	12.9	14.0	15.0							
24	7.7	8.1	8.7	9.2	9.9	10.7	11.5	12.4	13.4	14.4	15.4	16.5	17.4						
26	8.9	9.3	9.9	10.5	11.2	12.0	12.8	13.7	14.7	15.7	16.7	17.7	18.5	19.2					
28	9.9	10.4	10.9	11.5	12.2	13.0	13.9	14.8	15.7	16.7	17.6	18.5	19.2	19.7	20.0				
30	10.6	11.1	11.6	12.2	12.9	13.7	14.5	15.4	16.3	17.1	18.0	18.7	19.3	19.7	19.7	19.3			
32	10.8	11.3	11.8	12.4	13.1	13.8	14.6	15.4	16.2	17.0	17.7	18.3	18.7	18.9	18.7	18.0	16.8		
34	10.5	10.9	11.4	12.0	12.6	13.3	14.0	14.7	15.4	16.0	16.6	17.0	17.2	17.1	16.7	15.7	14.2	12.2	
36	9.5	9.9	10.4	10.9	11.4	12.0	12.6	13.2	13.8	14.3	14.6	14.9	14.8	14.5	13.7	12.5	10.7	8.4	5.5

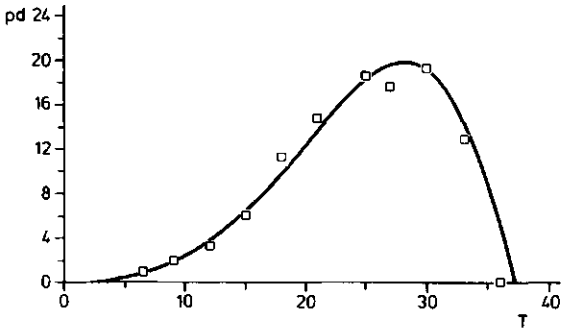

Fig. 1. Potential doublings per day of *Erwinia amylovora* in a shaking culture in relation to temperature. Billing's (1974) observed values (□) and the outcome of curve fitting by means of Equation 6 are shown. T = temperature (°C), pd = potential doublings per day in vitro (day^{-1}).

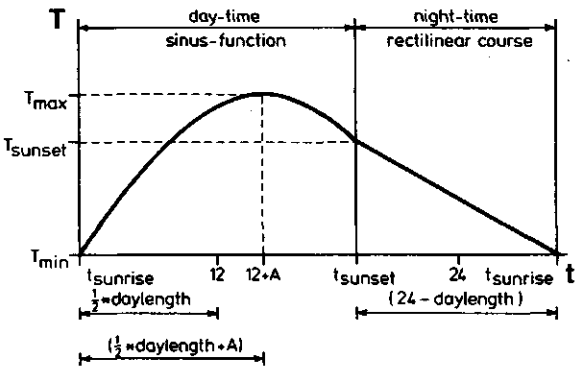

Fig. 2. Simulated temperature course during a period of 24 hours. t = solar time (hours), T = temperature (°C).

Results

For $0 < T < 36$ °C, the relation between T and pd (Fig. 1) was approximated by the equation:

$$pd = 20 * \sin(4.2 * 10^{-4} * T^{2.46}) \qquad r^2 = 0.99 \qquad (6)$$

The corrected PD values, for the latitude of Billing's East Malling Research Station are given in Table 2 and Fig. 3. Fig. 3 is composed of optimum curves with constant T_{min} values and optimum curves with constant T_{max} values. The PD course in the diagonal plane $T_{min} = T_{max}$ equals the pd course of Fig. 1.

The outcome of the sensitivity analyses of day-of-the-year and latitude is illustrated in Fig. 4 and 5. The curves in these figures are optimum curves valid for $T_{min} = 10$ °C.

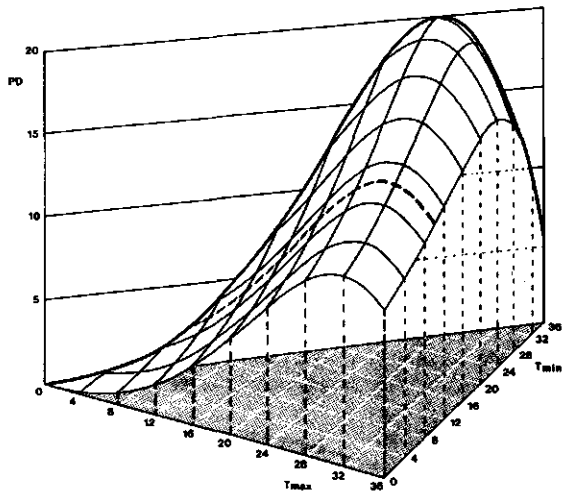

Fig. 3. Estimated potential doublings per day (PD) of *Erwinia amylovora* on June 1, at 50° North latitude, in relation to daily minimum and maximum temperatures. T_{min} = daily minimum temperature (°C), T_{max} = daily maximum temperature (°C), PD = estimated potential doublings per day (day^{-1}).

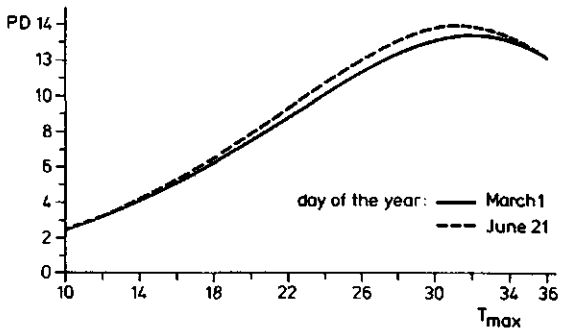

Fig. 4. Estimated potential doublings per day on March 1 and June 21, at 50° North latitude, calculated for T_{min} = 10 °C. T_{max} = daily maximum temperature (°C), PD = estimated potential doublings per day (day^{-1}).

The curves correspond with the dotted curve in Fig. 2. Optimum curves with other values for T_{min} showed similar variation. Fig. 4 and 5 show that day-of-the-year and latitude have little effect on PD values.

Discussion

The differences between Table 1 and Table 2 reveal that Billing's PD estimations were 0 to 5 day^{-1} (0 to 80 %) too low. In Billing's fire blight prediction systems, the PD

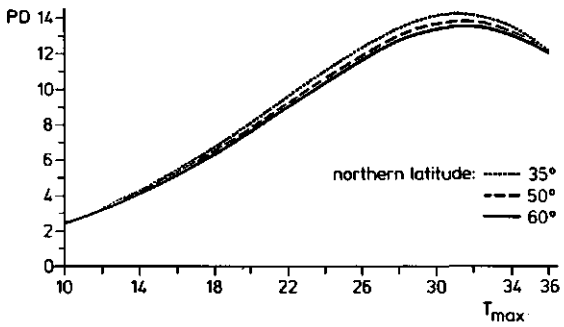

Fig. 5. Estimated potential doublings per day at three latitudes, on June 1, calculated for T_{min} = 10 °C. T_{max} = daily maximum temperature (°C), PD = estimated potential doublings per day (day^{-1}).

values are used to estimate the duration of the incubation period. An incubation period is expected to be completed when the number of accumulative potential doublings (after infection) exceeds a certain threshold. This threshold is dependent on rainfall. When Billing's table is used, the PD values are underestimated. Thus, it takes a longer time for the accumulative PD value to exceed the threshold, and therefore the duration of the incubation period will be overestimated. In a warning system this may lead to warnings that are too late for effective pruning out of diseased tree parts.

Samenvatting

Herziening van Billings potentiële-verdubbelingentabel voor bacterievuurvoorspelling

Billing maakte in haar systemen waarin de ontwikkeling van bacterievuur wordt voorspeld, gebruik van de parameter 'potentiële verdubbelingen per dag' (= PD) van de bacterievuur veroorzakende bacterie *Erwinia amylovora*. Uit herberekeningen blijkt echter, dat de PD-waarden in Billings tabel onderschat worden. PD-onderschattingen leiden in haar systemen tot overschattingen van de duur van incubatieperioden. Een gecorrigeerde PD-tabel werd samengesteld. Uit gevoeligheidsanalyses bleek dat de dag van het jaar en de breedtegraad een gering effect hebben op de waarden in de PD-tabel.

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CHAPTER 3

Neth. J. Pl. Path. 94 (1988) 213-220

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

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Accepted 1 February 1988

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

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

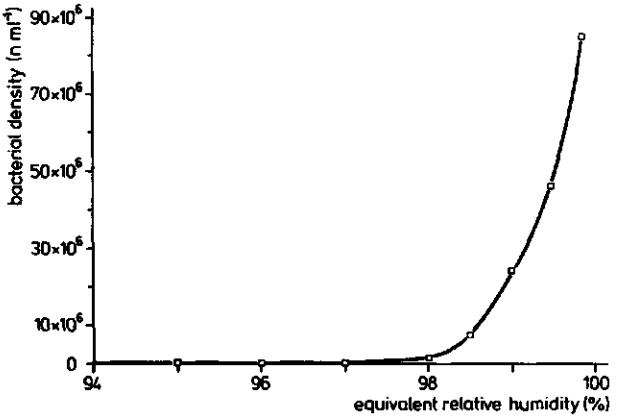

Fig. 1. Approximate numbers of cells of *Erwinia amylovora* per ml in a 24-hour-old culture in beef-extract-peptone medium, of which the different relative humidity equivalents were produced by varying the concentrations of diluted sugars (after Shaw, 1935).

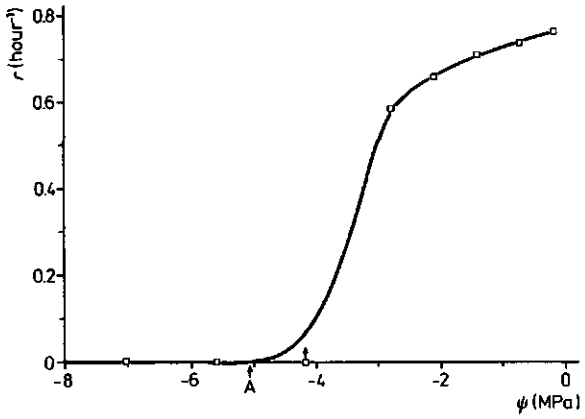

Fig. 2. Relationship between relative multiplication rate (r) of *Erwinia amylovora in vitro* and water potential (ψ) (derived from data by Shaw, 1935).

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:

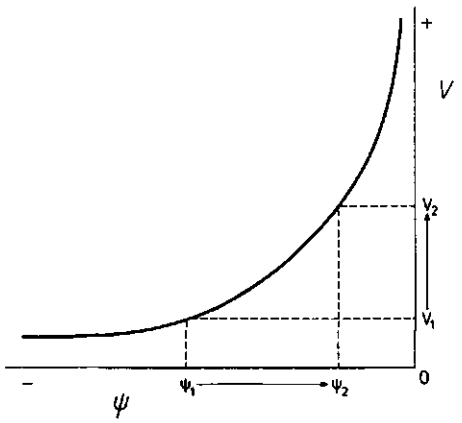

Fig. 3. An explanatory diagram of the hypothetical relationship between water potential (ψ in MPa) and the volume of fresh ooze of *Erwinia amylovora* per unit dry weight of that ooze (V in ml g^{-1}). The figure is not based on experimental data.

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

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.

Acknowledgements

The help of Professor J.C. Zadoks (Laboratory of Phytopathology, Wageningen, the Netherlands) to write this paper is gratefully acknowledged, as well as the linguistic assistance of Dr R.E. Stuckey (University of Kentucky). Thanks are due also to Ir H.D. Frinking, Dr M.A. Ruissen (Laboratory of Phytopathology, Wageningen, the Netherlands) and Dr E. Billing for critically reading the text.

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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CHAPTER 4

Neth. J. Pl. Path. 95 (1989) Supplement 1: 169-174

A possible role in pathogenesis for the swelling of extracellular slime of Erwinia amylovora at increasing water potential

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Accepted 11 May 1989

Abstract

The volume of the cells of *Erwinia amylovora* (Burrill) Winslow et al. hardly changes with changing water potential, but its extracellular slime swells strongly with increasing water potential. When bacterial slime has accumulated in intercellular spaces of the host plant and the water potential rises, the slime will swell. If this slime cannot escape, it will exert a pressure on the surrounding plant cells. This 'swelling pressure' can rise to $\Delta\psi$, being the change in water potential (up to 3 MPa). The pressure may lead to compression of soft host cells and to formation of large slime-filled holes in the plant tissue. Moreover, the swelling slime may force its way to the outside of the plant (exudation) or to healthy parts. Cork barriers, being formed by the plant after infection, may be breached if the mechanical pressure is high and the cork barrier is incomplete or not yet fully developed. Then, the resistance reaction of the plant, the attempt to seal off, is not effective. The swelling pressure would explain how the extracellular slime may function as a virulence factor.

Additional keywords: compartmentalization, fire blight, pear, swelling pressure

Introduction

Previous studies support the contention that the extracellular slime of *Erwinia amylovora* (Burrill) Winslow et al. plays an important role in the pathogenesis of fire blight (e.g. Goodman et al., 1978; Billing, 1984). Suhayda and Goodman (1981) showed that the extracellular slime may cause wilting by blocking the xylem vessels. From ultrastructural studies it appeared that the bacterial slime causes plasmolysis of xylem parenchyma cells, severe pertubation of the plasmalemma of those cells and formation of cavities in host tissue (Eden-Green, 1972; Huang and Goodman, 1976; Goodman and White, 1981). How the extracellular slime exerts its effect has not yet been established. In this paper an attempt is made in explaining how the extracellular slime could cause some of the ultrastructural changes in the host. The explanation is based on the ability of extracellular slime to shrink and swell strongly at changing water potential. Volume changes at varying water potential were investigated in two experiments. In the first experiment changes in cell volume of *E. amylovora* were studied, and in the second experiment attention was focussed on volume changes of bacterial slime. The emphasis of this paper, however, is not on the experiments, but on hypotheses put forward in the 'Discussion'.

Materials and methods

Cell volume in dependence of water potential. A virulent strain of *E. amylovora* (PD 269; PD = culture collection of the Plant Protection Service, Wageningen, the Netherlands) was grown at room temperature in a liquid medium (LM; pH 7.3) containing 10 g sucrose, 3 g peptone (Oxoid L37) and 1 g yeast extract (Oxoid L21) per liter distilled water. In order to obtain different levels of water potential, 0, 25, 50 and 75 g sucrose was added to 0.9 % (w/v) NaCl-solutions of 100 ml. The solutions were made free of particles by filtration and sterilized. To each solution, 100 μl of a 1-day-old LM culture of *E. amylovora* was added. Then the volumes of the bacterial cells were determined by means of a Coulter Counter (Coulter Electronics Ltd, model ZF, connected to a Coulter Channelyzer), using a tube with a diaphragm of 30 μm diameter. The water potentials of the four suspensions were measured by means of an osmometer (electronic semi-micro osmometer, Knauer, type M).

Biomass volume in dependence of water potential. For the production of a bacterial biomass with a water potential of almost 0 MPa, a suspension of *E. amylovora* was plated on water saturated nutrient sucrose agar (Lelliott, 1968), and incubated at 20 °C. The high percentage of sucrose in the agar (5%) caused abundant production of extracellular slime (Bennett and Billing, 1978). After 3 days, bacterial biomass (= bacterial cells and extracellular slime) was scraped off the agar. For volume and water potential determinations four metal cups (0.4 ml) were used, which fit in the sample chamber (Wescor Sample Chamber C-52) of a psychrometer. The cups were weighed, filled with bacterial biomass and weighed again. Previous determination of the dry matter percentage of the biomass enabled calculation of its dry weight per cup. The four cups were placed in a vacuum desiccator with silica gel for 0, 1, 2 and 3 h, respectively, to lower the water potential of the slime. After slight stirring, the water potentials of the four samples were measured by means of a calibrated Spanner (Peltier type) psychrometer (Wiebe et al., 1971). Then, the volumes of the four cups with biomass, and afterwards without biomass, were measured by means of a pycnometer (after Hubbard, Kimble no. 15110) with mineral oil (Phillips Petroleum Company, Soltrol 170). These data allowed computation of the volume of fresh biomass per gram dry weight in dependence of water potential. The experiment was repeated using another virulent strain, PD 754 from the collection of the Plant Protection Service, Wageningen.

Results

Cell volume in dependence of water potential. Fig. 1 shows that the volume of the bacterial cells decreased about 20 % when the water potential was lowered from 0 to −5 MPa. Probably, the volume changes were restricted by the firmness of the bacterial cell wall. The elastic properties of cell walls are described by the volumetric elastic modulus, ϵ, which determines the inverse of the slope of the water potential − volume curve of Fig. 1 (Baker, 1987). This measure of the cell wall to resist pressure is defined by the following equation:

$$\epsilon = \Delta\psi \frac{V}{\Delta V}$$

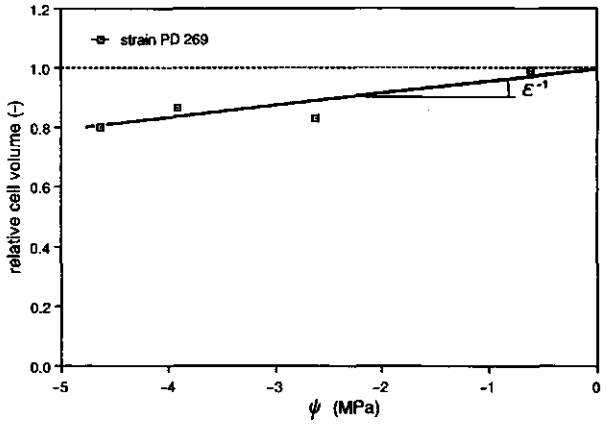

Fig. 1. Relative volume of *Erwinia amylovora* cells in dependence of the water potential (ψ). The relative volume of the cells at water saturation level (ψ = 0 MPa) is set to 1. The slope of the line equals the inverse of the volumetric elastic modulus (ε).

where ε is the volumetric elastic modulus (Pa), $\Delta\psi$ the change in water potential (Pa), V the volume at ψ = 0 MPa and ΔV the volume change induced by change in water potential. ε of elastic cells without walls is less then 0.1 MPa, whereas ε of plant cells with walls ranges from 3 to 60 MPa (Zimmermann, 1978). In Fig. 1 ε equals 19 MPa.

Volume of bacterial biomass in dependence of water potential. The bacterial biomass (cells and extracellular slime) shrank strongly when water potential decreased from 0 to −3 MPa (Fig. 2). This strong decrease in volume resembles what is often seen in the field: after exudation the bacterial ooze dries and shrinks and remains on the plant's surface as a very thin and shiny layer.

Comparison of Figs 1 and 2 shows that the strong shrinking of the biomass can be ascribed for a minor part to the cells and for a major part to the extracellular slime. Politis and Goodman (1980) had a similar experience when they had difficulties with making electron micrographs of the extracellular slime, because it shrank enormously during dehydration, whereas the bacteria approximately kept their shape and size.

Discussion

The issue is: do these changes in volume of the extracellular slime under the influence of water potential have a function in pathogenesis? This is probably the case. When bacterial slime has accumulated in the host plant (in the intercellular spaces or in the xylem) and the water potential in the host rises rapidly (for instance during a shower at the end of a sunny day), the slime will swell strongly. If this swelling slime cannot escape, it will exert a pressure on the surrounding plant cells. The 'swelling pressure' can rise to $\Delta\psi$, (the water potential rise; Schouten, 1988). Inside host plants of *E. amylovora*, the water potential usually fluctuates between 0 and −3 MPa (Klepper, 1986; Schouten, unpublished results), so that $\Delta\psi$ and the swelling pressure can reach

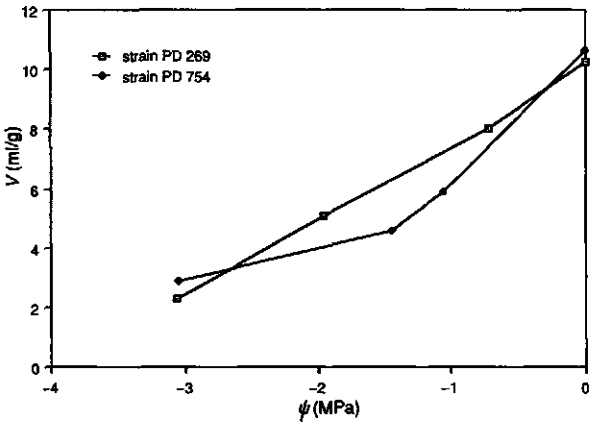

Fig. 2. The volume (V) per gram dry weight of fresh extracellular slime and cells of two virulent strains of *Erwinia amylovora* in dependence of water potential (ψ).

values of 2 to 3 MPa. For comparison: the pressure in an automobile tire is approximately 0.2 MPa. A prerequisite for swelling pressure is that extracellular slime can be produced *in vivo* at water potential levels below 0 MPa, which probably is the case (Schouten, 1988).

Increase of the swelling pressure can have several effects:

1. Plant cells are compressed and plasmolyzed and may be damaged, leaking nutrients, which become available for bacterial growth. Particularly, this can be the case for cells which lack firmness, such as parenchymal cells. Hignett and Roberts (1985) showed that isolates of *E. amylovora* induced electrolyte leakage of young pear fruit slices associated with multiplication of the bacteria. Prevention of contact between bacterial inoculum and plant tissue by means of a membrane filter prevented such loss of electrolytes and bacterial growth;
2. Cells are torn apart so that large slime-filled holes are formed in the plant's tissue;
3. The slime forces its way through the intercellular spaces of succulent and soft tissue and invades healthy parts of the host;
4. The slime forces its way through the intercellular spaces of the plant and the lenticells to the outside of the host (exudation, ooze droplet and strand formation);
5. The slime can breach developing cork barriers, being formed by the plant after infection to confine the disease.

In trees different barriers exist. Shigo (1984) showed in a general sense that, when trees are injured or infected, they defend themselves by walling off the damage. At the time of infection, the following prefabricated walls already exist: type 1 walls resist the vertical spread of disease, type 2 walls (the year rings) the radial spread, and type 3 walls the lateral spread. As a response to infection, new cambium appears which generates type 4 walls, which isolate diseased from healthy wood. Type 1 walls are relatively weak, type 2 and 3 walls are stronger, and type 4 walls are the strongest (Shigo and Marx, 1977). Shigo's ideas on compartmentalization can be applied to fire blight. The 3-dimensional structure of fire blight cankers (long, not deep and rather narrow) reflects the strength of type 1, 2 and 3 walls. The cork barriers formed in wood

and bark after infection by *E. amylovora* (Hockenhull, 1974) can be considered as type 4 walls. When the swelling pressure becomes strong enough to breach a 'wall', the compartmentalization no longer holds, and the bacterial mass will spread through the plant.

A prerequisite for progress of the disease is that the type 4 wall is broken before it is fully developed, because an established cork barrier will not allow water transport from the healthy to the diseased part of the plant where the bacterial mass needs water for growth and swelling. If, in contrast, the cork barrier is formed in time, the resistance barrier is effective. The tissue of young shoots is so soft (walls of types 1, 2 and 3 are so weak) that spread of the disease is often faster then the formation of cork barriers (type 4 walls). This is certainly the case for fast growing pear shoots. In branches, the existing walls are stronger, and effective confining is often possible in apple and hawthorn (Hockenhull, 1974; personal observations), and sometimes in pear trees. In tree trunks the existing walls are still stronger, and effective girdling (formation of so-called determinate cankers; Beer and Norelli, 1977) often occurs, even in pear trees. Sometimes, the type 1, 2 and 3 walls are breached, while the cork barriers are not yet fully developed, enabling the bacteria to spread (formation of indeterminate cankers).

In conclusion, breaking through compartmental walls is probably caused by swelling pressure of extracellular slime at increasing water potential. With extracellular slime *E. amylovora* may spread more easily and rapidly.

Acknowledgements

I thank Professor J.C. Zadoks, Dr M.A. Ruissen, Ir H.D. Frinking (Department of Phytopathology, Wageningen Agricultural University, the Netherlands) and Ir J.D. Janse (Department of Bacteriology, Plant Protection Service, Wageningen, the Netherlands) for critical reading of the manuscript and stimulating remarks. I am grateful to Dr C.J. Bos and E.F. Holub (Department of Genetics, Wageningen Agricultural University, the Netherlands) for kindly placing the Coulter Counter at my disposal.

