Prediction infection risk on the basis of weather-related factors and *Erwinia amylovora* colonization in apple and pear flowers

Bubán T.[1], Rutkai E.[2], Dorgai L.[2] and Thomson, S. V.[3]

[1]Research and Extension Centre for Fruit-growing, H-4244 Újfehértó, Hungary
e-mail: bubant@ujfehertokutato.hu
[2]Bay Zoltán Institute for Biotechnology, H-6726 Szeged, Hungary
e-mail: dorgai@bay.u-szeged.hu
[3]Department of Biology, Utah State University, Logan, Utah 84322-5305, USA
e-mail: shermt@ext.usu.edu

Abstract: Current infection risk prediction models utilize environmental parameters and field records, but do not take into account the estimated inoculum potential within the orchard. The object of this study was to survey the accuracy of three simple prediction methods under Hungarian climatic conditions, which could easily be used by the farmers. We also tested whether the accuracy of infection risk predictions can be improved by taking into consideration the incidence and/or rate of flower colonization by *Erwinia amylovora*.

After preliminary investigations in 1999–2001, data concerning the weather-related infection risk were recorded in 5 apple and 1 pear orchards in 2002, and in 12 apple and 1 pear orchards in 2003. The weather data were processed by the easy-to-use risk assessment models of the mean temperature prediction line (MTL), Smith's Cougarblight 98C and Billing's integrated system (BIS), and by the Maryblyt™ 4.3 computer-assisted model for reference. The population size of *E. amylovora* in the flower samples was estimated within an order of magnitude by PCR.

For all years and orchards tested, Maryblyt indicated 35 days on which there was an acute infection risk. The same days were indicated by all 3 methods in 23 cases (66%), 8 days were indicated by 2 methods (23%) and 4 days were indicated by 1 method only. A similarly good correlation was found for prediction of the date of the first massive infection risk: in 2003, for instance, there was a perfectly consistent prediction by all 4 models in 9 of the 13 participating orchards. A coincidental forecast was provided by 3 of the 4 models in the other 4 orchards.

The results indicate that any of the risk assessment models could provide an increased accuracy of the actual infection risk prediction if combined with an estimation of the incidence of *Erwinia amylovora* colonization in the open flowers. We found no convincing differences in the size of the epiphytic population in flowers of cultivars possessing high or low susceptibility to *Erwinia amylovora*.

We conclude that the easy-to-use methods tested could be used by the farmers to recognize weather-related risks, especially when coupled with an estimation of the proportion of the pathogen-infested flowers. This local prediction would provide rapid information (faster than the regional forecast systems) specifically for a given orchard.

Key words: *Erwinia amylovora*, infection risk prediction, Maryblyt™4.3, Billing's integrated system (BIS), mean temperature prediction line (MTL), Smith's Cougarblight 98C model

Introduction

> **Fire blight risk assessment systems are working hypotheses based on a combination of knowledge, speculation and trial and error – *Paul Steiner***

'Fire blight', as a danger to fruit production, was recognized by Cox in 1817 (cit. *Bonn* and *van der Zwet,* 2000). Nevertheless, the prevention of this bacterial disease has remained an unsolved problem. The only exception seems to be the situation in Israel: "authorities in the Fruit Board of Israel and in the Ministry of Agriculture and Rural Development and growers' representatives declared that the Fire-Man project was so successful that fire blight is no longer a problem in Israel!" (*Shtienberg et al.,* 2002). These latter authors state that fire blight can be managed by their project, but "the fight against *E. amylovora* is never-ending."

One of the preconditions for management of the disease is an accurate forecast of the infection risk. There are a number of risk assessment systems, e.g. Firescreen (*Tsiantos and Psallidas,* 1996), Feuerbra and Anlafbra (*Berger et al.,* 1996), FireWorks (*Gouk et al.,* 1999), the Fire Blight Control Advisory (FBCA) system (*Shtienberg et al.,* 2002), the computer-assisted Maryblyt model (*Steiner,* 1990a), the mean temperature prediction line (MTL; *Thomson et al.,* 1982), Smith's Cougarblight 98C model (*Smith,* 1993, 2000) and Billing's integrated system (*BIS, Billing,* 1996, 1999).

The incidence and severity of the disease, however, vary from season to season and from orchard to orchard. Consequently, reliable disease management in a given orchard should be based on weather-related risks in conjunction with field risk records, supplemented by an estimation of the incidence of flowers colonized with *E. amylovora*. Field records, however, are often inadequately managed. Besides blossom phenology, other records should be included: host species/cultivar susceptibility; tree age; soil nitrogen level; irrigation dates and level; blight incidence; diseased alternative hosts nearby; dates of high insect activity; the presence of beehives near flowering host trees; and bloom profusion. "These are ideal lists which are usually far from complete, but the reliability of a risk assessment may depend on the quality of the field data" (*Billing,* 1996). Furthermore, the surroundings of an orchard may also affect the manifestation of fire blight. An example of this is given by *Benedettini et al.* (2002). While compiling a fire blight risk map in the Emilia Romagna region of Italy, they found a correlation of $R^2 = 0.7066$ between disease cases and the distance of pear orchards from main roads; there was a significant relation between the percentage of disease cases and different soil groups; the absolute number of cases increased considerably in the year after a hail event, but there was no correlation ($R^2 = 0.2757$) between the territorial distribution of the disease cases and the closeness to rivers.