Samenvatting

Een mogelijke rol in de pathogenese voor de zwelling van het extracellulaire slijm van Erwinia amylovora bij toenemende waterpotentiaal

Uit *in vitro* experimenten bleek dat bij een wisselende waterpotentiaal de cellen van de bacterie *Erwinia amylovora* (Burrill) Winslow et al. nauwelijks krimpen of zwellen, maar dat het extracellulaire slijm van deze bacterie wel sterk van volume verandert. Als er veel bacterieel slijm gevormd is in de intercellulaire holten van de waardplant en de waterpotentiaal plotseling stijgt, bijvoorbeeld na een regenbui, dan zal het slijm sterk zwellen. Als dat slijm echter niet kan ontsnappen, zal het een druk uit gaan oefenen op de omringende plantecellen. De 'zweldruk' kan oplopen tot de mate van waterpotentiaalverandering (tot 3 MPa). De zwelling kan vermoedelijk leiden tot in elkaar drukken van zachte plantecellen en vorming van holten in aangetast planteweefsel. Verder kan waarschijnlijk het zwellende slijm door intercellulaire

holten zich een weg banen naar buiten (exudatie) of in gezond, zacht weefsel van de waard binnendringen. Als de druk van het extracellulaire slijm op het omringende planteweefsel hoog wordt, kunnen vermoedelijk ook mechanische barrières, zoals in aanleg zijnde kurkwanden, die de plant aan het vormen was om de ziekte in te sluiten, doorbroken worden. Na doorbreking van deze zones breidt de ziekte zich verder uit in de plant.

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CHAPTER 5

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

Submitted to Netherlands Journal of Plant Pathology, author Henk J. Schouten.

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 which evidence bacterial pressure are discussed.

Additional keywords: fire blight, extracellular slime, 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. Some research workers, a.o. 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 then 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 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; personal observations) 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, 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 *E. amylovora* is simulated dynamically by means of a computer model. Also the effect of extracellular bacterial slime on the pressure is simulated.

The simulation model

Assumptions

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 by means of factor f_T:

$$r = r_{max} * f_T \quad (1)$$

and

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

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 limiting 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, ψ, affects r as described in Schouten (1988), Fig. 1. This relationship is derived from *in vitro* and *in vivo* experiments by Shaw (1935).

$$r = r_{max} * f_T * f_\psi \qquad (3)$$

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

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

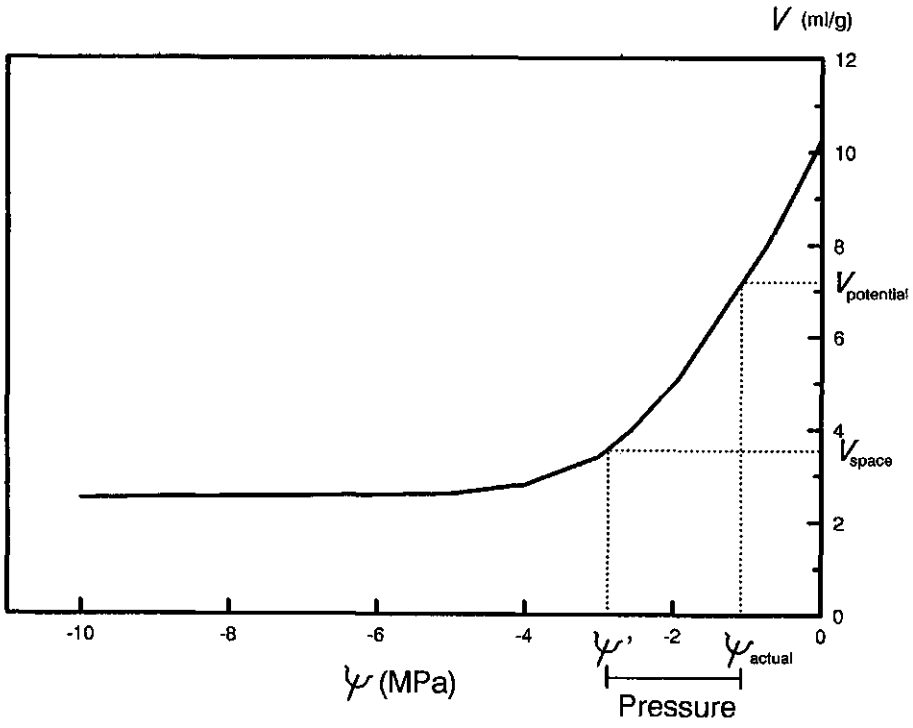


Fig. 1. The volume (V) per gram dry weight of fresh biomass of *Erwinia amylovora* in dependence of water potential, ψ (see text). Derived from Schouten (1989).

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, ψ_{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 ψ' represents the water potential at which the biomass completely fills the intercellular space, but without exerting pressure (Fig. 1).

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

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 ψ_{actual}, but ψ_{actual} - pressure (Schouten, 1988), and Equation (3) becomes:

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

omitting the subscript 'actual' in ψ_{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, 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 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 ψ', 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

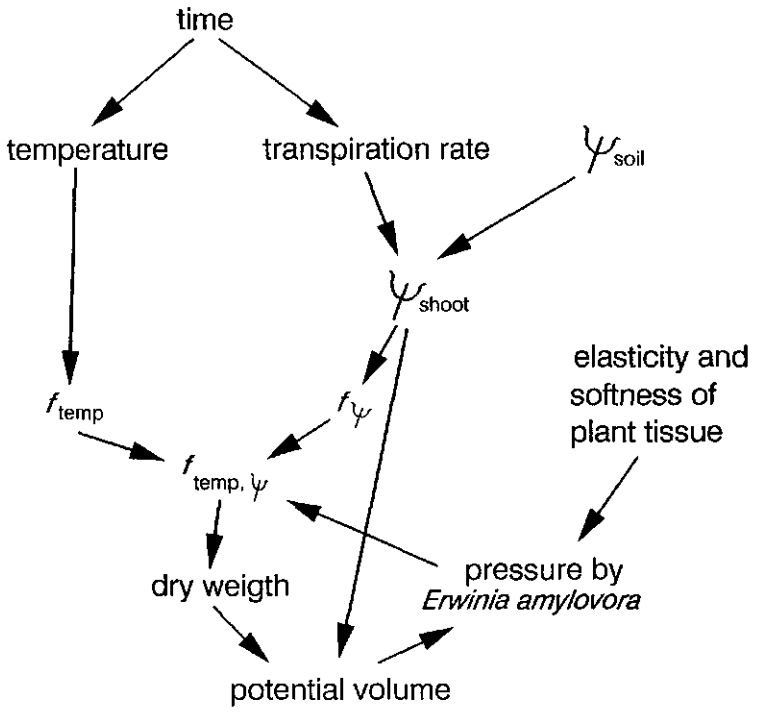


Fig. 2. Relational diagram of the major variables in the simulation model.

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 hours. 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.

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 hours). 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, ϵ, 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 ψ_{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 ψ_{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 equals 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 hours). 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 hours (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 ψ_{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 ψ_{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 intercellular 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

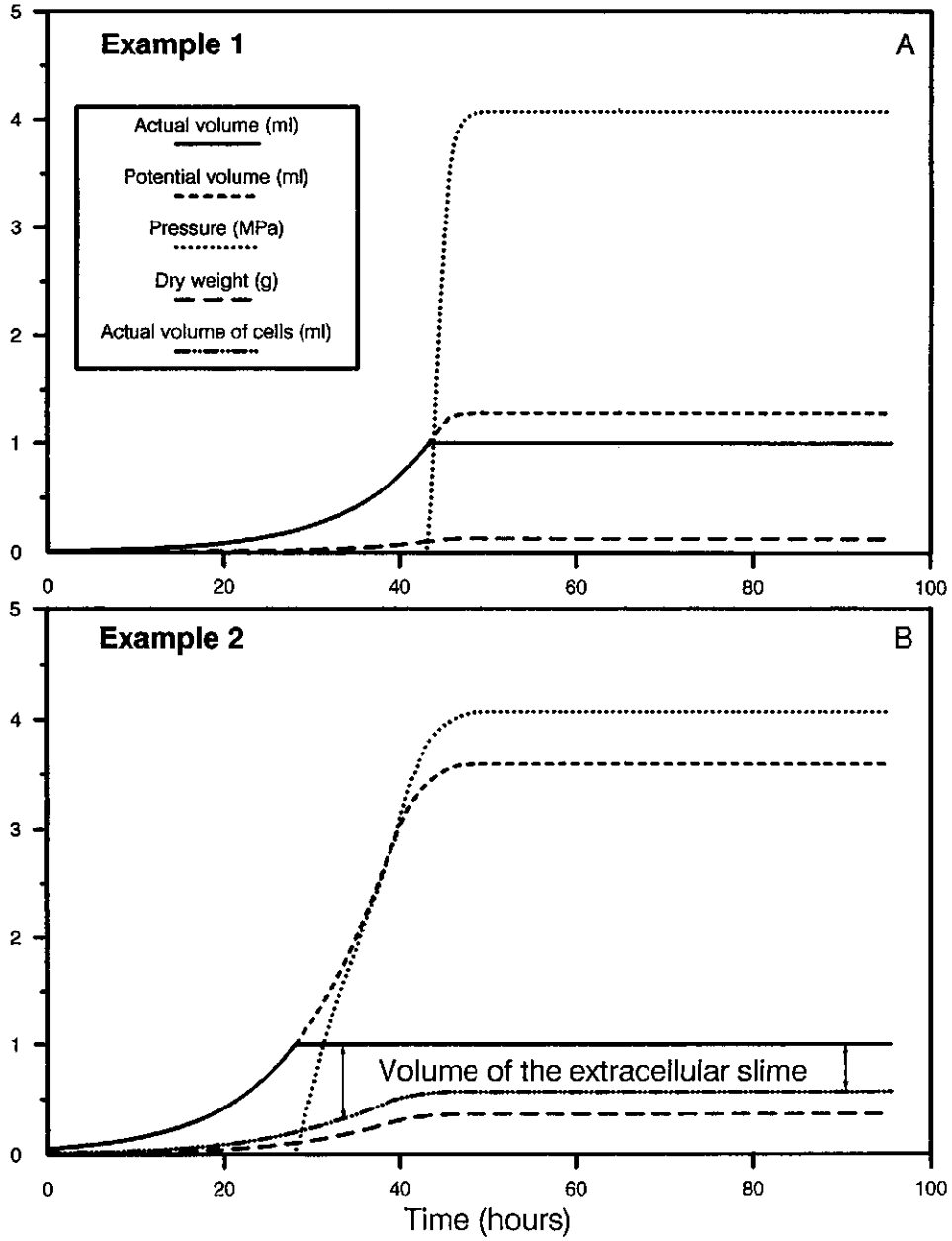
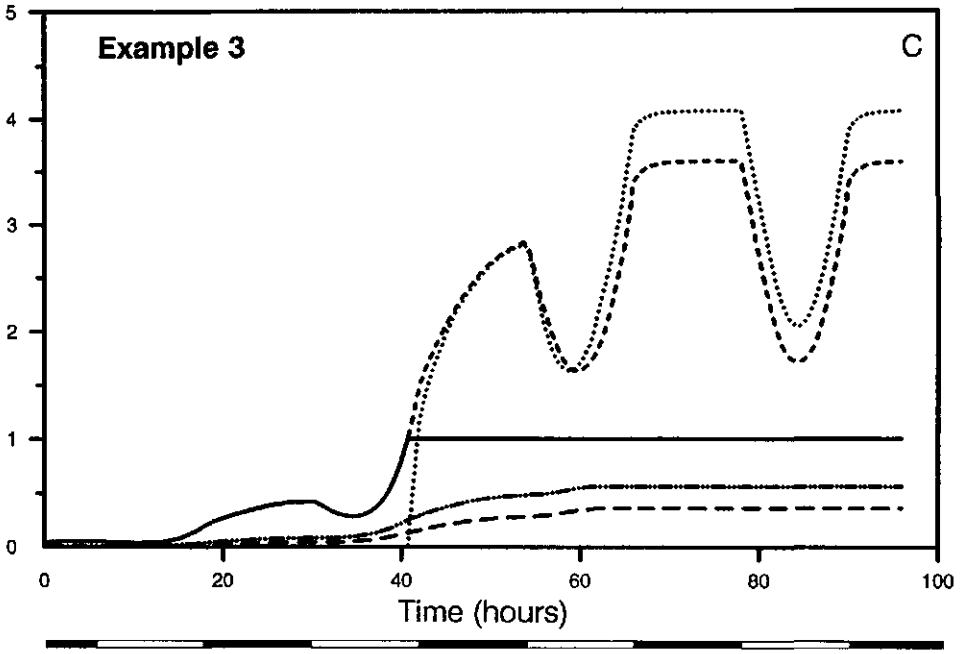
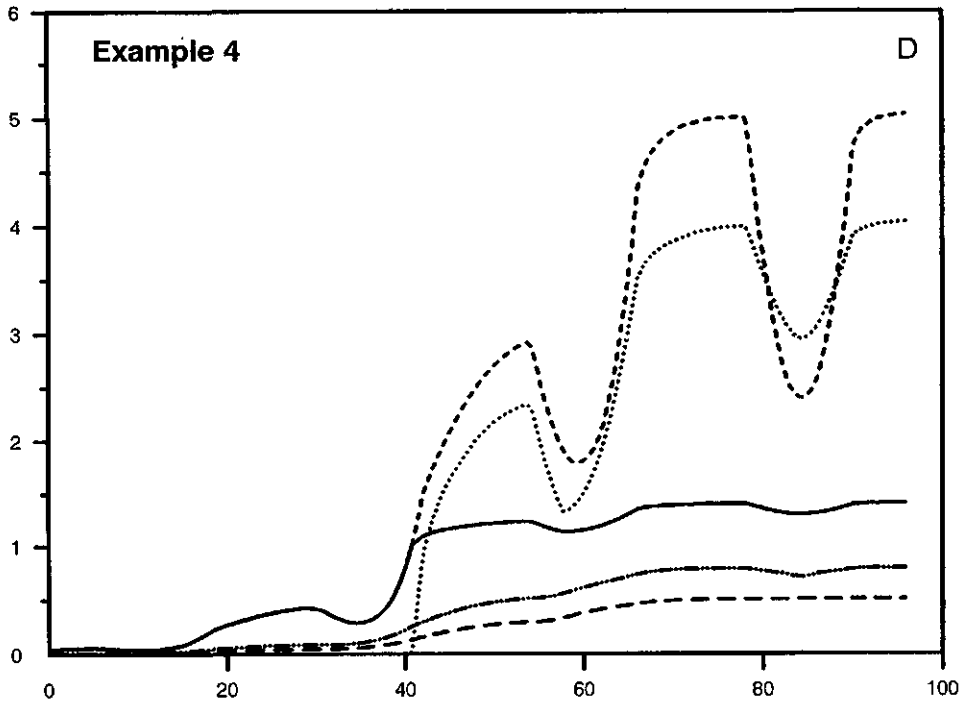


Fig. 3. Simulated volume and dry weight of *Erwinia amylovora* in an intercellular space of a host, and simulated pressure exerted by the bacterial biomass on surrounding plant tissue (see text). The volume of the bacterial biomass without compression is called 'potential volume'. The initial bacterial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

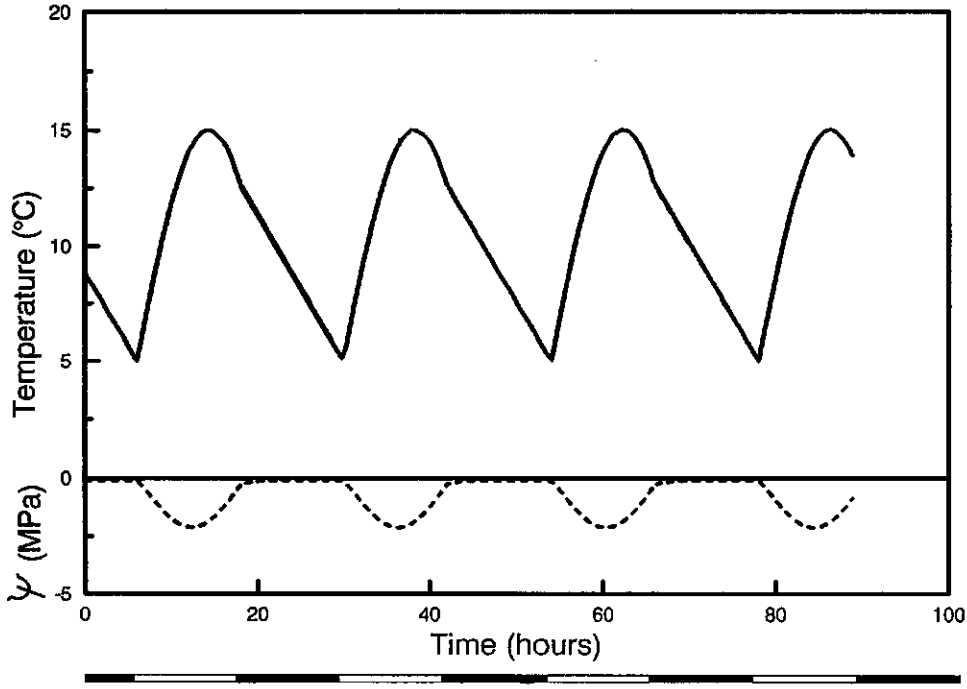


Fig. 4. Simulated courses of temperature and water potential ψ for a shoot of a fruit-tree. These courses were used as inputs to the simulation runs in Examples 3 and 4, Figures 3C, D. The horizontal bar indicates the day-night rhythm.

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. 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.

Samenvatting

Simulatie van druk veroorzaakt door vermenigvuldiging en zwelling van Erwinia amylovora in intercellulaire holten van waardplantweefsel

Erwinia amylovora, het pathogeen dat bacterievuur veroorzaakt, groeit met name in intercellulaire holten van de waardplant. Als een *E. amylovora* masse een intercellulaire holte gevuld heeft en doorgaat met vermenigvuldigen of zwellen, kan een druk ontstaan vanwege de beperkte ruimte. Door voortgaande groei van de bacteriële biomassa kan weefsel van de waardplant scheuren, zodat de bacteriële massa verder in de plant kan dringen, in die richtingen die het minste weerstand bieden. Theoretisch is de druk door de bacterie gelijk aan het verschil tussen de actuele waterpotentiaal van het waardplantweefsel en de waterpotentiaal waarbij de bacteriële biomassa de intercellulaire holte juist zou vullen zonder druk uit te oefenen. De groei en druk van de bacteriële biomassa werden dynamisch gesimuleerd.

Uitkomsten van de simulatie wijzen erop dat het extracellulaire slijm van *E. amylovora* sterk slinkt door waterafgifte als zich druk opbouwt. Door dit slinken is verdere produktie van bacteriële droge stof mogelijk. Het extracellulaire slijm bevindt zich dan rondom de bacteriecellen als een geconcentreerde substantie, met een sterk vermogen tot zwellen als weefsel van de waardplant zou scheuren. Verder geven resultaten van de simulatie aan dat de druk uitgeoefend door de bacterie 's avonds en 's nachts zijn hoogste waarden bereikt, omdat dan de waterpotentiaal van de waardplant het hoogst is.

Enkele bacterievuursymptomen die wijzen op bacteriële druk worden besproken.

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CHAPTER 6

Multiplication of *Erwinia amylovora* in fruit-trees. 1. A simulation study on limitations imposed by temperature and water, weather and soil.

Submitted to Netherlands Journal of Plant Pathology, author Henk J. Schouten.

Abstract

Temperature and water may limit multiplication of the fire blight causing bacterium *Erwinia amylovora* in shoots of fruit-trees. To gain insight into these limitation under field conditions, two simulation models were designed: a short-term model for immediate effects of weather and soil water potential, and a long-term model for effects of rain and soil profiles on the limitations imposed by temperature and water on bacterial multiplication.

In the Netherlands, in the month of June, when the soil is moist and the weather 'average', water hardly limits *E. amylovora* multiplication in shoots but, according to the short-term model, temperature reduces then the multiplication by about 60 %. When the soil is dry and the potential transpiration rate of the trees high, water may limit *E. amylovora* multiplication in shoots considerably. According to the long-term model, rain has a delayed effect on multiplication. The effect of a rain shower increases gradually in the course of time and reaches its maximum 2 to 30 days after the rain, in dependence of soil moisture content before the rain, amount of rain, and soil profile. Calculations were made for three soil profiles representing three typical fruit growing areas in the Netherlands. The results suggest different effects of rain on the behaviour of fire blight according to soil profile.

Additional keywords: fire blight, relative multiplication rate, water potential, rain, soil profile.

Introduction

Fire blight is a quarantine disease in the Netherlands. Dutch regulations prescribe that plants with fire blight (*Erwinia amylovora* (Burrill) Winslow *et al.*) have to be removed and destroyed (Anonymous, 1983, clause 8). This implies that no fire blight experiments can be done in the field. Only an inventory of host plants found to be diseased in spite of the regulations (Schouten, 1991a) was made in some parts of the Netherlands and analyzed statistically. The only trial field, isolated in the South-Western part of the Netherlands, where experiments on fire blight are allowed (van der Scheer, 1984), was not well suited for pathophysiological experiments. An alternative is to simulate fire blight development by means of computer models, although validation of these models remains difficult.

The present models were designed to gain insight into the effect of temperature and water on the multiplication of *E. amylovora* in shoots of fruit-trees. The effects of temperature and water were simulated for different weather conditions, values of soil water potential, and soil profiles. The input parameters of the simulation models were meteorological variables as usually measured in weather stations, and commonly used soil entities and characteristics.

Two models were built: one short-term model for immediate effects of the environment, and one long-term model for delayed effects. The simulated period was 24 hours in the short-term model and 40 days in the long-term model. In the short-term model, the water potential of the soil was assumed to be constant during the 24 hours' period, but in the long-term model the moisture profile of the soil was simulated dynamically.

Two models

The short-term model

Effect of water potential on the relative multiplication rate. As before (Schouten, 1991b), it is assumed that water potential, ψ, affects the relative multiplication

rate, r, of *E. amylovora* according to Schouten (1988; Fig. 2). This relationship is derived from *in vitro* and *in vivo* experiments by Shaw (1935).

$$r_\psi = r_{max} * f_\psi \quad (1)$$

with

$$0 \leq f_\psi \leq 1 \quad (2)$$

where: r = relative multiplication rate (hour^{-1}), the number of daughter bacteria per mother bacterium per hour. The subscript ψ denotes that r is affected by water potential; r_{max} = maximum value of r; f_ψ = variable relating r_ψ and r_{max}.

Under humid conditions, when ψ approaches 0, r_ψ attains its maximum value, so that f_ψ approaches 1. Under dry conditions, when $\psi < -5$ MPa, the bacteria do not multiply, and f_ψ equals 0.

Effect of temperature on the relative multiplication rate. The relationship between temperature (T) and f_T ($= r_T/r_{max}$) is derived from *in vitro* experiments (Billing, 1974) following Schouten (1987b).

$$f_T = \sin(4.2*10^{-4} * T^{2.46}) \quad (3)$$

with

$$0 \leq f_T \leq 1 \quad (4)$$

The argument of the sine function is expressed in radians, and temperature in °C. At optimal temperature (T = 28 °C) f_T equals 1.

Regression analysis by Schouten (1987a) showed that temperature and rainfall affected the development rate of fire blight multiplicatively, hence:

$$r_{\psi,T} = r_{max} * f_{\psi} * f_T \quad (5)$$

Output variables. The objective was to simulate the effect of water and temperature on the relative multiplication rate of *E. amylovora* in the intercellular space of a shoot of a fruit-tree. The output variables of the short-term model were $f_{\psi,T}$ and $\bar{f}_{\psi,T}$ (= $f_{\psi,T}$ averaged over a 24 hours' period).

$$f_{\psi,T} = f_{\psi} * f_T \quad (6)$$

$$\bar{f}_{\psi,T} = \frac{1}{24} \int_0^{24} f_{\psi,T} * dt \quad (7)$$

where t = solar time (hours).
Note that $f_{\psi,T}$ neighter accounts for nutrition effects in intercellular spaces, nor for bacterial pressure and softness of host tissue, which can also affect the relative multiplication rate of *E. amylovora* in shoots (Schouten, 1991b).

Simulation of daily course of water potential. The daily course of water potential was simulated over a 24 hours' period for the calculation of f_{ψ}. The course of water potential in a shoot of a fruit-tree was simulated by means of a module derived from Powell and Thorpe (1977). This module, on water flows and water potentials in a tree, required the following inputs 1) water potential of the soil, and 2) actual daily transpiration of the tree. The water potential of the soil was assumed to be constant during the simulated 24 hours' period, and to equal the water potential of the shoot at night. The actual daily transpiration of the tree was assumed to equal the potential daily transpiration of the tree, which was derived from the potential daily evapotranspiration of an orchard (Table 1). For estimation of the potential daily evapotranspiration of an orchard the temperature course and daily global radiation were required, as shown in Fig. 1.

Simulation of daily course of temperature. The daily course of temperature of the shoot was simulated according to Schouten (1987b, Equations (2) to (5)), using

Table 1. Methods and parameter values, used to estimate the actual daily transpiration per fruit-tree.

Reference crop evapotranspiration	according to the Makkink formula, which seems to be preferable to Penman's equation and the Penman-Monteith equation (de Bruin, 1987)
Crop factor of fruit orchard in June	1.6 (Feddes, 1987, Table 3)
Daily evaporation of bare soil under tree rows	derived from Ritchie (1972) according to Feddes *et al.* (1978, Equation (3.31))
Daily transpiration of the trees relative to daily transpiration of grass between the tree rows	4 : 1 (assumption)
Tree density	3200 trees per ha (modern Dutch pear orchard)

the daily minimum temperature, daily maximum temperature, and day length as input parameters. It was assumed that the temperature of the shoot equalled air temperature, measured in a weather station. The temperature courses were converted into courses of f_T by means of Equation (3).

Input parameters. The short-term model was used to study the effects of four parameters on the relative multiplication rate of *E. amylovora* by temperature and water: 1) daily minimum temperature (T_{min}), 2) daily maximum temperature (T_{max}), 3) daily global radiation (R), and 4) water potential of the soil (ψ_{soil}). These parameters were used as inputs to the short-term model, as indicated in

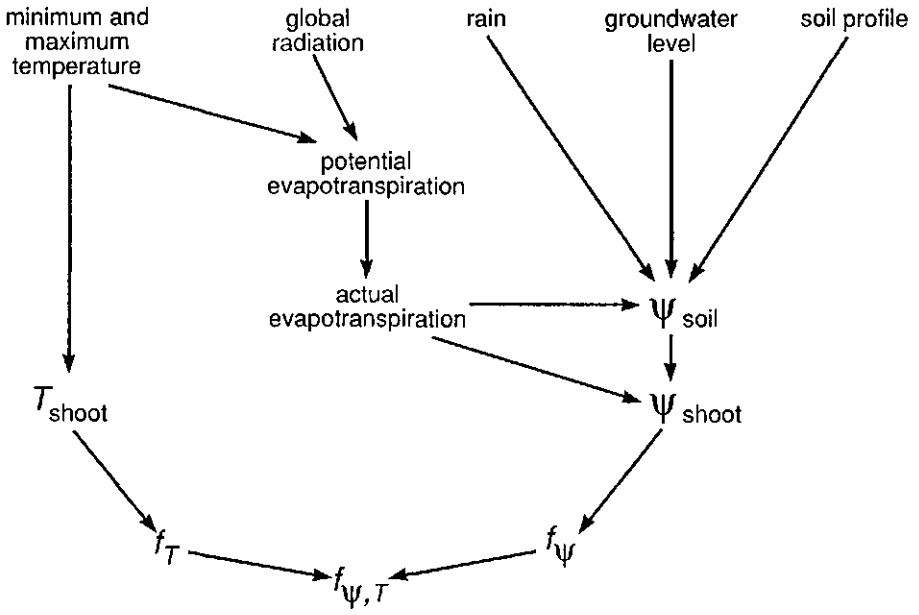


Fig. 1. Relational diagram of the major variables in the long-term model. Omission of the variables rain, ground water level, and soil type gives the relational diagram of the short-term model. T and ψ represent temperature and water potential, respectively, whereas f denotes the standardized relative multiplication rate of *Erwinia amylovora* in a shoot of a fruit tree, limited by T, by ψ, or by T and ψ simultaneously.

Fig. 1.

Examples of simulation runs. In order to gain insight into the daily courses of f_ψ, f_T and $f_{\psi,T}$ under different soil and weather conditions, the short-term model was run for 'average' soil water potential in combination with 'average' or sunny weather (Examples 1 and 2) and for a dry soil in combination with average, sunny, or cloudy weather (Examples 3, 4, and 5). The expression 'average' weather refers to the average values of T_{min}, T_{max}, and R measured by a synoptic weather station near Wageningen, the Netherlands, during the second half of June over the years 1974-1988. The exact input values of T_{min}, T_{max}, R and ψ_{soil} are

Table 2. Values of daily minimum and maximum temperature (T_{min}, T_{max}), daily global radiation (R), and water potential of the soil (ψ_{soil}), used as inputs to five example runs of the short-term model. The potential transpiration refers to an orchard, and is estimated according to Table 1.

	Example 1	2	3	4	5
weather	average	sunny	average	sunny	cloudy
soil	moist	moist	dry	dry	dry
T_{min} (°C)	10	10	10	10	5
T_{max} (°C)	20	30	20	30	10
R (J cm^{-2} day^{-1})	1630	2600	1630	2600	260
pot. transp. (mm day^{-1})	4.0	7.0	4.0	7.0	0.5
ψ_{soil} (MPa)	-0.01	-0.01	-1.6*)	-1.6	-1.6

*) wilting point (J.A. Kipp, Institute for Soil Fertility, Haren, the Netherlands; personal communication)

given in Table 2. The daily potential transpiration refers to an orchard, and equals daily reference crop evapotranspiration times crop factor minus daily evaporation of soil (Table 1).

Effect of water potential of the soil on $\bar{f}_{\psi,T}$. The effect of ψ_{soil} on the average $f_{\psi,T}$ over the simulated 24 hours' period ($\bar{f}_{\psi,T}$) was studied by running the short-term model repeatedly for a range of ψ_{soil} values (-1.6 MPa $< \psi_{soil} <$ 0 MPa), distinguishing the effects of sunny and 'average' weather.

The long-term model

The soil water profile was simulated dynamically by means of the model SWACROP (Feddes *et al.*, 1978). SWACROP is a FORTRAN program for simulation of the water balance of a cropped soil. In the model the soil is divided into compartments of equal size with a nodal point in the centre of each compartment. Variables, calculated for each time step during a run of

SWACROP, were a.o. water potentials of the nodal points, water flows from compartments to neighbour compartments, and water uptake from each compartment by plant roots. The rate of water uptake by plant roots was a function of the quotient of the potential transpiration rate and the number of soil compartments with roots, and also a function of the matrix potential of the considered compartment (Feddes *et al.*, 1978; Fig. 7). For each simulated day, the soil moisture profile was converted into a weighted average of the water potentials of the soil compartments, using the rates of water uptake by plant roots from the compartments as weights.

$$\psi_{soil} = \frac{1}{\sum S_z} * \sum_{z=1}^{L} (\psi_z * S_z) \qquad (8)$$

where: ψ_{soil} = weighted average of the water potentials of the soil compartments on the considered day (MPa);

S_z = rate of water uptake by roots from layer z (mm/day);

L = rooting depth, expressed in number of compartments with roots;

ψ_z = water potential (MPa) of soil compartment z. The water potential is composed of matrix potential and gravitational potential, ignoring osmotic and pneumatic potential (Feddes *et al.*, 1978). The gravitational potential, is assumed to be 0 at the soil surface.

ψ_{soil} was an input parameter of the short-term model, and thus functioned as the link between SWACROP and the short-term model. Calculating ψ_{soil} by means of SWACROP and Equation (8) for a series of days, and running the short-term model for each of these days, gave the long-term course of $f_{\psi,T}$.

The updated version of SWACROP, issued in March 1989, was used. This version has many options for dealing with the ground water table, weather, soil profiles, etc. (Wesseling *et al.*, 1989). The options chosen for the long-term model are given in Table 3.

Table 3. SWACROP options chosen and input parameter values (Feddes *et al.*, 1978; Wesseling *et al.*, 1989) used for dynamic simulation of the soil moisture profile in the long-term model.

Simulated period	from 15 June till 5 August
Potential evapotranspiration	constant over all runs; calculated for 'average' weather (Table 2), using Makkink's equation and a crop factor (Table 1)
Rain	no rain, except on the third simulation day (17 June); no irrigation
Ground water level	input parameter; constant during a run
Initial soil moisture profile	initial matrix potential constant over the rooting depth and under the roots linearly increasing to 0 at ground water level
Thickness of compartments	10 cm each
Water uptake by roots	according to Feddes *et al.* (1978; Fig. 7), with $\psi_1 = -10^{-5}$ MPa, $\psi_2 = -3*10^{-2}$ MPa, and $\psi_3 = -1.6$ MPa

Input parameters. Input parameters of the long-term model were (Fig. 1) 1. rain on the third simulation day, and no rain during the other days; 2. soil profile. The soils were characterized by means of soil moisture retention and hydraulic conductivity curves of Dutch top- and subsoils according to Wösten *et al.* (1986); 3. ground water level (Fig. 1).

The long-term model was run for three characteristic soils of Dutch fruit growing areas (Table 4). For T_{min}, T_{max}, and R constant average values were chosen (Table 2, 'average' weather).

Output variable. Simulated was the effect of rain on the course of $\bar{f}_{\psi,T}$. The output variable was $\Delta\bar{f}$, which equals $\bar{f}_{\psi,T}$ when it rains on the third simulation

Table 4. Three characteristic soils of Dutch fruit growing areas (J.A. Kipp, personal communication), used as example soils for runs of the long-term model. The soil codes (B8, O1, and O12) refer to soil moisture retention and hydraulic conductivity curves of Wösten *et al.* (1986).

Dutch fruit growing area	Betuwe	IJsselmeer polders	Zeeland
Soil type (code)	0 - 200 cm: clay (O12)	0 - 200 cm: sandy loam (B8)	0 - 60 cm: sandy loam (B8) 60 - 200 cm: sand (O1)
Groundwater level (cm)	80 - 100	140 - 180	80 - 160
Rooting depth (cm)	60	120	60

day minus $\bar{f}_{\psi,T}$ when there is no rain during the simulation period.

Results

The short-term model

Examples of simulation runs. The simulated courses of the factors f_ψ, f_T, and $f_{\psi,T}$ ($= f_\psi * f_T$) for 'average' weather and moist soil (Example 1 in Fig. 2A) indicate that at night, when ψ_{shoot} approaches ψ_{soil}, water does not limit multiplication of *E. amylovora*. At noon, the transpiration rate of the tree reaches its maximum value, so that ψ_{shoot} and f_ψ reach their lowest values, but even then water hardly limits multiplication of *E. amylovora* ($f_\psi = 0.91$), because of good water uptake from the soil by the roots. Temperature limits *E. amylovora* multiplication far more, even at noon, so that $f_{\psi,T}$ follows f_T closely in Example 1.

For Example 2 (Fig. 2B) the daily courses of f_ψ, f_T, and $f_{\psi,T}$ were simulated for sunny weather and a moist soil. As in the previous example, f_ψ approaches 1 at

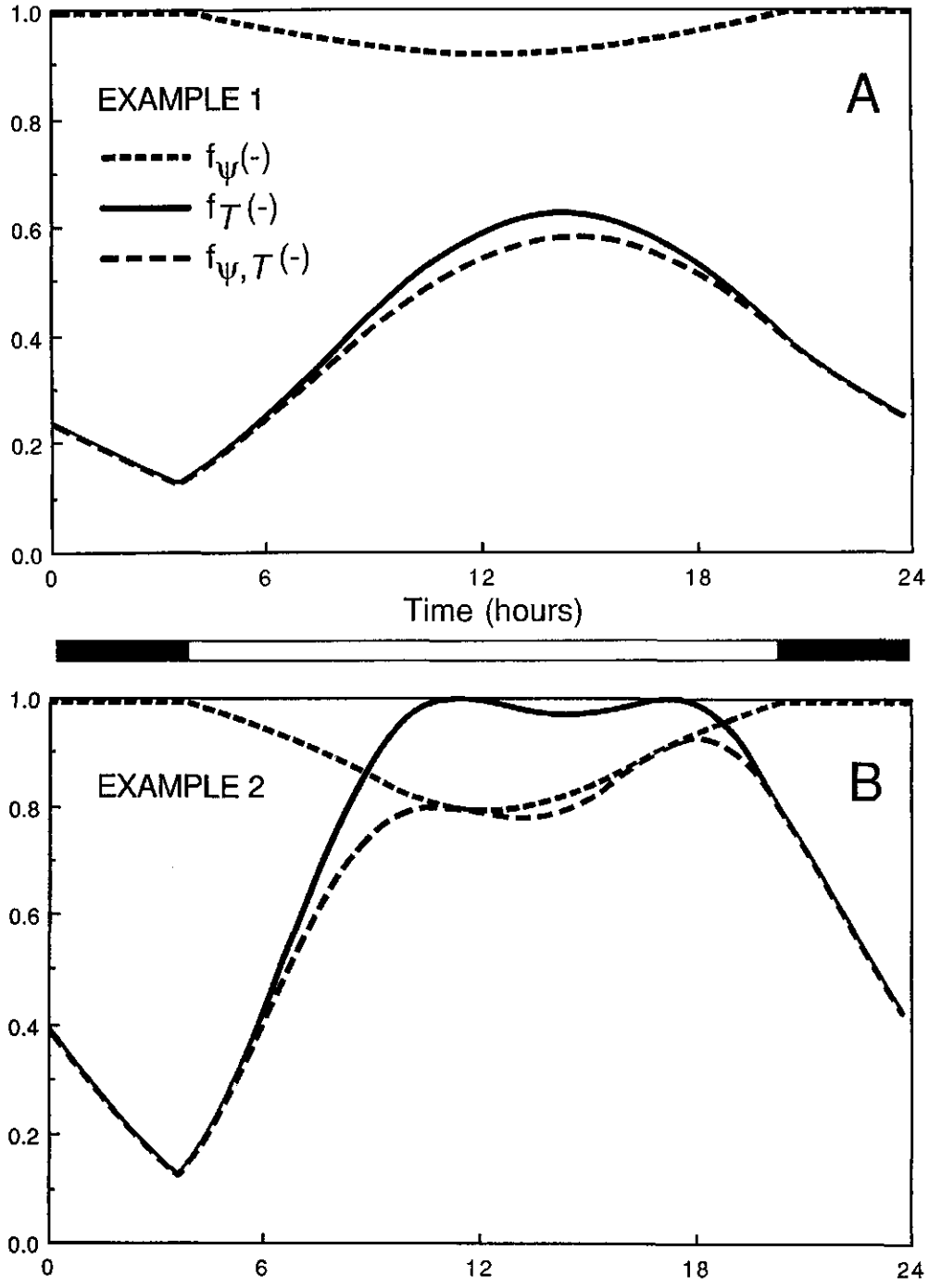
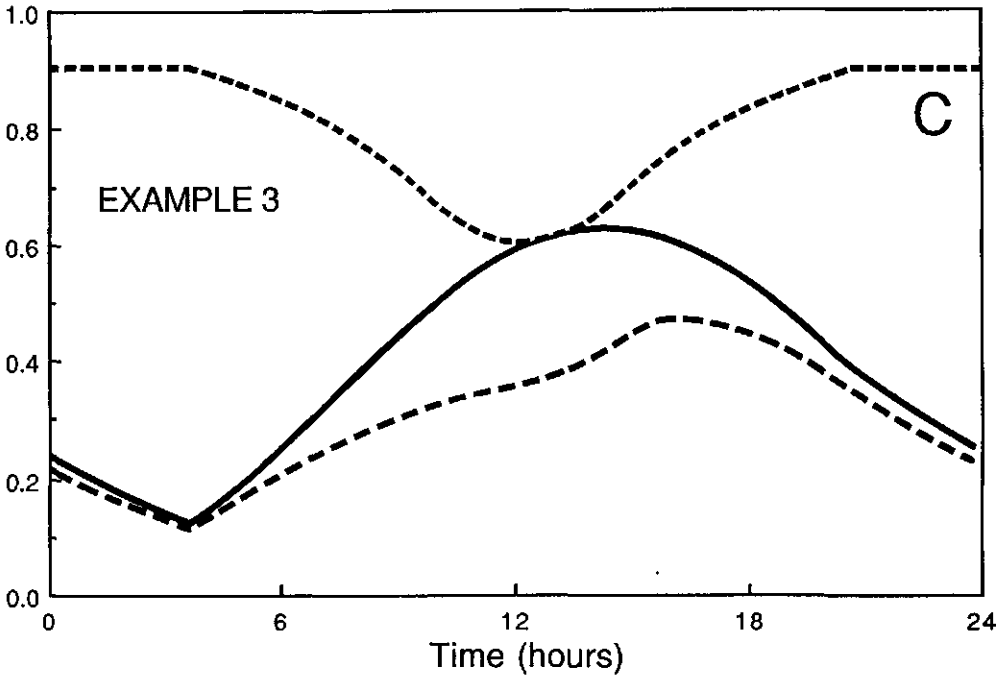
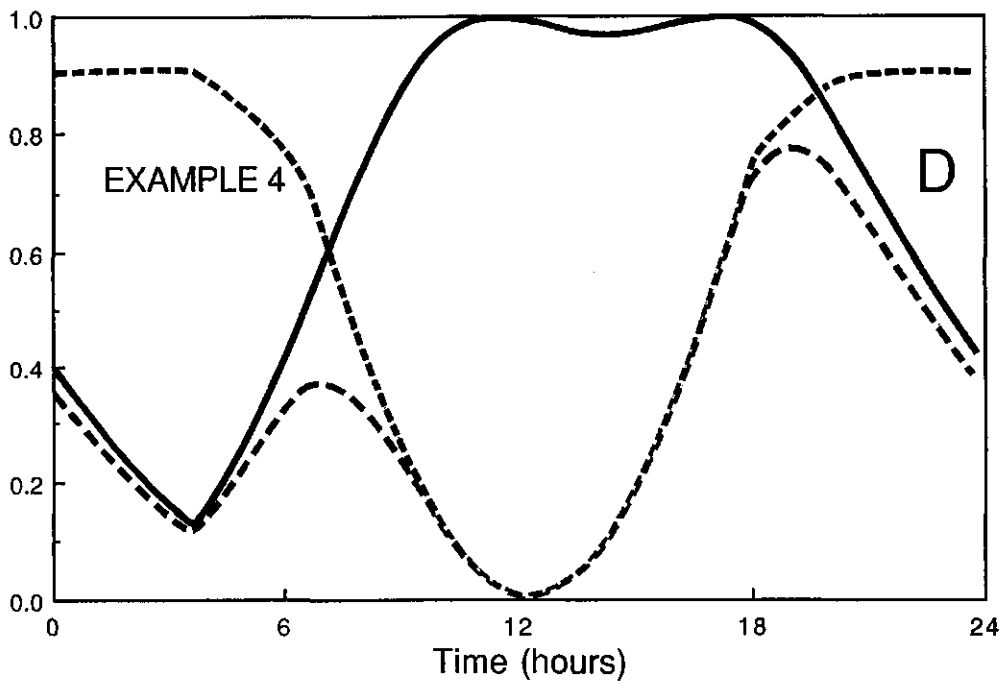


Fig. 2. Five simulated examples, illustrating effects of weather and soil water potential on the daily courses of f_ψ, f_T, and $f_{\psi,T}$. Table 2 gives the input parameter values used in the five examples. The day-night rhythm is displayed by horizontal white-black bars.


1.0
0.8
0.6
0.4
0.2
0.0
EXAMPLE 3
C
0
6
12
18
24
Time (hours)


1.0
0.8
0.6
0.4
0.2
0.0
EXAMPLE 4
D
0
6
12
18
24
Time (hours)

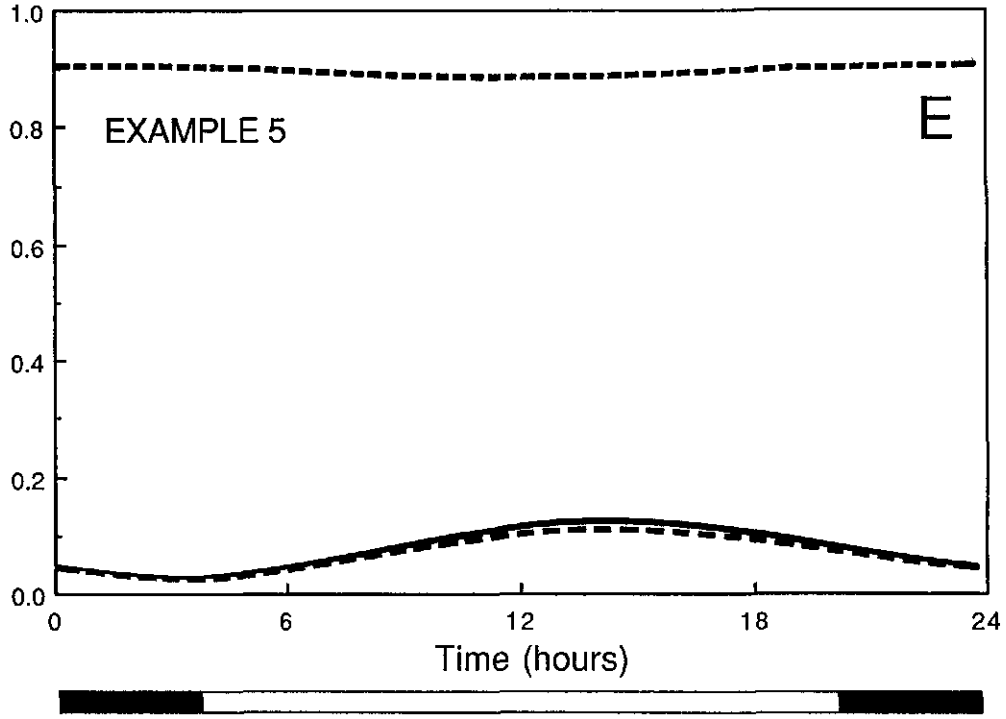


Fig. 2E

night time, but at midday water limits *E. amylovora* multiplication indeed, because of the high transpiration rate of the tree. In the afternoon the global radiation decreases, followed by a decrease of temperature, so that the transpiration rate slows down. Consequently, ψ_{shoot} and f_ψ increase. Temperature and f_T are higher than in the previous example during the whole 24 hours' period except at dawn, when temperature has its minimum value (10 °C). At about noon temperature reaches the optimal value for multiplication of *E. amylovora* (28 °C), so that f_T equals 1. At 14:20 hour, temperature equals T_{max} (30 °C), which is above the optimal temperature. Then, f_T is below 1. Somewhat later, temperature is optimal again, giving rise to a second peak in the f_T course. The daily course of $f_{\psi,T}$ in this example is not only greatly affected by the f_T course, but also by the f_ψ course. Note that temperature strongly affects the transpiration rate (de Bruin,

1987), and thereby f_ψ, so that temperature influences $f_{\psi,T}$ directly via f_T and indirectly via f_ψ.

In Examples 3, 4, and 5 the soil is so dry that the trees are about wilting at noon in case of average weather (ψ_{soil} = -1.6 MPa; J.A. Kipp, personal communication; Feddes *et al.*, 1978). In Example 3 the factors are simulated for average weather (Fig. 2C). The f_T course of Example 3 equals that of Example 1, because of identical temperature courses. The f_ψ course, however, differs greatly from that in Example 1. At night f_ψ does not approach 1, as in Example 1 and 2, because *E. amylovora* multiplication is noticeably reduced at ψ = -1.6 MPa, but not at ψ = -0.01 MPa (Schouten, 1987b). During daytime, f_ψ has lower values in Example 3 than in Example 1.

The factors at sunny weather and dry soil are simulated in Example 4 (Fig. 2D). f_ψ and thus $f_{\psi,T}$ fall nearly to 0 at about noon. Later, ψ, f_ψ and $f_{\psi,T}$ increase (because of decreasing transpiration rate), $f_{\psi,T}$ reaches a maximum value and decreases again during the evening and night because of decreasing temperature. This example indicates that a dry soil, combined with a high potential transpiration rate, reduces *E. amylovora* multiplication in the tree dramatically.

At dry soil and low potential transpiration rate (Example 5, Fig. 2E) f_ψ has high values, but in spite of this the bacterial multiplication may be slow because of low temperatures, which coincide with low potential transpiration rates.

Effect of water potential of the soil on $\bar{f}_{\psi,T}$. Fig. 3 shows the dependence of $\bar{f}_{\psi,T}$ on water potential of the soil (ψ_{soil}) during sunny weather and 'average' weather. $\bar{f}_{\psi,T}$ represents $f_{\psi,T}$, averaged over a 24 hours' period with constant ψ_{soil} over that period. The graph indicates that $\bar{f}_{\psi,T}$ is higher at sunny weather than at 'average' weather, as in Figures 2A and 2B. The high values of $\bar{f}_{\psi,T}$ during sunny weather are caused by the high T_{max} value via a high $\bar{f}_T$ value. The $\bar{f}_T$ values (f_T averaged over a 24 hours' period) at sunny and at average weather are also rendered in Fig. 3 as thin horizontal lines. When ψ_{soil} decreases, $\bar{f}_{\psi,T}$ decreases somewhat at average weather. This decrease is caused by decreasing f_ψ, as $\bar{f}_T$ is constant. The small decrease of $\bar{f}_{\psi,T}$ indicates that this variable is not very sensitive to ψ_{soil} at average weather. At sunny weather, however, $\bar{f}_{\psi,T}$ decreases strongly at decreasing ψ_{soil}. When the soil is dry, water uptake by the roots is slow, so that ψ_{shoot} is low at high potential transpiration rates. The low f_ψ during the day compensates the high f_T, to give a moderate $\bar{f}_{\psi,T}$.

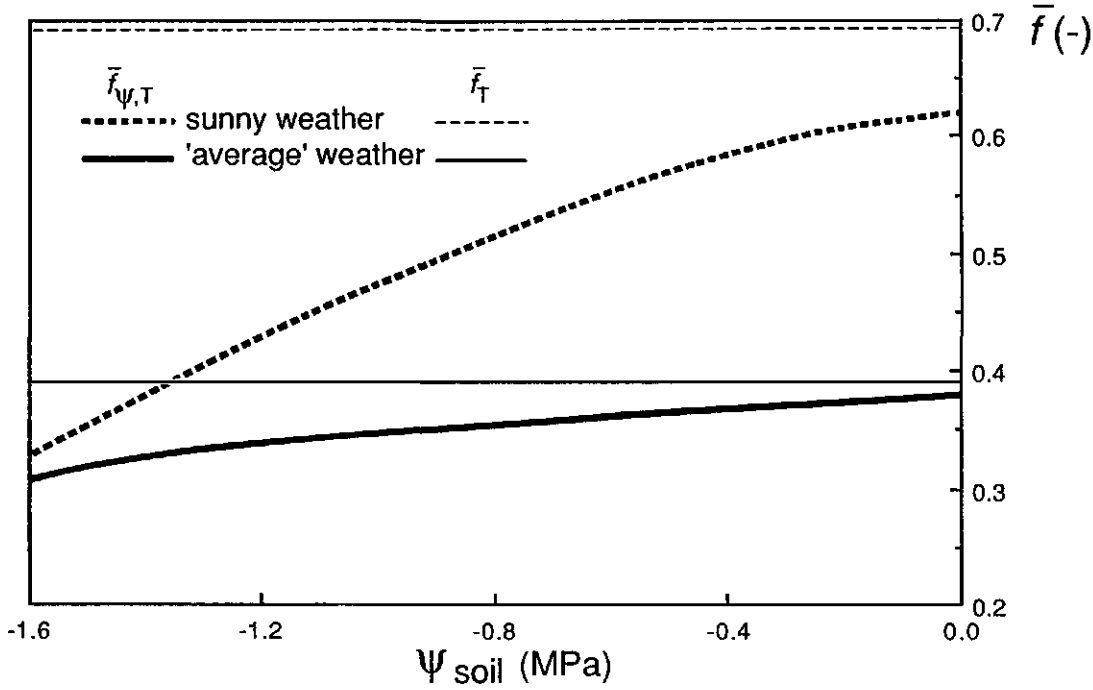


Fig. 3. Standardized relative multiplication rates $\bar{f}_{\psi,T}$ and $\bar{f}_T$ in simulated dependence of water potential of the soil (ψ_{soil}) at sunny weather (T_{min} = 10 °C, T_{max} = 30 °C, R = 2600 J cm^{-2} day^{-1}) and at 'average' weather (T_{min} = 10 °C, T_{max} = 20 °C, R = 1630 J cm^{-2} day^{-1}). $\bar{f}_{\psi,T}$ (heavy lines) and f_T (thin lines) represent $f_{\psi,T}$ and f_T, respectively, averaged over a 24 hours' period.

The two $\bar{f}_{\psi,T}$ curves in Fig. 3 are not parallel, which means that the sensitivity of $\bar{f}_{\psi,T}$ to ψ_{soil} depends on weather. In statistical terms: there is an interaction between ψ_{soil} and weather with respect to the effect on $\bar{f}_{\psi,T}$.

The long-term model

Fig. 4 shows the time course of $\Delta\bar{f}$, the standardized relative multiplication rate of *E. amylovora* when it rains during one day, indicated by an arrow, minus the standardized relative multiplication rate when there is no rain during the

simulated period. The graph indicates that for the chosen conditions (clay; ground water level = 80 cm; ψ_{soil} = -0.04 MPa on day = 0; average weather) the effect of rain on $\Delta\bar{f}$ starts immediately, increases gradually in course of time, reaches its maximum from 25 days after a 1 mm rain fall to 30 days after a 40 mm rain fall, and decreases finally. In case of little rain, evapotranspiration exhausts the amount of rain water earlier than in case of much rain, so that the tops of the curves in Fig. 4 move slightly to the right at increasing rain fall.

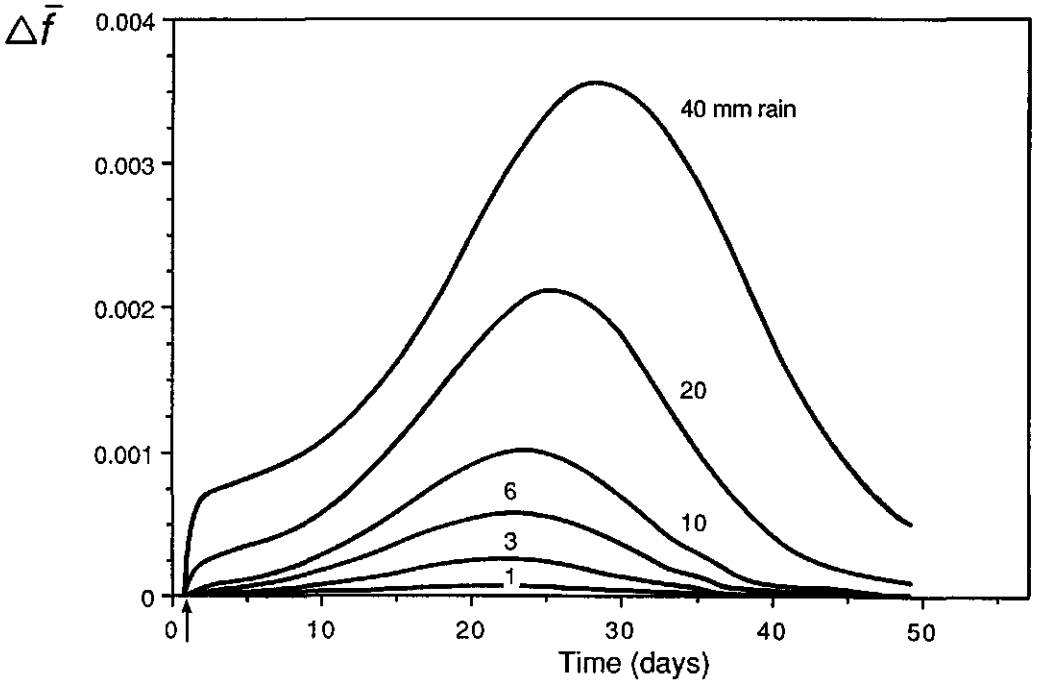


Fig. 4. Simulated effect of a day's rain on $f_{\psi,T}$ in the course of time at 'average' weather. $\Delta\bar{f}$ represents the standardized relative growth rate of *E. amylovora* when it rains during the one day indicated by an arrow, minus the standardized relative growth rate when there is no rain during the simulated period. The different curves refer to different amounts of rain. Soil: clay (see Table 4, fruit growing area Betuwe); Groundwater level = 80 cm; ψ_{soil} = -0.04 MPa on day = 0.

The effect of rain on $\bar{f}$ looks small ($\Delta\bar{f}$ < 0.004 in Fig. 4). This agrees with Fig. 3 where an increase of ψ_{soil} from -0.04 MPa to nearly 0 MPa hardly raises $\bar{f}$. Moreover, the increase of ψ_{soil} after a rain shower is often small, because the

amount of water from a rain shower is usually small compared to the water holding capacity of soil integrated over the rooting depth. But because the effect of a rain shower lasts for a long period (more than 40 days), the total effect of a short rain fall is significant. In Fig. 4, the area under the curve of the 40 mm rain fall equals 0.1 day, which represents the accumulated effect on $\bar{f}$. At sunny weather the accumulated effect would be stronger (Fig. 3).

Obviously, the effect of rain has a strong delay. This is mainly caused by the hydraulic conductivity of the soil, which is small for a clay soil (Fig. 5A), so that it takes a long time before rain water is spread over all rooted layers, and water uptake by the tree reaches its maximum.

Fig. 6A depicts time courses of $\Delta\bar{f}$ at different initial soil moisture contents when a fixed amount of water is added to the soil (30 mm). Three phenomena in Fig. 6A draw attention: 1. The dryer the soil, the higher the top of the curve; 2. the dryer the soil, the earlier $\Delta\bar{f}$ reaches its maximum; 3. the areas under the three curves are approximately equal. The reason for the first phenomenon (the dryer the soil, the higher the top) is that the matrix potential of the soil, and by that ψ_{soil} and $\bar{f}_{\psi,T}$, raises more in case of a dry soil than in case of a moist soil: the curve which relates soil water content to matrix potential of the soil is steeper at low than at high soil moisture contents (Fig. 5B). The explanation for the second phenomenon (the dryer the soil, the earlier $\Delta\bar{f}$ reaches its maximum) is that the plant roots absorb from a moistened soil not only water which comes from the 30 mm rain fall, but also water which was present before the rain. Moreover, the roots absorb water ascending from the ground water table by capillary suction. The moist soil had a higher ground water level than the dryer soils. The uptake of rain water is then less fast, and so the rain has a longer effect. The third phenomenon (the areas under the curves are approximately equal) indicates that an isolated precipitation of 30 mm in a continuum of dry weather has the same effect on $\Delta\bar{f}$, integrated over time ($\int \Delta\bar{f}\, dt \approx 0.08$ days), over a wide range of soil moisture contents.

Whereas Fig. 6A refers to clay and rather undeep rooting (60 cm), Fig. 6B refers to sandy loam and deep rooting (120 cm). The stronger delay of the rain effect is caused by the deeper rooting. A part of the delay by deeper rooting is compensated by higher hydraulic conductivity of sandy loam as compared to clay (Fig. 5A). The areas under the curves are virtually equal again (the curve which refers to an initial ψ_{soil} of -0.04 MPa is not complete for reasons of scale), and

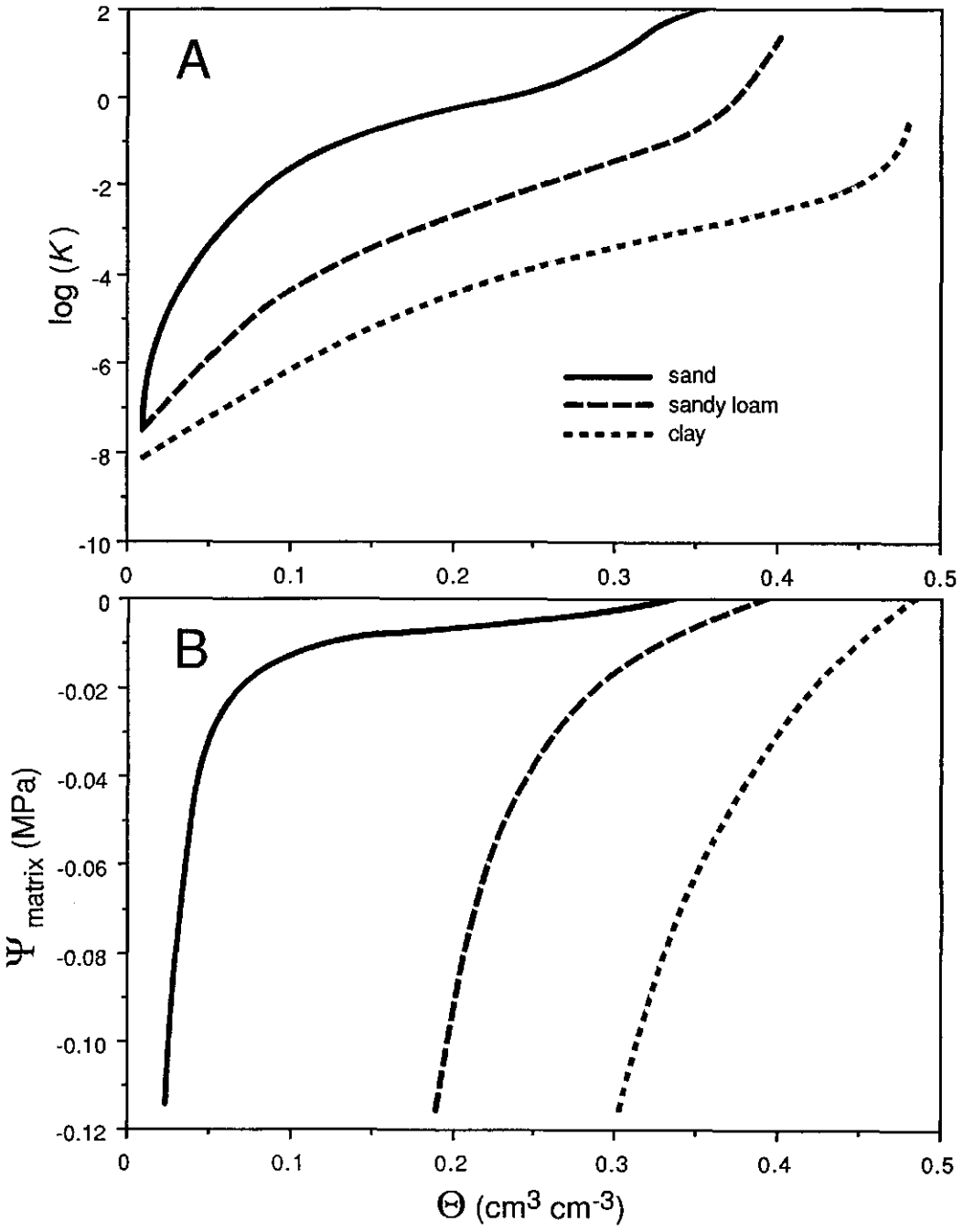
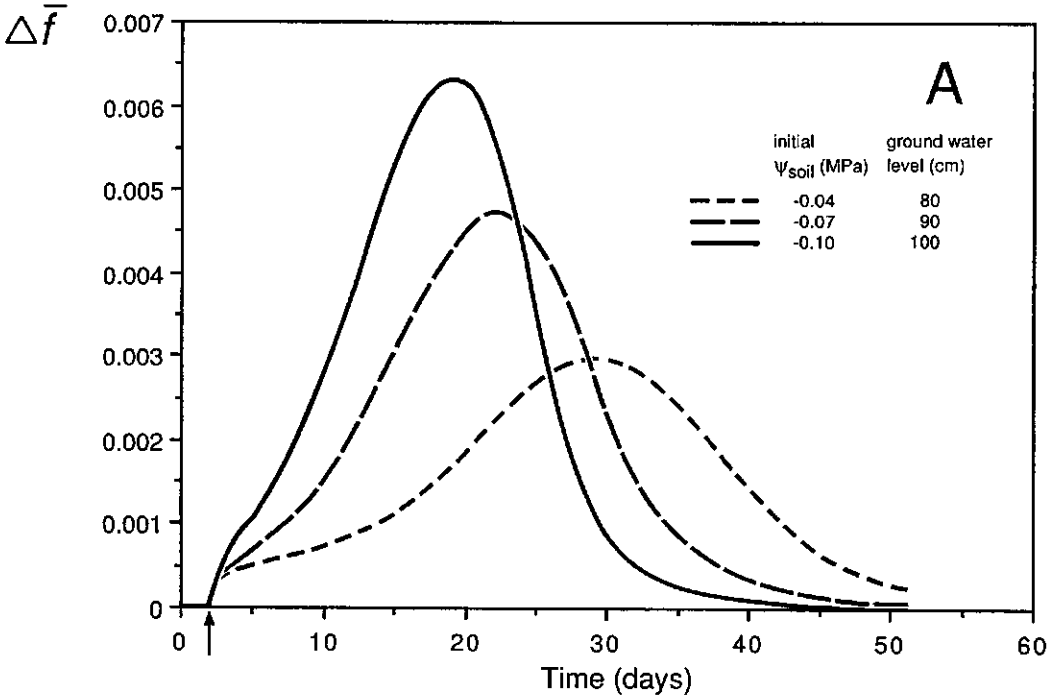
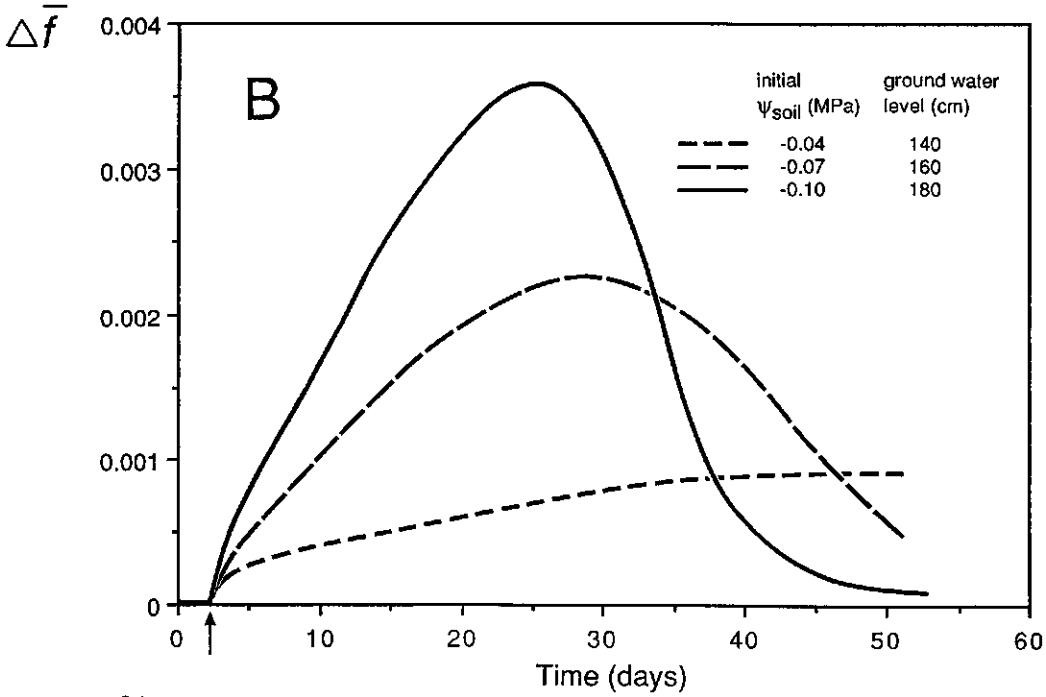


Fig. 5. Hydraulic conductivity (K) and matrix potential (ψ_{matrix}) of the soil, in relation to soil water content (Θ) (from Wösten *et al.*, 1986).


$\Delta\bar{f}$
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
A
initial ψ_{soil} (MPa)
ground water level (cm)
-0.04 80
-0.07 90
-0.10 100
0 10 20 30 40 50 60
Time (days)


$\Delta\bar{f}$
0.004
0.003
0.002
0.001
0
B
initial ψ_{soil} (MPa)
ground water level (cm)
-0.04 140
-0.07 160
-0.10 180
0 10 20 30 40 50 60
Time (days)

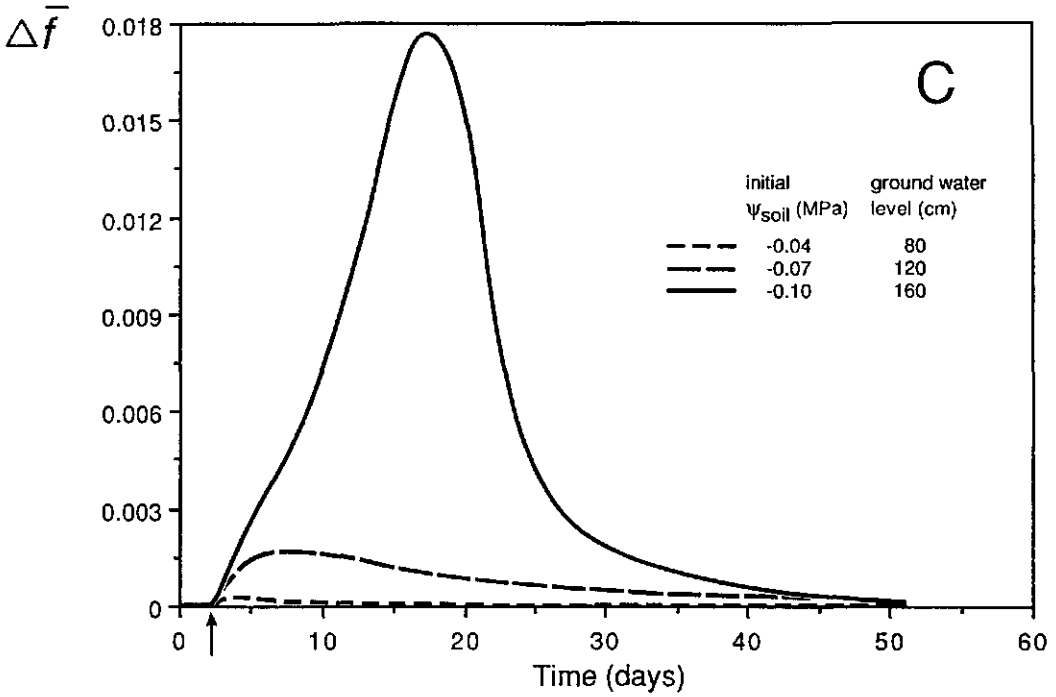


Fig. 6. Simulated time courses of $\Delta\bar{f}$ for a 30 mm rainfall on the day indicated by an arrow. T_{min}, T_{max}, and R have average values continuously (T_{min} = 10 °C, T_{max} = 20 °C, R = 1630 J cm^{-2} day^{-1}). Fruit growing areas: A. Betuwe; B. IJsselmeer polders; C. Zeeland (see Table 4).

approximately equal the areas under the curves of Fig. 6A.

Fig. 6C, which refers to a profile with sandy loam as topsoil and sand as subsoil, differs strongly from Figures 6A and B. The first phenomenon (the dryer the soil, the higher the top) holds even more convincingly, but the other two phenomena do not hold at all. The top soil of Fig. 6C with an initial ψ_{soil} of -0.1 MPa has a low hydraulic conductivity. Moreover, the dry top soil has a large water holding capacity (Fig. 5B), so that the rain water barely flows into the subsoil, but remains in the topsoil where it can be optimally absorbed by the roots. The area under the curve equals 0.25 days, whereas the areas under the curves in Figures 6B and B equalled about 0.08 days.

The hydraulic conductivity of the moister soil in Fig. 6C (initial ψ_{soil} = -0.04 MPa) is high, so that rain water sinks fast through the thin root layer into the sand, where no roots are present (Table 4). Therefore, rain has only a short-lasting and small effect.

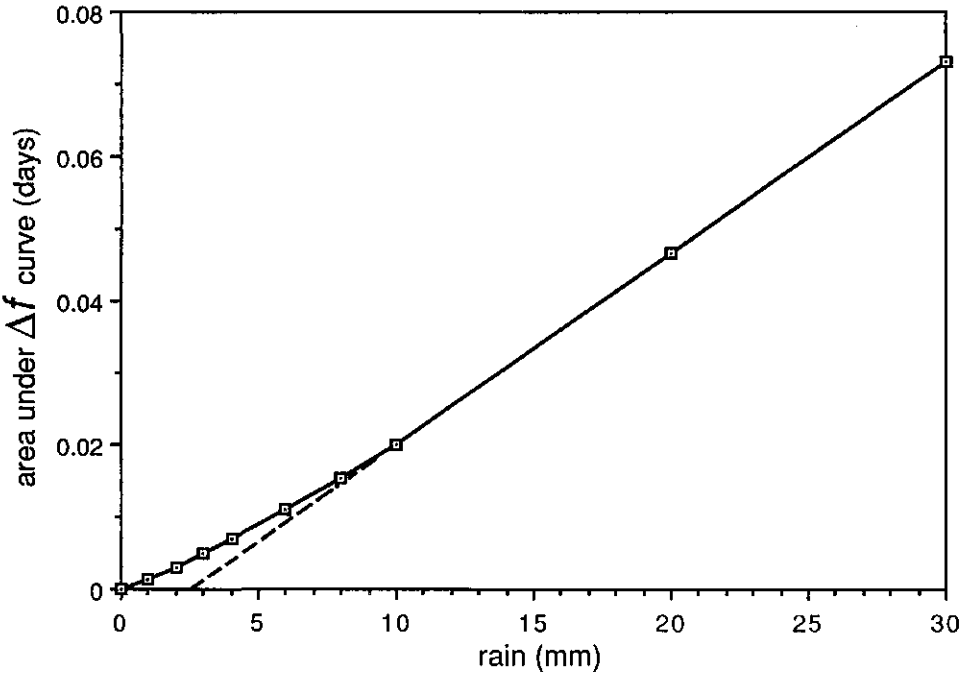


Fig. 7. Effect of the amount of rain on $\Delta\bar{f}$, integrated over the simulation period.

Fig. 7 shows an example of the simulated effect of amount of rain on $\bar{f}$. The curve is approximately linear, which means that an increase from 0 to 1 mm rain raises $\bar{f}_{\psi,T}$ about as much as an increase from 20 to 21 mm rain, a counter-intuitive result. A close look to the graph reveals that at small amounts of rain the curve is even less steep than at high amounts of rain. This is caused by interception of rain by leaves, so that only a part of the rain reaches the soil. The maximum interception rate by the leaves equals about 2 mm rain per day (Feddes *et al.*, 1978, Fig. 32).

Discussion

The short-term and long-term models were designed to simulate effects of temperature and water on multiplication of *E. amylovora* in shoots. Temperature and water affect *E. amylovora* in shoots not only directly but also indirectly via the growth rate of the host (Barlow, 1975). Fast growing, succulent tissue is more susceptible to fire blight than tough tissue (van der Zwet and Keil, 1979). Effects of temperature and water on growth rate, succulence, and softness of host tissue, and therewith on multiplication rate of *E. amylovora* in the intercellular space of that tissue, were not incorporated in the model. Differences in tree nutrition from the different soils were not taken into account.

Debatable in both short-term and long-term models is the assumption that the actual transpiration rate equals the potential transpiration rate. Especially at dry soils, the actual transpiration may be slower than the potential transpiration, because of closing of the leaf stomata. For that reason the simulated water potential of the shoot may be lower than actual water potentials of shoots. This would lead to underestimation of f_ψ when the soil is dry and the potential transpiration rate high. On the other hand f_ψ may be overestimated in case of dry soils, because a dry soil reduces the growth rate of shoots (tissues less succulent), and so decreases the relative multiplication rate of *E. amylovora* in the shoots. These over- and underestimations may partly compensate each other.

Regression analysis, using observed incubation periods of fire blight, and temperature and rain data from weather stations as inputs (Schouten, 1987a), revealed that both temperature and rain fall were positively correlated with development rate of fire blight in hosts. Temperature was more important than rain fall in explaining variation in the development rate. These regression results do not contradict the results in this paper, though the delay in the effect of rain comes as a surprise. The near-linear relationship between amount of rain and $\bar{f}$ demonstrated by simulation, may provide a refinement of the classical rainfall classification (rainfall = 0; $<$ 2.5 mm; $\geq$ 2.5 mm; Billing, 1976).

When the soil is dry (water potential of the soil below -1 MPa), limitations by water are important, so that progress of the disease through the host tissue may cease more often. Particularly in the Mediterranean area, where fire blight invaded several countries recently (Zutra *et al.*, 1986; Psallidas, 1990), soils are

often dry, but temperatures are high compared to Dutch circumstances. In those countries it might be useful to reduce irrigation in orchards with fire blight, so that 1) the multiplication rate of and pressure by *E. amylovora* in intercellular spaces of hosts is reduced (Schouten, 1991b), and 2) host tissues becomes tougher. Then, the diseased tissues can be effectively sealed off by the host plants, reducing oozing of the pathogen and damage to infected trees.

Acknowledgements

I am grateful to Ir J.A. Kipp (Institute for Soil Fertility, Haren, the Netherlands) for advice with respect to choices of soil parameter values, and to Ir B.J. van den Broek (The Winand Staring Centre, Wageningen, the Netherlands) for providing the model SWACROP and relevant literature. I am indebted to Prof. Dr J.C. Zadoks (Department of Phytopathology, Wageningen, the Netherlands) for critically reading the text and for linguistic corrections.

Samenvatting

Vermenigvuldiging van Erwinia amylovora *in fruitbomen.*
2. Een simulatiestudie naar beperkingen door temperatuur en vocht, weer en bodem.

Vermenigvuldiging van de bacterievuur veroorzakende bacterie *Erwinia amylovora* in scheuten van fruitbomen kan beperkt worden door temperatuur en vocht. Om inzicht in deze beperkingen te verkrijgen werden twee simulatiemodellen gebouwd: een korte-termijn model voor onmiddellijke invloeden van weer en waterpotentiaal van de bodem op de beperkingen door temperatuur en vocht, en een lange termijn-model voor invoed van regen bij verschillende bodemprofielen.

Bij een vochtige bodem en "gemiddeld" juni-weer in Nederland limiteert vocht de vermenigvuldiging van *E. amylovora* in scheuten nauwelijks, maar volgens het korte-termijn model reduceert de temperatuur deze vermenigvuldiging dan met 61 %. Als de bodem droog is en de potentiële transpiratiesnelheid hoog,

reduceert vocht de vermenigvuldiging van de bacterie in aanzienlijke mate. Het lange-termijn model geeft aan dat regen de vermenigvuldiging beïnvloedt, zij het na een zekere vertraging. Het effect van een regenbui neemt in de tijd geleidelijk toe, en bereikt 2 tot 30 dagen na de regendag zijn maximum, afhankelijk van het vochtgehalte van de bodem voordat het regende, de hoeveelheid regen en het bodemprofiel. Berekeningen werden uitgevoerd voor bomenprofielen van drie fruitgebieden in Nederland. De resultaten laten zien dat het bodemprofiel het gedrag van bacterievuur beïnvloedt.

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CHAPTER 7

Multiplication of *Erwinia amylovora* in fruit-trees. 2. A simulation study on sensitivity to weather.

Submitted to Netherlands Journal of Plant Pathology, author Henk J. Schouten.

Abstract

The sensitivity of an output variable of a model to changes of an input parameter value can be analyzed in various ways. Some methods of sensitivity analysis are described, and applied to a simulation model which has daily minimum and maximum temperatures (T_{min} and T_{max}, respectively), and daily global radiation as input parameters, and standardized relative multiplication rate of *Erwinia amylovora* in shoots in fruit-trees, averaged over a 24 hours' period, as output variable. Values of the input parameters were obtained from a weather station near Wageningen, the Netherlands, and refer to the second half of June, 1974-1988.

According to the model, the output variable was twice as sensitive to T_{max} as to T_{min}. Because of this difference in sensitivity, and because the standard deviation of T_{max} was larger than that of T_{min}, the variation of the output variable due to T_{max} was three times larger than that due to T_{min}. The sensitivity to daily global radiation was negligible when the soil was moist.

Additional keywords: fire blight, temperature, global radiation, water potential, relative multiplication rate, sensitivity analysis.

Introduction

Temperature has a predominant effect on the epidemiological development of fire blight (*Erwinia amylovora* (Burr.) Winslow *et al.*) in rosaceous plants.

Obviously, it affects insect activity and therewith dispersal of fire blight (Free, 1970; de Wael, 1988; Billing, 1990); it influences strongly the epiphytic colonisation of flowers (Zoller and Sisevich, 1979; van der Zwet *et al.*, 1988), the internal colonization of the host, and the incubation period of fire blight (Billing, 1976). Billing (1976) showed that also water affects the development of fire blight.

To gain more insight into the effects of environment on temperature and water potential within fruit-trees, and thus on the multiplication rate of *E. amylovora* in these fruit-trees, two simulation models were built (Schouten, 1991): a short-term model to investigate immediate effects of weather and soil water potential, and a long-term model to study delayed effects of rain and soil. In this paper the short-term model is used. The impact of several weather parameters on multiplication of *E. amylovora* within trees, considering only limitations by temperature and water, was quantified by means of sensitivity analyses.

The model

Sensitivity analyses were applied to a model simulating effects of weather and soil moisture on temperature and water potential in a shoot of a fruit-tree and therewith on multiplication of *E. amylovora* in the intercellular space of that shoot (Schouten, 1991, 'the short-term model'). The output variable of the simulation model was the standardized relative multiplication rate of *E. amylovora* in the shoot, averaged over a 24 hours' period. This standardized relative multiplication rate was obtained by calculating the hourly values of the relative multiplication rate, averaging them over the 24 hours' period, and dividing the average by the relative multiplication rate at optimal temperature and water potential. In the paper in which the simulation model was described (Schouten, 1991), the standardized relative multiplication rate was denoted by $\bar{f}_{\psi,T}$, but in this paper it is represented by the simpler symbol y.

The model required as inputs daily minimum temperature (T_{min}), daily maximum temperature (T_{max}), daily global radiation (R), and water potential of the soil. The sensitivity of the output variable to T_{min}, T_{max}, and R was studied, whereas the water potential of the soil was kept constant (-0.01 MPa) over all

simulation runs. During a simulation run T_{min}, T_{max}, and R had constant values, because the simulation period was 24 hours, but they varied from run to run.

Sensitivity analyses

Impact of input parameters on the magnitude of the output variable

By running the simulation model repeatedly, with different values of the input parameter x for each run, the effect of changes in the x value on the output variable y was traced. The 'sensitivity' of y to x is:

$$S = \frac{\delta y}{\delta x} \tag{1}$$

where: S = sensitivity coefficient;
δy = the change in y in response to infinitesimal alteration δx in x.

It is common practice to express the alterations δx and δy not in absolute but in relative terms:

$$S_{\%} = \frac{\delta y}{y} / \frac{\delta x}{x} = \frac{\delta \log y}{\delta \log x} \tag{2}$$

In economics, this coefficient is called elasticity coefficient.

Impact of input parameters on the variance of the output variable

A measure for impact of x *on variation in* y. To quantify the impact of x on y, not only the sensitivity of y to x was investigated, but also the natural variation in x was taken into account. If y would be equally sensitive to changes in input parameters x_1 and x_2, but x_1 is rather constant whereas x_2 fluctuates strongly, then the variance of y should be attributed to x_2 rather than to x_1.

To gain insight into the influence of the considered input parameter on variation in y, different sources of variation in y have been distinguished:

$\sigma_{y,\infty}$ = standard deviation of y as found in orchards. Variation in y is not only caused by variation in T_{min}, T_{max}, and R, but also by variation in numerous other variables, e.g. soil water potential, leaf area index, and rootstock.

$\sigma_{y,n}$ = standard deviation of y, caused by simultaneous variation in n considered input parameters (T_{min}, T_{max}, and R; $n = 3$). T_{min}, T_{max}, and R vary simultaneously in mutual dependence. Other variables (e.g. soil water potential, leaf are index) are assumed to be constant;

$\sigma_{y,1}$ = standard deviation of y, caused by variation in only one input parameter ($n = 1$).

The impact of x on variation in y was quantified by means of S_σ, defined as:

$$|S_\sigma| = \frac{\sigma_{y,1}}{\sigma_{y,n}} \qquad (3)$$

When an increase of x causes a decrease of y, S_σ is negative. Because the right hand term cannot be negative, S_σ is rendered as an absolute value.

The more the value of S_σ deviates from 0, the more the considered input parameter explains variation of the output variable. Note, however, that S_σ is not a measure of the importance of x in determining the magnitude of the output variable y, in contrast to S and $S_\%$ according to Equations (1) and (2).

Estimation method 1. It was not possible to estimate the real standard deviation $\sigma_{y,\infty}$ by means of the model. But $\sigma_{y,3}$ could be estimated by varying the parameter values of T_{min}, T_{max}, and R simultaneously, in mutual dependence, as found in reality. This was attained by using meteorological data from a weather station near Wageningen, the Netherlands, from 15 to 29 June of the years 1974-1988. Global radiation measurements were missing on four out of these 15 x 15 = 225 days, so that 221 complete daily records remained. For each of the 221 daily

records the corresponding response y was calculated, running the simulation model. The standard deviation of these calculated y-values was the estimate of $\sigma_{y,3}$.

To estimate $\sigma_{y,1}$ (a measure of variation in y, caused by variation in one weather parameter), the simulation model was run 221 times, using the 221 measurements of one considered weather parameter as input values, and giving the other two weather parameters constant average values. The standard deviation of the 221 obtained y-values was the estimate of $\sigma_{y,1}$.

Estimation method 2. Rather than estimating $\sigma_{y,1}$ by means of running the simulation model 221 times, this standard deviation can be approximated by means of two runs of the model, using $\bar{x}+\sigma_x$ and $\bar{x}-\sigma_x$ as input values:

$$\sigma_{y,1} \approx \frac{|y_{\bar{x}+\sigma_x} - y_{\bar{x}-\sigma_x}|}{2} \tag{4}$$

where: $\bar{x}$ = the mean of x;
σ_x = the standard deviation of x.

This approximation requires that the relationship between x and y is a linear one, which is often not the case in simulation models. Furthermore, for unbiased estimation of $\sigma_{y,1}$ using Equation (4), the frequency distribution of x has to be approximately symmetric. When both requirements are satisfied, y also has a symmetric distribution.

$\sigma_{y,1}$ can be substituted into Equation (4), giving

$$S_\sigma \approx \frac{(y_{\bar{x}+\sigma_x} - y_{\bar{x}-\sigma_x})}{2*\sigma_{y,3}} \tag{5}$$

Estimation method 3. S_σ can also be estimated by means of regression analysis. Coefficients of a multiple regression equation were estimated, using T_{min}, T_{max},

and R from the 221 weather records as explanatory variables, and the simulated y as dependent variable:

$$y = a + b_1 * T_{min} + b_2 * T_{max} + b_3 * R \quad (6)$$

where: a = an estimate of the intercept;
b_1, b_2, b_3 = regression coefficients.

The estimated regression coefficients were used to approximate $\sigma_{y,1}$:

$$\sigma_{y,1} \approx b * \sigma_x \quad (7)$$

so that

$$S_\sigma \approx \frac{b * \sigma_x}{\sigma_{y,3}} \quad (8)$$

In statistical a context, $b*\sigma_x/\sigma_y$ is called standard regression coefficient (Snedecor and Cochran, 1980).

An important advantage of the regression method is that S_σ-values could be estimated of interaction terms, e.g. $T_{max}*R$, and of higher-order terms, e.g. $(T_{max})^2$. One has to be aware, however, that estimations of regression coefficients are based on correlations between x and y, rather than on real cause-effect relations, so that this method may be misleading with respect to causal relationships.

Results

Impact of input parameters on the magnitude of the output variable

Table 1 shows calculated sensitivity coefficients S of y for T_{min}, T_{max}, and R. To calculate an S value, only one input parameter was varied at a time, while the

other input parameters were kept constant (*ceteris paribus*). According to the model, y is most sensitive to T_{max} (S = 0.033 °C^{-1}; Table 1), and only half as sensitive to T_{min}. The reason for this is that the curve relating relative multiplication rate of *E. amylovora* (vertical axis) to temperature (horizontal axis; Schouten, 1987, Fig. 1) is steeper at the mean T_{max} value than at the mean T_{min} value, so that changes of T_{max} affect the relative multiplication rate of *E. amylovora*, and thus y, more than changes of T_{min} would do. The output variable y is hardly sensitive to global radiation (Table 1): High values of R reduce y only slightly ($S < 0$). Strong radiation increases the transpiration rate of fruit-trees (de Bruin, 1987), and therewith lowers the water potential of the shoot, and y (Schouten, 1991). With a dry soil y would have been more sensitive to R (Schouten, 1991).

Table 1. Sensitivity of y to daily minimum and maximum temperature (T_{min} and T_{max}), and daily global radiation (R), applying the *ceteris paribus* rule. y represents the standardized relative growth rate of *Erwinia amylovora* (see text). S represents the sensitivity coefficient according to Equation (1), replacing the differential quotient by the corresponding difference quotient ($\Delta x = \pm 0.1 * \bar{x}$). The values for $\bar{x}$ are based on n = 221 observations. The value of Δy is based on 2 simulation runs.

	mean	$y_{1.1*\bar{x}}$	$y_{0.9*\bar{x}}$	Δy	Δx	$S = \Delta y/\Delta x$
T_{min} (°C)	10.1	0.388	0.353	0.035	2.02	0.017
T_{max} (°C)	19.7	0.436	0.306	0.129	3.94	0.033
R (J cm^{-2} day^{-1})	1630	0.367	0.371	-0.0043	326	-1.3E-5

The choice for a Δx of 10 % in Table 1 instead of e.g. 5 % or 25 % was arbitrary. A more complete image is given in Fig. 1, in which the relationships between the input parameters and y are depicted over ranges of input values. These input ranges equal the ranges of the values as measured at the weather

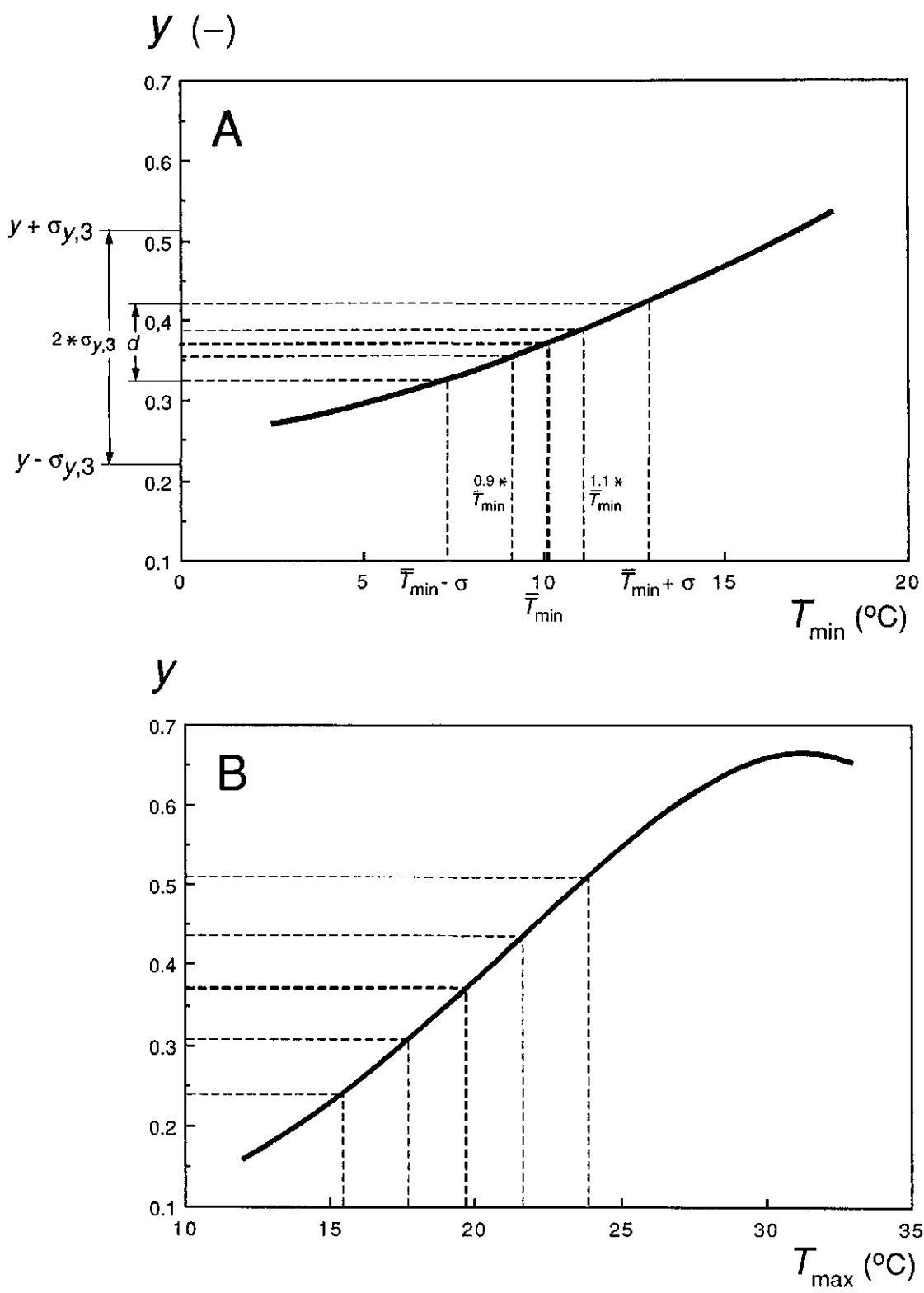
station. The sensitivity coefficient S according to Equation (1) and given in Table 1 equals the slope of the straight line between the points ($0.9*\bar{x}$; $y_{0.9*\bar{x}}$) and ($1.1*\bar{x}$; $y_{1.1*\bar{x}}$). As expected from the S values in Table 1, this slope is smaller in the T_{min} - y graph (Fig. 1A) than in the T_{max} - y graph (Fig. 1B), and slightly negative in the R - y graph (Fig. 1C). Fig. 1 shows also that the three curves approximate straight lines, except at high values of T_{max}. This implies that S would hardly alter when not 10 % changes in the input parameters were chosen, but e.g. 5 % or 25 % changes.

Impact of input parameters on the variance of the output variable

Estimation method 1. Values of S_σ are given in Table 2, considering not only the sensitivity of y to the input parameters, but considering also the standard deviations of the input parameters. S_σ of T_{max} has the highest value, which means that among the three input parameters, variation in T_{max} has the highest impact on variation in y. According to the model, T_{max} is nearly three times as important as T_{min} in explaining variation in y, because first y is more sensitive to T_{max} than to T_{min} (Table 1), and second the standard deviation of T_{max} is larger than that of T_{min} (Table 2).

Estimation method 2. Unbiased estimation of S_σ using Equations (4) and (5) demands a linear relationship between x and y, and a symmetric distribution of x. T_{min} and R satisfy those requirements (Figures 1 and 2), so that their approximations of S_σ according to Equation (5) were fairly close to the $|S_\sigma|$ - values according to Equation (3), neglecting the minus sign of S_σ of R. T_{max}, however, does not fulfil the two conditions (Figures 1B and 2B). Therefore, the approximation of S_σ according to Equation (5) deviates somewhat from $|S_\sigma|$ according to Equation (3). The skewness of the T_{max} distribution is reflected in the distribution of y (Fig. 3).

$|S_\sigma|$ can be visualized in Fig. 1. $|S_\sigma|$ resembles $d/(2*\sigma_{y,3})$ in Fig. 1A when calculated according to Equation (5). Note that d has different values in Figures 1A, 1B and 1C, whereas $2*\sigma_{y,3}$ is constant.


y (–)
A
0.7
0.6
0.5
0.4
0.3
0.2
0.1
y + σy,3
2*σy,3
d
y - σy,3
0.9 * T̄min
1.1 * T̄min
0
5
T̄min - σ
10
T̄min
T̄min + σ
15
20
Tmin (°C)
y
B
0.7
0.6
0.5
0.4
0.3
0.2
0.1
10
15
20
25
30
35
Tmax (°C)

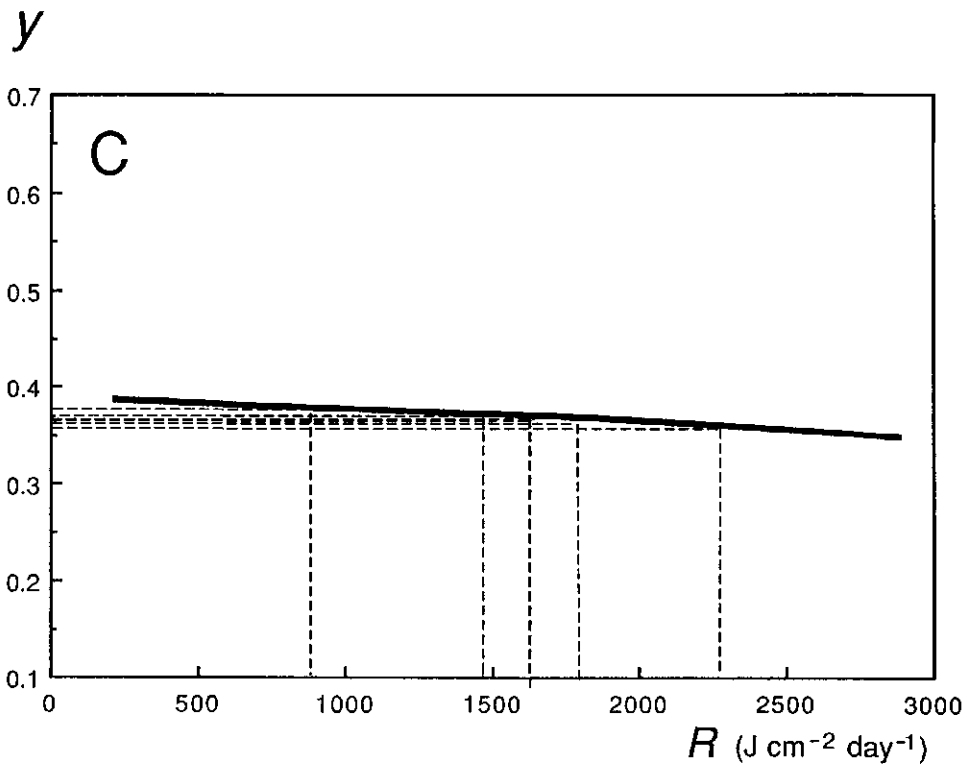


Fig. 1. Relationship between an input parameter and output parameter y, keeping other input parameters constant (*ceteris paribus*). S according to Equation (1) equals the slope of the straight line between the co-ordinate pairs ($0.9*\bar{x}$; $y_{0.9*\bar{x}}$) and ($1.1*\bar{x}$; $y_{1.1*\bar{x}}$). S_σ according to Equation (5) equals $d/(2*\sigma_{y,3})$ (Fig. 1A). Fig. 1B and 1C are similar to Fig. 1A. The five x values in each graph equal from left to right $\bar{x}$-σ, $0.9*\bar{x}$, $\bar{x}$, $1.1*\bar{x}$, and $\bar{x}$-σ, where σ represents the standard deviation of the input parameter x.

Estimation method 3. Table 3 shows standard regression coefficients, estimated by means of multiple regression analysis. These standard regression coefficients are approximations of S_σ. The initial set of input parameters consisted of simultaneously varying T_{min}, T_{max}, R, and their interaction terms ($T_{min} * R$, $T_{max} * R$, $T_{min} * T_{max} * R$). By means of multiple regression analysis with stepwise backward variable selection a final set was obtained, containing only input parameters which had high explanatory values simultaneously. The final set

Table 2. The impact of T_{min}, T_{max}, and R on variation in y. $|S_\sigma|$ represents the standard deviation of y when one x varies ($\sigma_{y,1}$), divided by $\sigma_{y,3}$, the standard deviation of y when the three input parameters vary simultaneously in mutual dependence ($\sigma_{y,3}$ = 0.146).

	σ_x	$\lvert S_\sigma \rvert$ Equation (3)	approximation of S_σ Equation (5)
T_{min} (°C)	2.8	0.335	0.335
T_{max} (°C)	4.3	0.862	0.933
R (J cm^{-2} day^{-1})	641	0.062	-0.059 *)

*) The minus sign indicates that increase of R leads to decrease of y.

Table 3. Regression coefficients of input parameters, selected by means of multiple regression analysis with stepwise backward variable selection (F-to-enter and F-to-remove equalled 40), explaining y (R^2 = 0.99, n = 221). The initial set of input parameters consisted of T_{min}, T_{max}, R, and their interaction terms. Only T_{min} and T_{max} were selected for the final set of input parameters. The standard regression coefficient equals S_σ according to Equation (8).

	regression coefficient	standard regression coefficient
T_{min} (°C)	0.017	0.32
T_{max} (°C)	0.027	0.80

consists of only two input parameters, T_{min} and T_{max}. Daily global radiation (R) and the interaction terms were eliminated. The values of the regression coefficients and standard regression coefficients echo the values of S in Table 1 and of S_σ in Table 2. This implies that multiple regression analysis with stepwise variable selection revealed the causal relationships.

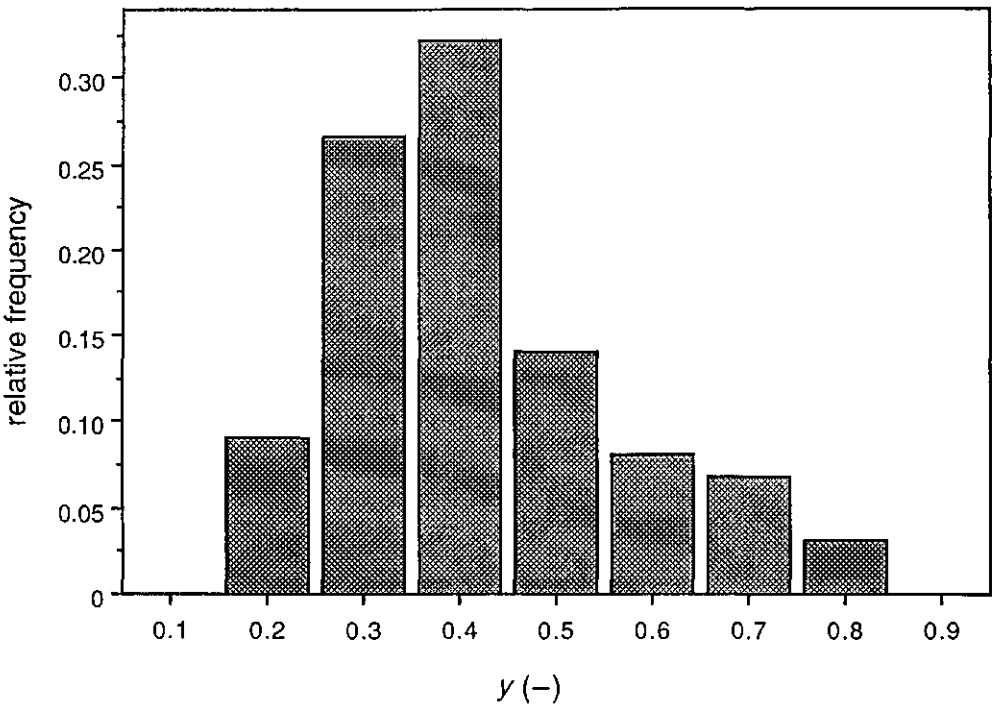
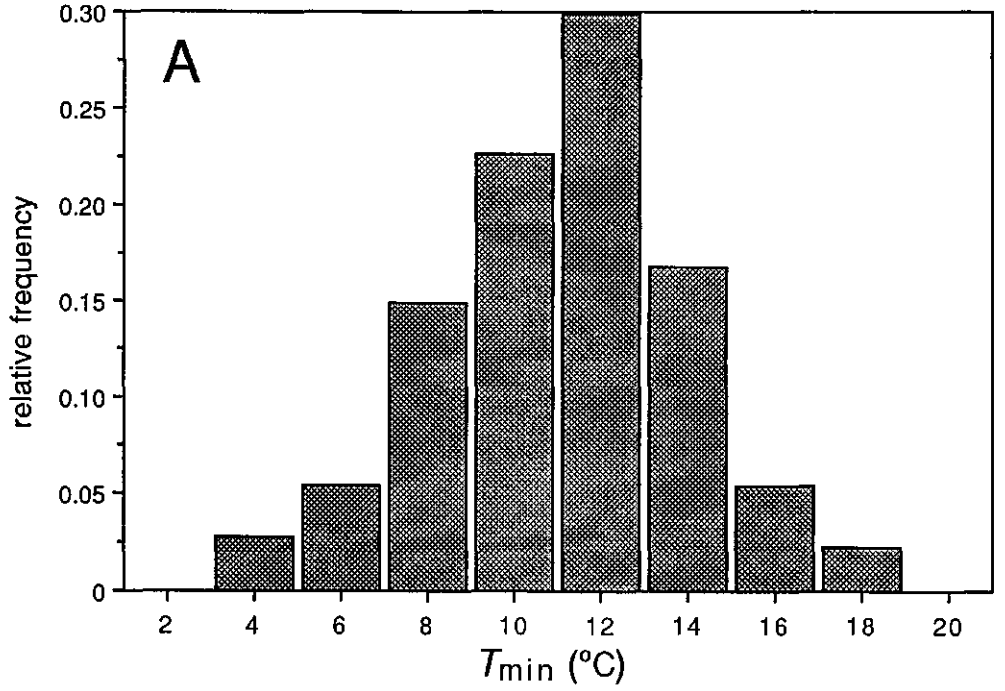


Fig. 3. The distribution of y when T_{min}, T_{max}, and R varied simultaneously in mutual dependence (n = 221).


A
relative frequency
0.30
0.25
0.20
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2
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6
8
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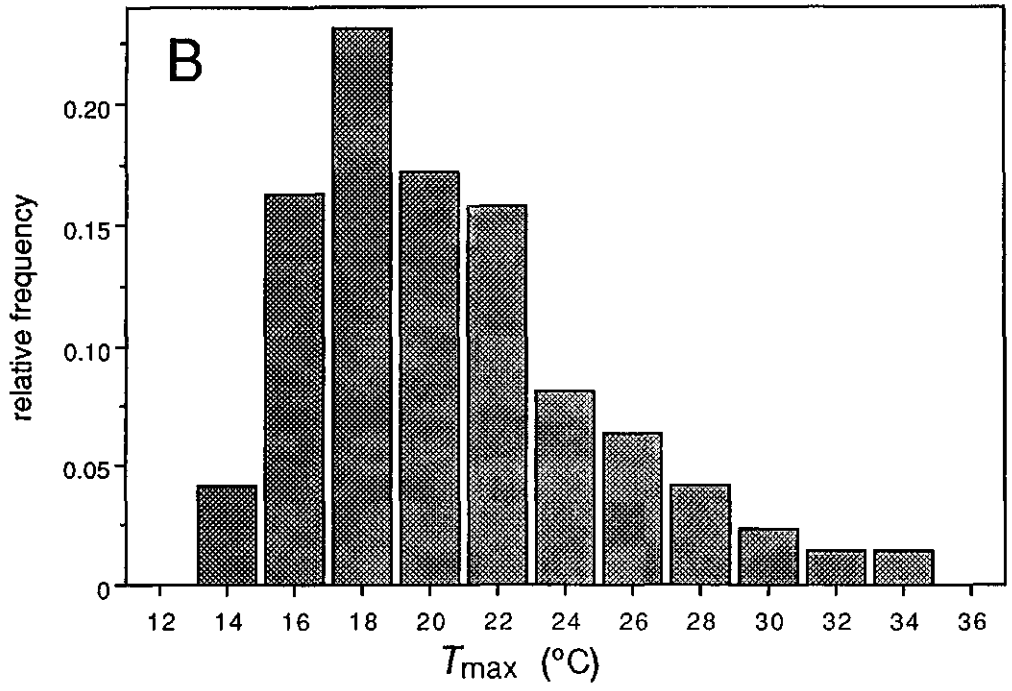
20
T_{min} (°C)


B
relative frequency
0.20
0.15
0.10
0.05
0
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16
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22
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28
30
32
34
36
T_{max} (°C)

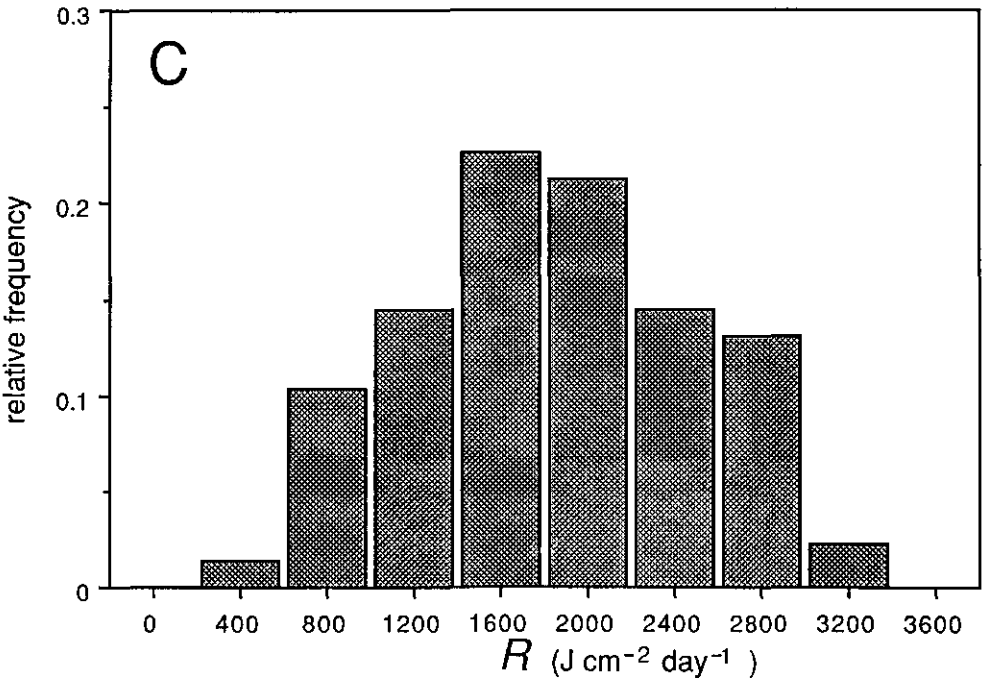

Fig. 2. The distributions of T_{min}, T_{max}, and R. The values on the horizontal axes equal the upper limits of the intervals. The daily records were obtained from a weather station near Wageningen, the Netherlands, and refer to the period 15 to 29 June of the years 1974-1988 (n = 221).

Discussion

One assumption of the model is that air temperature equals shoot temperature. Possible deviations from air temperature, because of e.g. heating by solar radiation at day-time, cooling by transpiration, and cooling by long wave radiation at night were not incorporated in the model. The effects of temperature and radiation on succulence and softness of the host (Barlow, 1975) were not simulated either, although van der Zwet and Keil (1979) showed that succulent and soft tissue is more susceptible to fire blight than tough tissue.

The sensitivity of the standardized relative multiplication rate of *E. amylovora* to soil water potential was studied by means of a simulation model (Schouten, 1991). In this paper the water potential of the soil is assumed to equal -0.01 MPa constantly, which represents a moist soil. When the soil is moist, the water potential in shoots of fruit-trees is continuously high, and thus limits *y* only to a small extent (Schouten, 1991). For that reason, daily global radiation, which affects the transpiration rate of fruit-trees, and therewith the water potential of shoots, has a negligible effect on *y* in this paper. Limitations of *y* by temperature dominate limitations by water, when the soil is moist.

Acknowledgement

The author is grateful to Prof. Dr J.C. Zadoks for critically reading the text and for linguistic corrections.

Samenvatting

Vermenigvuldiging van Erwinia amylovora *in fruitbomen.*
2. Een simulatiestudie naar gevoeligheid voor weersfactoren.

De gevoeligheid van een uitvoervariabele van een model voor verandering van de waarde van een invoerparameter kan op diverse wijzen worden geanalyseerd. Enkele methoden van gevoeligheidsanalyse worden beschreven, en toegepast op een simulatiemodel met dagelijkse minimum en maximum temperatuur (respectievelijk T_{min} en T_{max}) en dagelijkse globale straling als invoerparameters, en de genormaliseerde relatieve groeisnelheid van *Erwinia amylovora* in scheuten van fruitbomen, gemiddeld over een etmaal, als uitvoervariabele. De gebruikte waarden voor de invoerparameters zijn afkomstig van een weerstation bij Wageningen, en hebben betrekking op de tweede helft van juni van de jaren 1974 tot en met 1988.

Volgens het model is de uitvoervariabele twee maal zo gevoelig voor T_{max} als voor T_{min}. Vanwege dit verschil in gevoeligheid, en omdat de standaardafwijking van T_{max} groter was dan die van T_{min}, had T_{max} drie keer meer invloed dan T_{min} op variatie van de waarde van de uitvoervariabele. De gevoeligheid van de uitvoervariabele voor dagelijkse globale straling was bij een vochtige bodem verwaarloosbaar klein.

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PART 2

CHAPTER 8

The effectiveness of flowering prevention of hawthorns to control fire blight in pear orchards

Submitted to Netherlands Journal of Plant Pathology, author Henk J. Schouten.

Abstract

Since 1984, when a new Ministerial Regulation on fire blight came into force, there are in the Netherlands 20 'protected regions', where nurseries and pear and apple orchards are extra protected against fire blight. This policy is also necessary to meet the EC-requirements on fire blight. One of the measures in the protected regions is the prohibition of flowering of the native hawthorns (*Crateagus monogyna* and *C. leavigata*).

Five research areas of about 3 x 3 km² were chosen with hawthorns and pear orchards. Two of these areas were located in protected regions and three in non-protected regions. The more than 50,000 hawthorns in the areas were grouped into 1125 hawthorn objects.

2.3 % of the non-flowering and 16.4 % of the flowering (or berry carrying) hawthorn objects had fire blight at least once in 1987, 1988 and/or 1989. The flowering prohibition for hawthorn in protected areas was rather well observed, so that in protected areas a smaller proportion of hawthorn objects had fire blight

(4.1 %) than in non-protected areas (14 %). Moreover, there were less hawthorn objects per km² in the protected areas (13) than in the non-protected areas (26).

In protected areas 53 % and in non-protected areas 59 % of the pear orchards had fire blight during 1987, 1988 and/or 1989. The difference was not significant. The first reason for the ineffectiveness of the flowering prevention in hawthorn to control fire blight in pear orchards is the inadequate sanitation of the pear orchards. The second reason is that in this study fire blight hardly spread from hawthorn to pear, assuming that a new focus is most probably initiated by the nearest existing focus. Spread of fire blight within pear orchards and between pear orchards occurred frequently.

Additional keywords: Crataegus monogyna, Crataegus leavigata, Erwinia amylovora, Pyrus communis, epidemiology, bacterial disease, spread.

Introduction

Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) Winslow *et al.*, is a serious disease of pomaceous plants. It is very destructive to pear trees and less so to apple trees, hawthorn and several other members of the Rosaceae. In the Netherlands, fire blight was not found until August, 1966, when a focus was discovered in an isle in the southwest of the country. Immediately, strict eradication measures were started, which implied total destruction of the orchards with fire blight and removal and burning of all hawthorns within a radius of 8 km. More than 21 km of hawthorn hedges and about 175,000 solitary hawthorn bushes were destroyed. In 1971, fire blight was discovered again in the southwest in another isle, but also in the northwest of the Netherlands. Another eradication program was started, but in spite of this effort the disease spread gradually all over the Netherlands (Meijneke, 1967, 1984).

A containment policy was initiated in 1984 by means of a Ministerial Regulation (Anonymous, 1984): 20 'protected regions' were created (Fig. 1). The objective was to meet the EC-requirements on fire blight and to protect nurseries and pear and apple orchards in these regions against fire blight as far as possible. Only inside the protected regions it is allowed to grow plants which are

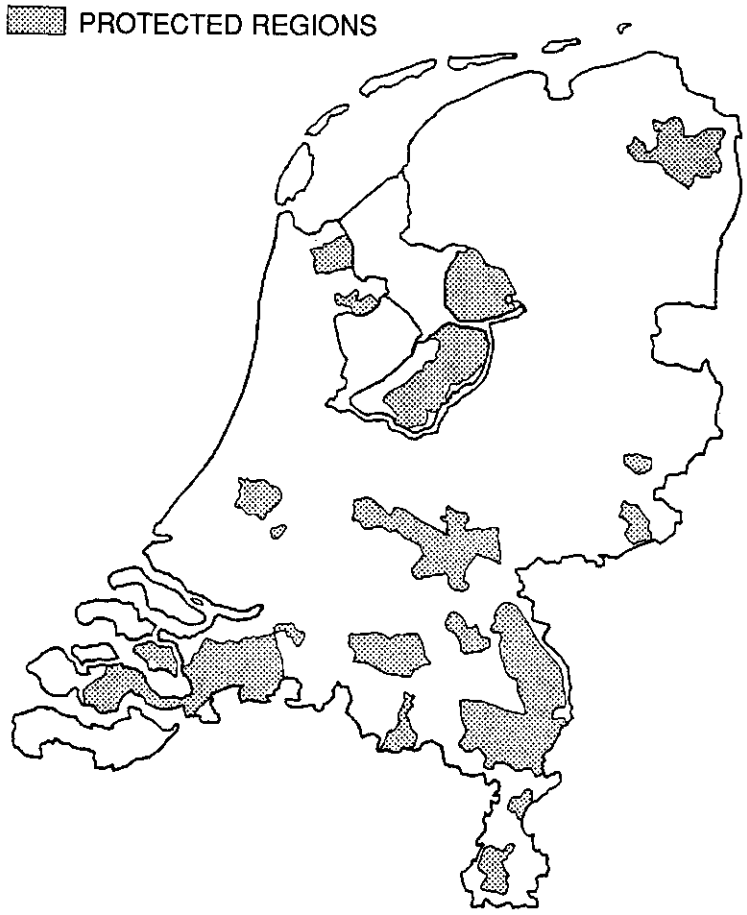

Fig. 1. 'Protected regions' in the Netherlands.

susceptible to fire blight. In order to 'ensure' that these protected regions are free from fire blight, the Plant Protection Service inspects all susceptible plants several times a year in the protected regions, including the plants in nurseries and private gardens, except pear and apple trees in orchards for which the responsibility is left to the fruit growers themselves. The native hawthorn (*Crataegus monogyna* and *C. leavigata*) is not allowed to bloom in the protected regions, as its blossom is the most important point of entry for the bacterium

(Wilson *et al.*, 1987). Prevention of flowering of hawthorn lowers the risk of hawthorn becoming a source of infection for orchards and nurseries (Billing, 1981). Flowering can be prevented by cutting down the hawthorn bushes at least once per three years or by clipping the hawthorn hedges annually. Outside the protected regions, fire blight control is less intensive and is restricted to zones around orchards. In these non-protected regions, hawthorn is allowed to bloom.

From the point of view of nature and landscape conservation the containment policy is strongly criticized. In particular, the blooming prohibition for hawthorns has aroused much opposition. Unfortunately, little quantitative information is available on, for instance, the effect of flowering hawthorns on fire blight in orchards and nurseries. For this particular part, the official policy, though reasonable at first sight, lacks scientific justification and support. To gain insight in the effect of the prevention of hawthorn bloom, the incidence of fire blight in hawthorns and pear orchards in protected regions was compared to the incidence in non-protected regions, thus providing policy makers with quantitative information. Several members of the Rosaceae are susceptible to fire blight, but in the Netherlands the disease is established primarily in hawthorns and pear trees. Other host species were taken into account only if they had fire blight. The growing of potential host plants in nurseries is only allowed in protected regions, so that a comparison to potential host plants in nurseries in non-protected regions is impossible. In this study the attention was focused on hawthorns and pear orchards.

Materials and methods

Five areas with hawthorns and pear orchards were chosen (Fig. 2). Two of these areas were situated in large protected regions and the other three in non-protected regions. Each area had a size of about 3 x 3 km^2. The 50,940 hawthorns in these five areas were grouped into 1125 'hawthorn objects' as defined in Table 1. The state of each hawthorn object was characterized as to number of hawthorns per object, presence of blossom or berries (using a score of 0 to 2 per object), presence of fire blight (using a score of 0 to 3 per hawthorn), and co-ordinates on Ordnance Survey maps, scale 1:10,000. All hawthorn objects were inspected three times a year in 1987, 1988 and 1989, and changes of state were noted.

Table 1. Definitions.

Hawthorn object:	A solitary hawthorn (*Crateagus monogyna* or *C. leavigata*) or a group of hawthorns, with a maximum distance of 50 meters between the hawthorns (e.g. a hedge or a mixed planting). If a hawthorn object is longer than 100 meter, the object is split into two or more objects.
Focus in pear orchard:	One pear tree or a group of adjacent pear trees with fire blight. Diseased trees belong to different foci when these trees are separated by five or more healthy trees within a row or by one or more healthy rows.
Focus in hawthorn:	Any hawthorn object with fire blight is called a focus.
New focus:	A focus, discovered for the first time. Note that all hawthorns were inspected three times a year by officers of the Plant Protection Service and/or by the author, and that the pear orchards were inspected by the respective growers.
Old focus:	A previously discovered and still existing focus. Though an attempt was made to remove the focus at first discovery, the attempt failed according to later inspection.

Similarly, all pear orchards were located on the 1:10,000 maps. In addition, 1:2500 maps were made of these orchards, using blow-ups of the Ordnance Survey maps. In the winter of 1987-1988 the owners of all 135 pear orchards were visited to investigate the orchards' histories concerning fire blight. The pear growers were visited again in 1988 and 1989, three times a year. The fire blight foci were marked on the orchard maps and their co-ordinates determined. Each focus was characterized: new or old (for definitions see Table 1), number of diseased trees per focus, subdivided into trees which showed symptoms for the first time, and trees which were also diseased before.

Fig. 2. The five research areas.

The data thus gathered were organized in databases, manipulated by means of PASCAL-programs, and analyzed statistically.

Results

Fire blight in hawthorn

According to the Ministerial Regulation on fire blight, hawthorns are not allowed to bloom in protected regions. The regulation was rather well observed (Table 2), since in the protected areas 19 % of the hawthorn objects flowered abundantly and 9 % had some flowers, but in the non-protected areas a much higher proportion of the hawthorn objects flowered (Table 2).

Table 2. Frequencies and proportions of hawthorn objects in protected and in non-protected areas, august 1989. Flowering scale: - = no flowers or berries; ± = few flowers or berries; + = a moderate or large number of flowers or berries per hawthorn object.

Frequencies		Flowering				Proportion flowering (+)
		-	±	+	Σ	
Protected area	-	261	85	534	880	0.61
	+	176	23	46	245	0.19
	Σ	437	108	580	1125	

Flowering had a clear effect on fire blight incidence: using the data of all five areas, protected and non-protected, 16.4 % of the blooming hawthorn objects were blighted and out of the non-blooming objects only 2.3 % showed fire blight in 1987, 1988 or 1989. Also in either research area the effect of flowering was obvious (Table 3). The frequencies of Table 3 were subjected to categorical data analysis (Grizzle *et al.*, 1969) using flowering and research area as input

Table 3. Frequencies of blighted and flowering hawthorn objects in the five research areas. The names of the research areas are given between brackets. For flowering scale and time, see Table 2. Flowering had a highly significant effect ($P < 0.001$) on fire blight in hawthorn objects.

Protected areas:

(Maurik)

fire blight \ flowering	-	± or +	Σ
-	85	31	116
+	1	1	2
Σ	86	32	118

(Ovezande)

fire blight \ flowering	-	± or +	Σ
-	83	36	119
+	7	1	8
Σ	90	37	127

Non-protected areas:

(Terwolde)

fire blight \ flowering	-	± or +	Σ
-	192	289	481
+	14	14	28
Σ	206	303	509

(Velddriel)

fire blight \ flowering	-	± or +	Σ
-	25	93	118
+	1	7	8
Σ	26	100	126

(Nisse)

fire blight \ flowering	-	± or +	Σ
-	26	136	162
+	3	80	83
Σ	29	216	245

variables and fire blight as response variable. This multivariate statistical analysis revealed that flowering had a highly significant effect ($P < 0.0001$) on fire blight in hawthorn objects.

Table 4. Frequencies and proportions of hawthorn objects with and without fire blight in 1987, 1988 and/or 1989.

Frequencies		Fire blight			Proportion with fire blight
		-	+	Σ	
Protected area	-	761	119	880	0.14
	+	235	10	245	0.041
	Σ	996	129	1125	

Table 4 shows that the hawthorn objects in the protected areas had less frequently fire blight than those in the non-protected areas. Probably, this is mainly caused by the flowering prohibition for hawthorn in the protected areas, together with the lower susceptibility of non-flowering hawthorn objects compared to flowering objects. On average, there were 13 hawthorn objects per km^2 in the protected areas and 26 in the non-protected areas. In the non-protected areas there were (0.14 x 26 / (0.041 x 13) =) 7 times more diseased hawthorn objects per km^2 than in the protected areas. Up to this point the Ministerial Regulation is successful. However, its objective is not only the sanitation of hawthorns, but also the protection of orchards and nurseries.

Fire blight in pear orchards

Pear orchards in protected areas had approximately as often fire blight as pear orchards in non-protected areas (Table 5). There was no significant difference. In contrast to the hawthorn situation, the blooming prohibition for hawthorns had no demonstrable effect on fire blight in pear orchards. The absence of a significant difference for pear orchards is curious and asks for an explanation. To find the answer, it is necessary to pay attention to fire blight foci in and around the pear orchards.

Table 5. Frequencies and proportions of pear orchards (> 0.2 ha) with and without fire blight in 1987, 1988 and/or 1989. No significant effect (P = 0.29) according Fisher's exact test (right tail).

Frequencies		Fire blight			Proportion with fire blight
		-	+	Σ	
Protected area	-	29	42	71	0.59
	+	26	29	55	0.53
	Σ	55	71	126	

Old foci in pear orchards

In Fig. 3 new and old foci are rendered separately. There should hardly be any old focus: pear growers try to eliminate foci by careful pruning. Fig. 3 shows,

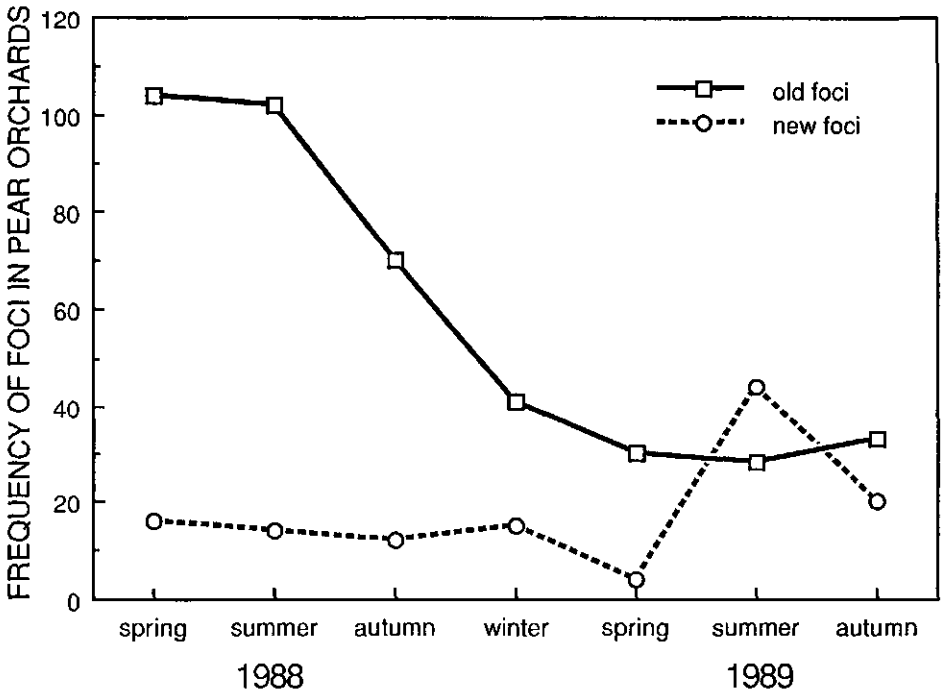

Fig. 3. Total frequencies of fire blight foci ('old' and 'new') in pear orchards over 1988 and 1989 in the considered areas. The terms 'old foci' and 'new foci' are defined in Table 1.

however, that there were far more old foci than new foci. Obviously, fire blight removal was unsatisfactory. Poor sanitation explains why the proportion of pear orchards with fire blight in protected areas did not differ significantly from that in non-protected areas. Sanitation of the environment of pear orchards will be ineffective if the pear orchards themselves are not sanitated adequately.

Fig. 3 shows a decline of the frequency of old foci, but probably there were more old foci in 1989 than depicted. The graph is based on the assumption that treatment of a focus by a grower implies removal of that focus, unless fire blight will be detected again in that group of trees. Because the observations were discontinued in 1989, the number of foci showing fire blight again (= old foci)

in 1990 is not known. So the decline in the frequency of old foci is overestimated.

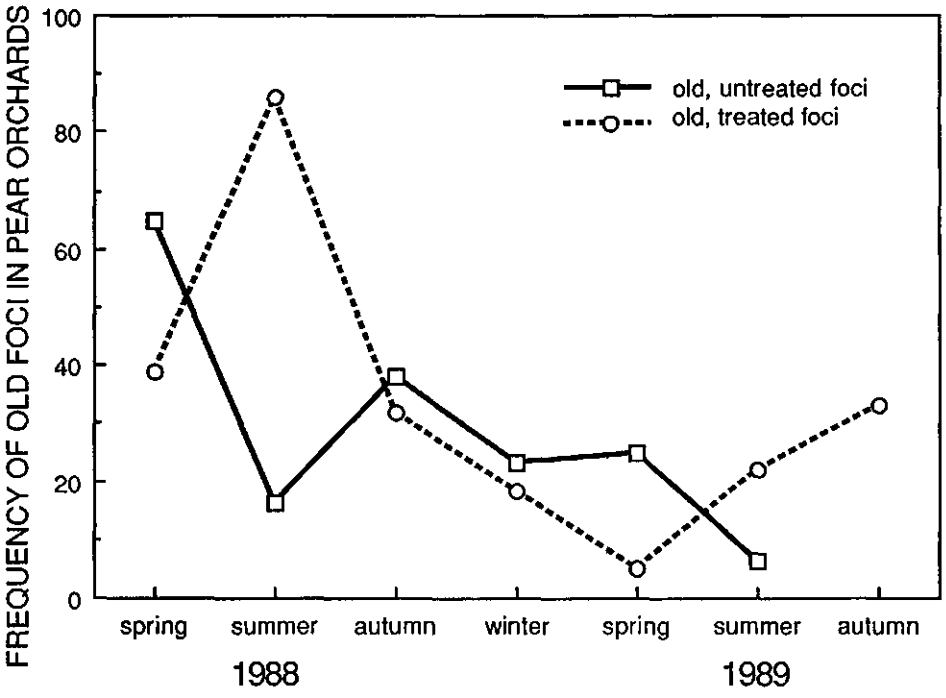

Fig. 4. Frequencies of old foci in pear orchards, 1988 and 1989. See Table 1 for definition of the term 'old focus'. Old, untreated foci = old foci which had not been treated at that season; the bacterium was still present, as could be seen later. Old, treated foci = old foci which had been treated by the pear grower. Note that the sum of these two frequencies equals the frequency of old foci in Fig. 2.

In Fig. 4 the old foci are divided two groups: old, untreated foci (old foci which had not been treated during that season, although the bacterium was still present) and old, treated foci (old foci which had been treated again by the grower). First the old, untreated foci will be discussed. Some pear growers did not treat old fire blight foci for months. In most cases, they overlooked the fire blight, but sometimes they did have no time to inspect and treat again. Fig. 5 shows the number of trees involved. Especially in spring time, 1988 and 1989,

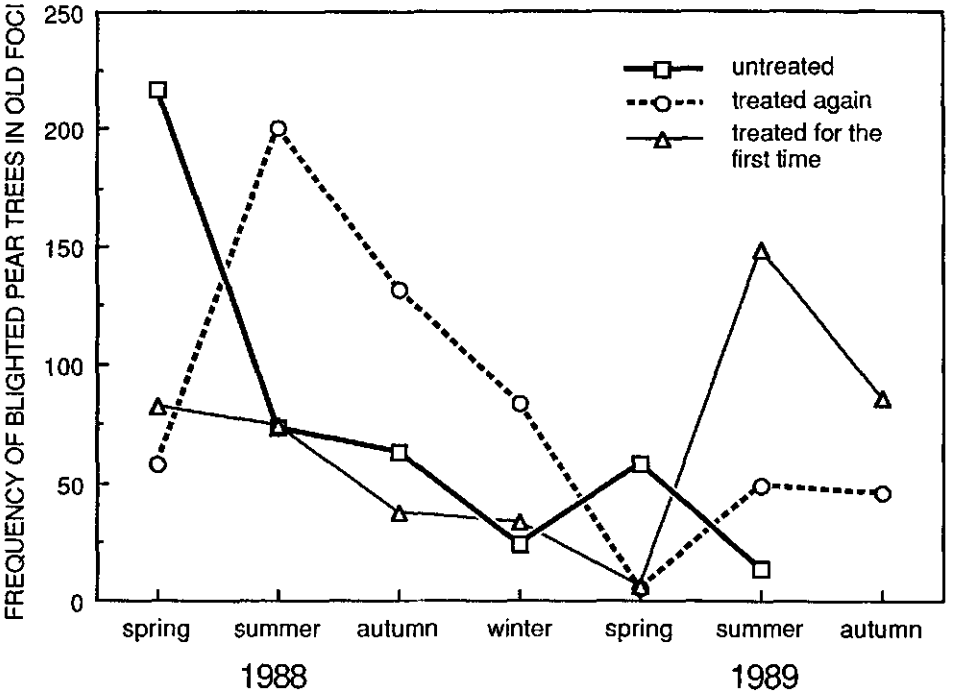

Fig. 5. Frequencies of blighted pear trees in old foci.

there were many untreated diseased trees. During these springs the untreated trees might have served as sources of inoculum for blossom infection in pear and hawthorn.

Many foci, which had already been treated, had to be treated again for either of two reasons. The first reason was that tree sanitation by pruning and sawing was not good enough, so that the bacterium remained present in these trees. This group of trees is called 'treated again'. It was a large group, especially in 1988. Affected shoots and branches were removed and burned, but the bacterium had migrated deep into the trees or had infected other shoots of the same tree, and consequently the trees remained diseased. The second reason for the large amount of old, treated foci was that old foci expanded by infecting healthy neighbour trees. In Fig. 5 these infected neighbour trees are called 'treated for the first time'. Expansion of old foci was relatively important in 1989.

New foci

New foci appeared regularly in pear orchards (Fig. 3). In order to gain insight in the infection sources of these new foci, a 'nearest focus' assumption was formulated, in accordance with theory of dispersal gradients (Gregory, 1968; Glasscock, 1971). The probability that a new focus originates from the nearest focus on record is supposed to be higher than the probability that it originates from a focus at a larger distance. The nearest fire blight focus was assumed to

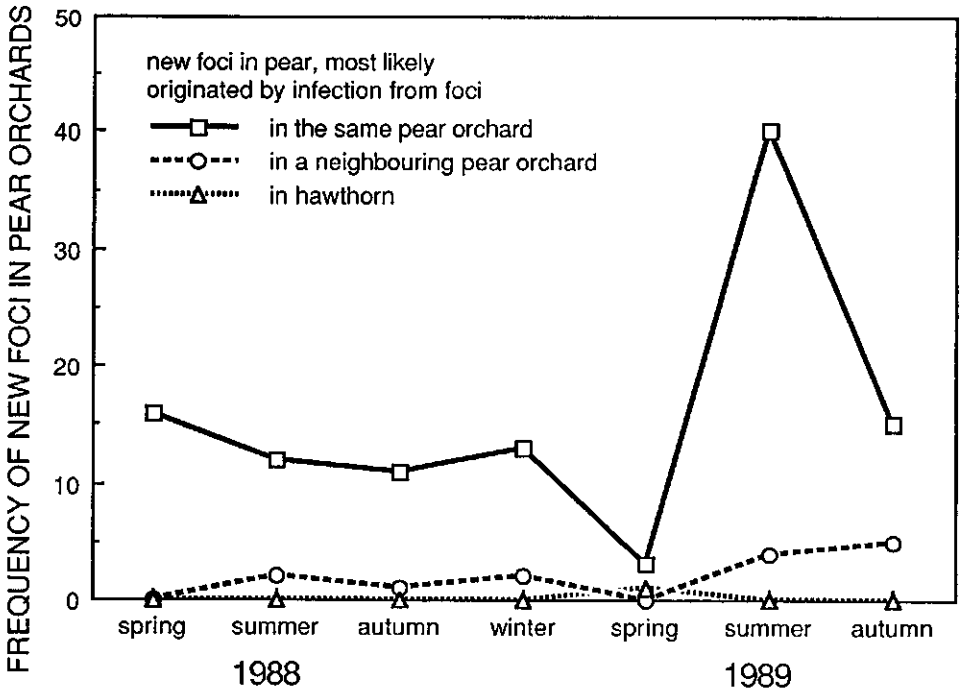

Fig. 6. Frequencies of new foci in pear orchards, 1988 and 1989. It was assumed that the nearest focus led to formation of the new focus.

be the infection source of the new focus. Fig. 6 indicates that the major part of the foci originated from other foci in the same orchard. A much smaller part originated from foci of a neighbouring orchard, and probably only one focus in pear could be ascribed to contamination by a diseased hawthorn object. Apparently, pear trees were rarely contaminated by hawthorn, neither in the protected areas nor in the non-protected areas.

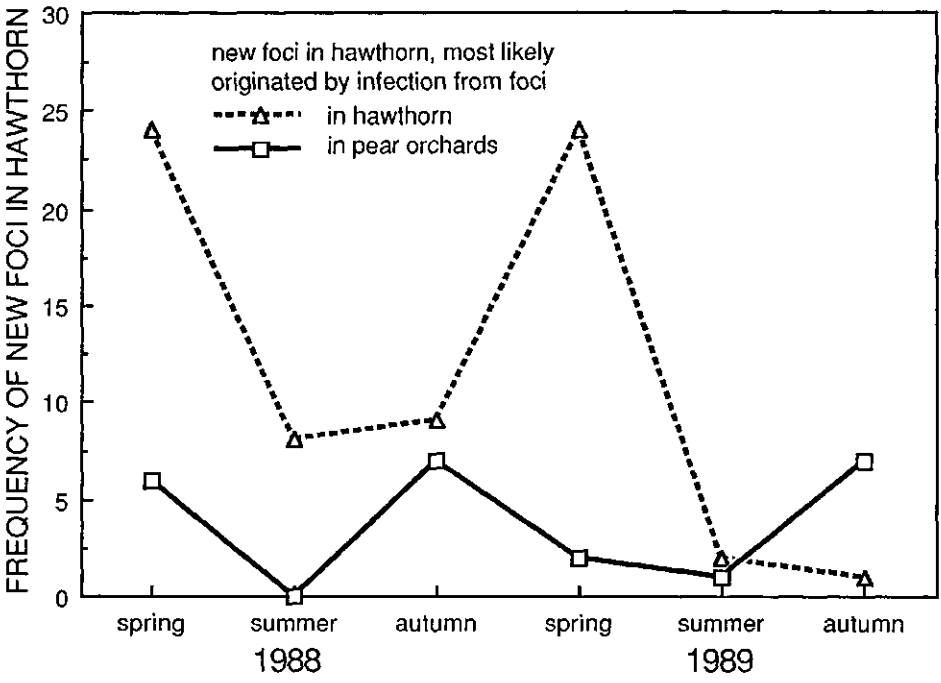

Fig. 7. Frequencies of new foci in hawthorn, 1988 and 1989. It was assumed that the nearest focus led to formation of the new focus.

According to the 'nearest focus' assumption, most foci in hawthorn (Fig. 7) originated from contamination by other foci in hawthorn, but several foci in hawthorn originated from foci in pear. It is concluded that fire blight spread mainly within either host genus and less between the host genera. The primary reason is probably the spatial separation of the genera: blighted pear trees were usually surrounded by healthy pear trees, so that new foci may be expected first

in the orchards containing old foci. Additional reasons might be (1) the temporal separation of pear trees and hawthorns, as the main blossom periods only partly overlap, (2) the flower constancy of several flower visiting insects, such as bees (de Wael, 1988), and (3) possibly some differential virulence of *E. amylovora* to different host species (Norelli *et al.*, 1984).

Discussion

Statistics. In a strict, formal sense one basic assumption underlying the statistical analyses has not been met. Hawthorn objects and pear orchards within an area are not statistically independent, because of spread of fire blight among the orchards and objects. Moreover, soil type and ground water table varied according to area, so that 'protection' was not the only differentiating factor. Because of these inevitable inadequacies, the statistics used must be seen as descriptive rather than explanatory tools. Explanations were given by biological reasoning at different explanatory levels (area - pear orchard - focus - tree).

Containment. The objective of this study was to test the effectiveness of the blooming prohibition for hawthorn, as formulated in the Ministerial Regulation on fire blight 1984, thus providing the policy makers with quantitative information. That information is needed for a regulation of fire blight control which balances damage to nature and landscape against damage to crops. In this study special attention was given to the strongly criticised prohibition of flowering of hawthorn in protected regions.

With respect to hawthorn, the flowering prohibition appeared to be effective. The proportion of diseased hawthorn objects in protected areas was much smaller than that in non-protected areas. The objective of the flowering prohibition, however, is not only sanitation of hawthorns, but also protection of orchards and nurseries. Unfortunately, the flowering prohibition for hawthorn did not appear to be effective in the control of fire blight in pear orchards. Pear orchards in protected areas had approximately the same incidence of fire blight as the pear orchards in non-protected areas. The primary reason is the unsatisfactory sanitation of the pear orchards. The bacterium had established itself in pear orchards in a focal pattern, and removal of the bacterium from the foci by means

of pruning and sawing away affected tree parts seemed to be difficult. The secondary reason for the ineffectiveness of the flowering prohibition to control fire blight in pear is that hardly any focus in pear originated from diseased hawthorns. A small proportion of the foci in pear originated probably from foci in neighbouring pear orchards, but most foci were initiated by foci in their own pear orchard. Flowering prevention of hawthorn is not effective to control fire blight in pear orchards.

The results presented here contrast with growers' experiences during the early years of the fire blight epidemic in the Netherlands and the official reports of that time, which all pointed to hawthorn as active infection sources of fire blight in pear (conform Glasscock, 1971 and Bech-Andersen, 1973). The cause of the discrepancy is not known. Possibly the following items contributed: 1. When fire blight is not endemic in pear, diseased hawthorns may play an important role for introduction of fire blight in pear. Glasscock (1971) and Baumm (1985) provided circumstantial evidence that diseased hawthorns, which bordered on healthy pear and apple orchards, served as sources of inoculum when there were severe hail storms during the shoot growth of pear or apple. When fire blight is endemic in pear, diseased hawthorns are relatively less threatening to pear, as the disease spreads more frequently within pear orchards and between pear orchards than from hawthorns to pear orchards. 2. Since the early 80's, officers of the Plant Protection Service have inspected intensively the hawthorns in the protected regions and in the 500 meter zones around orchards in the non-protected regions, so that fire blight in hawthorns has been early detected and removed there. Probably, this early removal of fire blight in hawthorn prevented in most cases spread of the disease to pear orchards. 3. The climatical conditions during the three years of study were not particularly conducive to fire blight. They were moderate fire blight years. The present study does not permit a generalisation to years particularly favourable to spread of fire blight. The study does point to the need of better eradication of fire blight in pear orchards.

Acknowledgements

The author is very grateful to M. van Teylingen and many other officers of the Plant Protection Service for co-organizing the field work and collecting the bulk

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

Samenvatting

De doeltreffendheid van bloeipreventie van wilde meidoorns voor beheersing van bacterievuur in pereboomgaarden

Sinds de 'Beschikking Bestrijding Bacterievuur 1984' zijn er in Nederland 20 'beschermde gebieden', waarbinnen de boomkwekerij en pere- en appelteelt extra beschermd worden tegen bacterievuur. Tevens wordt hiermee voldaan aan de EG-regeling op dit gebied. Eén van de maatregelen in de beschermde gebieden is het verbod wilde meidoorns te laten bloeien.

Om na te gaan wat de invloed is van bloeiende meidoorns op bacterievuur in pereboomgaarden, werden er vijf onderzoeksgebieden van ongeveer 3 x 3 km² gekozen. Twee bevonden zich in beschermd gebied en drie in niet-beschermd gebied. De ruim 50.000 wilde meidoorns binnen de onderzoeksgebieden werden gegroepeerd tot 1125 meidoornobjecten, en werden in 1987, 1988 en 1989 drie keer per jaar geïnspecteerd op o.a. bloei en bacterievuur. Tevens werden de eigenaars van de 135 pereboomgaarden in de onderzoeksgebieden drie keer per jaar bezocht.

Bloei van wilde meidoorns beïnvloedde de aantasting van die meidoorns sterk: In 1987, 1988 en 1989 had 16,4 % van de bloeiende (of bes dragende) meidoornobjecten één of meer keer bacterievuur, en van de niet-bloeiende meidoornobjecten had 2,3 % bacterievuur. Men hield zich redelijk aan het bloeiverbod voor wilde meidoorn in beschermde gebieden, wat tot gevolg had dat meidoornobjecten daar minder vaak bacterievuur hadden: 4,1 % van de meidoornobjecten in de beschermde onderzoeksgebieden had bacterievuur en in de niet-beschermde onderzoeksgebieden had 14 % bacterievuur. Bovendien waren er in de beschermde onderzoeksgebieden minder meidoornobjecten per km² (13) dan in de niet-beschermde onderzoeksgebieden (26).

In 1987, 1988 en/of 1989 had 53 % van de pereboomgaarden in de beschermde gebieden bacterievuur en in de niet-beschermde gebieden had 59 % van de pereboomgaarden bacterievuur. Er was geen significant verschil, hoewel

er in de onderzochte niet-beschermde gebieden 7 maal zo veel zieke meidoornobjecten per km² waren als in de beschermde gebieden. Dit resultaat is merkwaardig en vraagt om een verklaring. De eerste reden is dat bacterievuur onvoldoende werd weggehaald uit pereboomgaarden. De bacterie overleefde vaak in haarden in pereboomgaarden, hoewel de telers de bacterievuursymptomen naar hun mening ruim hadden weggehaald uit die haarden. De tweede reden is dat verspreiding van bacterievuur van wilde meidoorn naar peer tijdens het onderzoek niet of nauwelijks aantoonbaar was, noch in de beschermde noch in de niet-beschermde gebieden. Verspreiding binnen perepercelen en tussen perepercelen trad wel duidelijk op. Verder bleek dat bacterievuur zich verspreidde van zieke perepercelen naar gezonde wilde meidoorns, zij het minder vaak dan tussen meidoornobjecten onderling.

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CHAPTER 9

GENERAL DISCUSSION

Practical implications

Recommendations to pear and apple growers. When infection has taken place, the air temperature is high (20 - 28 °C), and the soil moist and fertile, *E. amylovora* is able to colonize soft tissues such as cambium, young bast, and young wood at such a high speed that the formation of cork layers around the diseased tissue by the host plant cannot be completed in time. In that case, the bacteria pass the cork barriers before the diseased part is effectively girdled. This may have important consequences:

1. colonisation of the tree by the pathogen goes on;
2. bacterial biomass oozes out of the tree, and may function as inoculum to healthy plants;
3. the diseased parts do not dry, and there may be no noticeable outward change for several days. The exterior of diseased trunks and thick branches may look healthy for weeks;
4. when colonisation proceeds fast, the diseased cambium of trunks and thick branches have not a black but a red colour. The red colour of young tissue indicates that the invasion by the bacterium goes very fast. Where the cambium of branches is red, the outside of the bark may not yet show any discoloration (personal observations), so that fruit growers may be misled.

It is common practice that fruit growers inspect their trees and remove visibly diseased tree parts down to about half a meter under the outwardly blackening lesion. Fruit growers usually are not willing to remove tree parts which seem healthy to them. Rather than looking only at the outside of the tree, it may be an improvement of the sanitation practice to inspect how far the internal fire blight symptoms have spread towards the trunk.

The internal fire blight symptoms can be recognized by means of cutting into the tree with a disinfected knife, so that the colour of the cambium and the young bark can be judged. In case of reddish discoloration, the diseased tree part has to be removed at one meter below the discoloration at least. Such a procedure may imply total removal of the tree. Blackened tissue with a sharp transition to pale tissue points to effective girdling of the diseased part by the tree. Removing the diseased part a few decimeters below the blackening lesion may be sufficient. Effective girdling is frequently the case in apple and hawthorn, but less often in pear and fast growing cotoneasters. If growers see that cambium is discoloured, they will be motivated to remove branches and trunks which look healthy from outside.

Because incising a tree with a contaminated knife is a form of inoculation, it is important to disinfect the knife. Disinfecting the tree surface at the incision site is useful too. A small plant sprayer, filled with an antiseptic, is handy for this purpose.

One has to be aware that the underpressure in the xylem vessels leads to the suction of air or external liquid into the vessels when they are damaged during cutting, pruning, or sawing. Bacteria may be sucked in also. 'Painting' the wounds with an aseptic covering agent is necessary, but may not kill the bacteria which have already been sucked into the xylem vessels. Wound covering is not common for fire blight removal in the Netherlands, but is, in the author's opinion, essential.

Good sanitation of affected orchards and diseased trees is not only beneficial to the trees and orchards involved, but also to still healthy orchards, flowering hawthorns, and other host plants in nurseries, gardens, and nature.

When the soil is dry (water potential of the soil below -1 MPa), water limitation is important, and progress of the disease through the host tissue may be hampered. Particularly in the Mediterranean area, where fire blight invaded several countries recently (Zutra *et al.*, 1986; Psallidas, 1990), soils are often dry, but temperatures are high compared to dutch circumstances. In those countries it might be useful to reduce irrigation in orchards with fire blight, so

that 1) the multiplication rate of and pressure by *E. amylovora* in intercellular spaces of hosts is reduced, and 2) host tissues becomes tougher. Then, the diseased tissues can be effectively sealed off by the host plants, reducing both the oozing out of the pathogen and the damage to infected trees.

Recommendations to policy makers. The results of the study on the effectiveness of flowering prevention of hawthorn for fire blight control in pear has been extensively discussed with and by many people who are concerned with the fire blight regulation. A new fire blight policy is proposed and discussed at the moment (autumn 1990; Anonymous, 1990). The draft regulation takes into account the results of the study reported in Chapter 8. Let it suffice here to state that flowering prevention of hawthorn is overdone for control of fire blight in pear, provided that diseased hawthorns are (partly) removed. Sanitation of pear orchards should get more attention.

Exploration - analysis - synthesis - application

Part 1 started with an exploratory chapter on the effect of weather on the development rate of fire blight under field conditions. Temperature and water seemed to influence fire blight strongly. The relationship temperature - r (r = relative multiplication rate of *E. amylovora*) and water - r were analyzed in more detail in the Chapters 2 and 3. In contrast to the exploratory study of Chapter 1, no field data were used in the Chapters 2 and 3, but data obtained under controlled conditions in laboratories, allowing a sharp analysis. The role of water potential in the pathogenesis of fire blight was analyzed further in Chapter 3, applying a physical, quantitative approach. The analysis went into more detail in Chapter 4, leaving epidemiology aside and taking a physiological approach. Laboratory experiments were carried out to investigate the influence of water potential on the extracellular slime of *E. amylovora* and therewith on the pathogenesis of fire blight.

From Chapter 5 onwards, the results of the previous chapters were integrated into simulation models. Whereas the simulation study of Chapter 5 still referred to the

physiological level (processes in the host tissue), the short-term model of Chapter 6 referred to a higher integration level (epidemiology; effect of weather on the relative multiplication rate of *E. amylovora* within shoots of fruit trees). The long-term model of Chapter 6 was a synthesis of the short-term model and SWACROP, a model for the water balance of a cropped soil, integrating the effects of weather and soil.

Unfortunately, the simulation models could hardly be validated because fire blight is a quarantine disease. The simulation results agreed with common experience with fire blight. The simulation studies gave insight into underlying relationships.

The insight obtained from the epidemiological and physiological studies was applied to the fruit growing practice and to the dutch fire blight control policy.

Integration into simulation models versus statistical analysis of correlations

Simulation models should be considered as complex hypotheses needing validation and verification. By means of modelling, effects of e.g. environmental factors on development of fire blight can be studied, although in the author's opinion the reliability of predictions by these simulation models is less than that by regression equations obtained from field experiments. Usually, regression equations adequately describe correlations between one or more input variables at the one side and an output variable at the other side, and they often provide good prognoses if circumstances do not differ much from the experimental circumstances. In spite of their ability to predict, regression equations are sometimes misleading with respect to causal relationships. For prediction purposes, however, one is not so much interested in underlying processes and causal relationships as in reliable prognoses. Simulation models, in contrast, provide in the author's opinion more insight into underlying processes, and into the structure of the system in which the processes are linked to one another. Simulation models are appropriate for explanation, whereas regression models are more appropriate for prediction.

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GENERAL SUMMARY

Part 1

Effects of water potential and temperature on multiplication of and pressure by Erwinia amylovora *in host plants*

Analysis of field data from Eve Billing, England, on the duration of the incubation period of fire blight revealed that temperature and rainfall were positively and interactively correlated with the development rate of fire blight. Values of standard regression coefficients suggest that temperature had more impact on the variation in the development rate than rainfall.

Billing studied the effect of temperature on the multiplication rate r of *E. amylovora* by means of laboratory experiments. Instead of r, she used the variable 'potential doublings per day', represented by PD ($PD = r / \ln(2)$). Reconsideration of her calculations of PD revealed, however, that the PD-values in Billing's table were underestimated. The relationship temperature - r was recalculated, and corrected PD-table presented.

Shaw studied the effect of water potential on r of *E. amylovora*. This relationship appears to be applicable to *E. amylovora* in plant tissues and in nectar of flowers.

Growth of *E. amylovora* in a restricted space, e.g. an intercellular hole, may create a pressure on the surrounding 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. Growth of *E. amylovora* can be caused by multiplication and by swelling of the bacterial biomass, due to absorbtion of water without increase of dry weight. The maximum 'multiplication pressure' equals the actual water potential minus the lowest water potential at which *E. amylovora* is able to multiply at absence of bacterial pressure. The maximum 'swelling pressure' equals the change in the water potential.

The volume of the cells of *E. amylovora* hardly changes with changing water potential, but the extracellular slime of *E. amylovora*, consisting mainly of extracellular polysaccharide, swells strongly with increasing water potential. The hypothesis of the swelling pressure would explain why the extracellular slime is a virulence factor.

The pressure, caused by multiplication and swelling of the bacterial biomass, may lead to compression of soft host cells, to tearing of host tissues, and to formation of large slime-filled holes in the plant tissue. Moreover, the expanding biomass may force its way to the outside of the plant or to healthy parts. Cork barriers, being formed by the plant after infection, may be broken through if the mechanical pressure is high and if the cork barrier is incomplete or not yet fully developed. Sealing off is then prevented. Strong tissue may be able to resist the pressure, so that symptom progression can be prevented.

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 bacterial pressure attains its highest values at evening and night.

To gain insight into the limitations imposed by temperature and water on multiplication of *E. amylovora* in shoots of fruit-trees under field conditions, two simulation models were designed: a short-term model for immediate effects of weather and soil water potential, and a long-term model for effects of rain and soil profiles. The relationships temperature - r derived from Billing and water potential - r derived from Shaw were incorporated into the models.

In the Netherlands, in the month of June, when the soil is moist and the weather 'average', water hardly limits *E. amylovora* multiplication in shoots but, according to the short-term model, temperature reduces then the multiplication by about 60 %. When the soil is dry and the potential transpiration rate of the trees high, water may limit *E. amylovora* multiplication in shoots considerably. According to the long-term model, rain has a delayed effect on multiplication.

The effect of a rain shower increases gradually in the course of time and reaches its maximum 2 to 30 days after the rain, in dependence of soil moisture content before the rain, amount of rain, and soil profile. Calculations were made for three soil profiles representing three typical fruit growing areas in the Netherlands. The results suggest different effects of rain on the behaviour of fire blight according to soil profile.

According to the short-term model, r of *E. amylovora* in shoots of fruit trees was twice as sensitive to the daily maximum temperature T_{max}, as to the daily minimum temperature T_{min}, during the second half of June under dutch conditions. Because of this difference in sensitivity, and because the standard deviation of T_{max} was larger than that of T_{min}, the variation of r due to T_{max} was three times larger than that due to T_{min}. The sensitivity to daily global radiation was negligible when the soil was moist.

Part 2

The effectiveness of flowering prevention of hawthorns to control fire blight in pear orchards

Since 1984, when a new Ministerial Regulation on fire blight came into force, there are in the Netherlands 20 'protected regions', where nurseries and pear and apple orchards are extra protected against fire blight. This policy is also necessary to meet the EC-requirements on fire blight. One of the measures in the protected regions is the prohibition of flowering of the native hawthorns (*Crateagus monogyna* and *C. laevigata*).

Five research areas of about 3 x 3 km² were chosen with hawthorns and pear orchards. Two of these areas were located in protected regions and three in non-protected regions. The more than 50,000 hawthorns in the areas were grouped into 1125 hawthorn objects.

2.3 % of the non-flowering and 16.4 % of the flowering (or berry carrying) hawthorn objects had fire blight at least once in 1987, 1988 and/or 1989. The

flowering prohibition for hawthorn in protected areas was rather well observed, so that in protected areas a smaller proportion of hawthorn objects had fire blight (4.1 %) than in non-protected areas (14 %). Moreover, there were less hawthorn objects per km^2 in the protected areas (13) than in the non-protected areas (26).

In protected areas 53 % and in non-protected areas 59 % of the pear orchards had fire blight during 1987, 1988 and/or 1989. The difference was not significant. The first reason for the ineffectiveness of the flowering prevention in hawthorn to control fire blight in pear orchards is the inadequate sanitation of the pear orchards. The second reason is that in this study fire blight hardly spread from hawthorn to pear, assuming that a new focus is most probably initiated by the nearest existing focus. Spread of fire blight within pear orchards and between pear orchards occurred frequently.

SAMENVATTING

ONDERZOEK AAN BACTERIEVUUR

Deel 1

Effecten van waterpotentiaal en temperatuur op vermenigvuldiging van en druk door Erwinia amylovora *in waardplanten*

Uit analyse van veldgegevens van Billing, Engeland, betreffende de duur van de incubatieperiode van bacterievuur bleek dat temperatuur en regen positief en interactief gecorreleerd waren met de ontwikkelingssnelheid van bacterievuur. Waarden van standaard regressiecoefficiënten duidden erop dat temperatuur meer invloed had op variatie in de ontwikkelingssnelheid dan regen.

Billing bestudeerde aan de hand van laboratoriumexperimenten het effect van temperatuur op de vermenigvuldigingssnelheid van de bacterievuur veroorzakende bacterie *Erwinia amylovora.* In plaats van de relatieve vermenigvuldigingssnelheid r gebruikte zij de variabele 'potentiële verdubbelingen per dag', weergegeven als PD ($PD = r / \ln(2)$). Uit herberekeningen blijkt echter dat de PD-waarden in Billings tabel onderschat worden. Een gecorrigeerde PD-tabel werd samengesteld.

Shaw bestudeerde de invloed van waterpotentiaal op r van *E. amylovora*. De relatie tussen waterpotentiaal en r kan zowel worden toegepast op de pathogenese in planteweefsel als op de epifytische ontwikkeling in nektar.

Groei van *E. amylovora* in een beperkte ruimte, bijvoorbeeld in een intercellulaire holte, creëert een druk, die tot scheuren van zacht weefsel kan leiden. Theoretisch is de druk door de bacterie gelijk aan het verschil tussen de

actuele waterpotentiaal van het waardplantweefsel en de waterpotentiaal waarbij de bacteriële biomassa de intercellulaire holte juist zou vullen zonder druk uit te oefenen. Groei van *E. amylovora* kan worden veroorzaakt door vermenigvuldiging (productie van droge stof) en door zwelling van de bacteriële biomassa, als gevolg van wateropname zonder toename van het drooggewicht. De maximale 'vermenigvuldigingsdruk' is gelijk aan de actuele waterpotentiaal minus de laagste waterpotentiaal waarbij *E. amylovora* zich nog kan vermenigvuldigen bij afwezigheid van bacteriële druk. De maximale 'zweldruk' is gelijk aan de verhoging van de waterpotentiaal.

Uit experimenten bleek dat bij een wisselende waterpotentiaal de cellen van *E. amylovora* nauwelijks krimpen of zwellen, maar dat het extracellulaire slijm van deze bacterie, dat voornamelijke uit extracellulaire polysaccharide bestaat, dan wel sterk van volume verandert. Zweldruk kan verklaren waarom het extracellulaire slijm van *E. amylovora* een virulentiefactor is.

De druk, veroorzaakt door vermenigvuldiging en zwelling van de bacteriële biomassa, kan vermoedelijk leiden tot in elkaar drukken van zachte plantecellen, tot scheuren van waardplantweefsel en tot vorming van holten in aangetast planteweefsel. Verder kan waarschijnlijk het zwellende slijm door intercellulaire holten zich een weg banen naar buiten of in gezond, zacht weefsel van de waard binnendringen. Als de druk van het extracellulaire slijm op het omringende planteweefsel hoog wordt, kunnen vermoedelijk ook mechanische barrières, zoals in aanleg zijnde kurkwanden, die de plant aan het vormen was om de ziekte af te grendelen, doorbroken worden. Na doorbreking van deze compartimentalisatie breidt de ziekte zich verder uit in de plant. Sterk weefsel kan de druk van de bacteriën vermoedelijk wel weerstaan, zodat uitbreiding wordt verhinderd.

Simulatiestudies wijzen erop dat het extracellulaire slijm van *E. amylovora* sterk slinkt door waterafgifte als zich druk opbouwt. Door dit slinken is verdere produktie van bacteriële droge stof mogelijk. Het extracellulaire slijm bevindt zich dan rondom de bacteriecellen als een geconcentreerde substantie, met een sterk vermogen tot zwellen als weefsel van de waardplant zou scheuren. Verder geven resultaten van de simulatie aan dat de druk uitgeoefend door de bacterie

's avonds en 's nachts zijn hoogste waarden bereikt, omdat dan de waterpotentiaal van de waardplant het hoogst is.

Om inzicht te verkrijgen in de beperkingen door temperatuur en vocht op vermenigvuldiging van *E. amylovora* in scheuten van fruitbomen werden twee simulatiemodellen gebouwd: een korte-termijn model voor onmiddellijke invloeden van weer en waterpotentiaal van de bodem op de beperkingen door temperatuur en vocht, en een lange termijn-model voor invoed van regen bij verschillende bodemprofielen. De relatie temperatuur - r, afgeleid van experimenten van Billing, en de relatie waterpotentiaal - r, afgeleid van experimenten van Shaw, werden in de modellen verwerkt.

Bij een vochtige bodem en "gemiddeld" juni-weer in Nederland limiteert vocht de vermenigvuldiging van *E. amylovora* in scheuten nauwelijks, maar volgens het korte-termijn model reduceert de temperatuur deze vermenigvuldiging dan met 61 %. Als de bodem droog is en de potentiële transpiratiesnelheid hoog, reduceert vocht de vermenigvuldiging van de bacterie in aanzienlijke mate. Het lange-termijn model geeft aan dat regen de vermenigvuldiging beïnvloedt, zij het na een zekere vertraging. Het effect van een regenbui neemt in de tijd geleidelijk toe, en bereikt 2 tot 30 dagen na de regendag zijn maximum, afhankelijk van het vochtgehalte van de bodem voordat het regende, de hoeveelheid regen en het bodemprofiel. Berekeningen werden uitgevoerd voor bomenprofielen van drie fruitgebieden in Nederland. De resultaten laten zien dat het bodemprofiel het gedrag van gedrag van bacterievuur beïnvloedt.

Volgens het korte-termijn model is r van *E. amylovora* in scheuten van fruitbomen twee maal zo gevoelig voor de dagelijkse maximum temperatuur T_{max} als voor dagelijkse minimum temperatuur T_{min}. Vanwege dit verschil in gevoeligheid, en omdat de standaardafwijking van T_{max} groter was dan die van T_{min}, had T_{max} drie keer meer invloed dan T_{min} op variatie van de waarde van r. De gevoeligheid voor dagelijkse globale straling was bij een vochtige bodem verwaarloosbaar.

Deel 2

De doeltreffendheid van bloeipreventie van wilde meidoorns voor beheersing van bacterievuur in pereboomgaarden

Sinds de 'Beschikking Bestrijding Bacterievuur 1984' zijn er in Nederland 20 'beschermde gebieden', waarbinnen de boomkwekerij en pere- en appelteelt extra beschermd worden tegen bacterievuur. Tevens wordt hiermee voldaan aan de EG-regeling op dit gebied. Eén van de maatregelen in de beschermde gebieden is het verbod wilde meidoorns te laten bloeien. Om na te gaan wat de invloed is van bloeiende meidoorns op bacterievuur in pereboomgaarden, werden er vijf onderzoeksgebieden van ongeveer 3 x 3 km² gekozen. Twee bevonden zich in beschermd gebied en drie in niet-beschermd gebied. De ruim 50.000 wilde meidoorns binnen de onderzoeksgebieden werden gegroepeerd tot 1125 meidoornobjecten, en werden in 1987, 1988 en 1989 drie keer per jaar geïnspecteerd op o.a. bloei en bacterievuur. Tevens werden de eigenaars van de 135 pereboomgaarden in de onderzoeksgebieden drie keer per jaar bezocht.

Bloei van wilde meidoorns beïnvloedde de aantasting van die meidoorns sterk: In 1987, 1988 en 1989 had 16,4 % van de bloeiende (of bes dragende) meidoornobjecten één of meer keer bacterievuur, en van de niet-bloeiende meidoornobjecten had 2,3 % bacterievuur. Men hield zich redelijk aan het bloeiverbod voor wilde meidoorn in beschermde gebieden, wat tot gevolg had dat meidoornobjecten daar minder vaak bacterievuur hadden: 4,1 % van de meidoornobjecten in de beschermde onderzoeksgebieden had bacterievuur en in de niet-beschermde onderzoeksgebieden had 14 % bacterievuur. Bovendien waren er in de beschermde onderzoeksgebieden minder meidoornobjecten per km² (13) dan in de niet-beschermde onderzoeksgebieden (26).

In 1987, 1988 en/of 1989 had 53 % van de pereboomgaarden in de beschermde gebieden bacterievuur en in de niet-beschermde gebieden had 59 % van de pereboomgaarden bacterievuur. Er was geen significant verschil, hoewel er in de onderzochte niet-beschermde gebieden 7 maal zo veel zieke meidoornobjecten per km² waren als in de beschermde gebieden. Dit resultaat is merkwaardig en vraagt om een verklaring. De eerste reden is dat bacterievuur onvoldoende werd

weggehaald uit pereboomgaarden. De bacterie overleefde vaak in haarden in pereboomgaarden, hoewel de telers de bacterievuur-symptomen naar hun mening ruim hadden weggehaald uit die haarden. De tweede reden is dat verspreiding van bacterievuur van wilde meidoorn naar peer tijdens het onderzoek niet of nauwelijks aantoonbaar was, noch in de beschermde noch in de niet-beschermde gebieden. Verspreiding binnen perepercelen en tussen perepercelen trad wel duidelijk op. Verder bleek dat bacterievuur zich verspreidde van zieke perepercelen naar gezonde wilde meidoorns, zij het minder vaak dan tussen meidoornobjecten onderling.

Curriculum vitae

Henk Schouten werd geboren op 24 januari 1961 te Andel, Noord-Brabant. Na het behalen van het VWO-diploma aan de 'Oude Hoven' te Gorinchem, werd in 1979 begonnen met de studie Planteziektenkunde aan de toenmalige Landbouwhogeschool te Wageningen. De doctoraalstudie omvatte de hoofdvakken Fytopathologie en Theoretische Teeltkunde, en het bijvak Meteorologie. Gedurende deze tijd verrichtte hij veldonderzoek naar de schade van bladvlekkenziekte (*Septoria tritici*) in wintertarwe, en simuleerde hij deze schade met behulp van een computermodel. Verder werkte hij op een weerstation en simuleerde hij dauwvorming. In 1985 werd het doctoraalexamen met lof behaald. Vervolgens werd op de vakgroep Fytopathologie van de Landbouwuniversiteit het onderzoek verricht dat geleid heeft tot dit proefschrift. Vanaf 1 januari is hij als onderzoeker resistentie-management verbonden aan het Centrum voor Plantenveredelings- en Reproduktieonderzoek.

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