In Hungary, the forecasting of the *E. amylovora* infection risk is based on a nation-wide monitoring network, which uses the computer-assisted Maryblyt model operated by the Plant and Soil Protection Services established in all 19 counties (*Németh,* 1999). These services receive the bloom phenology data together with meteorological data recorded in large commercial orchards and use them as input for the program. Each individual Plant Protection Service processes the data received from its own county and, in the event of an output of an acute infection risk a call for the use of streptomycin is published in the local newspapers and electronic media, for a particular area within the county, or even for the whole county. Since the number of orchards providing such data is limited (e.g. 110 apple and 19 pear orchards were involved in the whole country in the year 2000), a county-wide prediction is based on data recorded only in several orchards. Consequently, the environmental parameters in a given orchard could differ considerably from those in the few orchards providing the data for Maryblyt.

Simple methods, however, may be used by the farmers to predict the risk of infection in their orchards, in this way complementing the Maryblyt forecast. Such easy-to-use methods, MTL and Cougarblight, utilizing rainfall and temperature data, accurately predicted infections in the San Joaquin Valley of California, in good agreement with Maryblyt (*Holtz et al.,* 2002). Similar results were reported earlier by *Bubán et al.* (2002), using MTL, Cougarblight and BIS. During recent years, we have extended our previous study to a number of large commercial orchards in order to test the accuracy of three simple prediction methods (MTL, BIS and Cougarblight) under Hungarian climatic conditions.

Current prediction models utilize environmental parameters, but do not take into account the inoculum potential within the orchard. The stigma imprinting of pear and apple flowers on selective media can be used to determine the risk and to anticipate fire blight outbreaks within the orchards, and it can be used in conjunction with Maryblyt and Cougarblight (*Thomson et al.,* 2002). Another possibility is the rapid, semiquantitative estimation of the actual epiphytic population of *E. amylovora* in the flowers by PCR (*Dorgai and Bubán,* 2002). We used this PCR detection during the present study in order to establish whether the accuracy of predicting the risk of infection can be improved by taking into consideration the incidence and/or rate of flower colonization by the pathogen.

Material and methods

Orchards

The initial 3-year study was carried out between 1999 and 2001 in the apple orchard of the research station in Újfehértó (Orchard 5), situated in the north-east of Hungary. In 2002, 4 apple and 1 pear orchards, located in the eastern half of Hungary, joined in the project (Orchards 1-4 and 6, respectively). Another 7 commercial orchards became partners in our study in 2003 (Orchards 7–13), and one more (Orchard 2) replaced the previous co-operating partner with the same number.

Bloom phenology was monitored as suggested in connection with the use of the Maryblyt model: pink bud (PB), early bloom (B1), full bloom (BB), secondary bloom (B2) and petal fall (PF). Assessment of the infection risk was based on the blooming time of cv. Jonathan in Orchard 5 (1999–2001, Table 2), and on the blooming time of the most representative cultivars in the other orchards (2002, Tables 4–6; and 2003, Tables 10–16, respectively).

Weather data

The source of the daily records (temperature, rainfall, relative humidity and periods of leaf wetness) in Orchard 5 in each year of the study was an automated weather station (μMetos, Pessl Instruments Co., Austria), located very close to the young, high-density orchard investigated. A similar instrument was used by Orchard 2 in 2002, and by Orchards 4 and 6 in 2002 and 2003. Otherwise, minimum and maximum temperatures and daily rainfall were recorded by simple instruments (thermometer and rain gauge), and dew formation was observed empirically at about sunrise in the other orchards. The infection risk-forecasting models, including the Maryblyt model, were run on the weather data recorded in the given orchard.

Protective sprayings

Prediction of the days on which there was an infection risk, necessitating treatment with antibiotics was done by the

Maryblyt system (as a function of the nationwide monitoring network) operated by the regional Plant and Soil Protection Service located nearest to the given orchard. In general, streptomycin in the form of Erwin 25WP at a dosage of 0.5 kg/hectare was used during blooming time. The spray volume was usually 1000 litres/ha. Differences from this routine in a given orchard are indicated after the dates of spraying.

The spraying dates in 2002 were:

Orchard 1: April 24 and April 30
Orchard 2: No treatment with streptomycin
Orchard 3: April 27/28 and June 14 with Kasumin 2L, 4 l/ha
Orchard 4: April 29
Orchard 5: April 29 and May 3 with 600 l/ha
Orchard 6: April 24 and April 26 with 800 l/ha

Dates of sprayings were in 2003:

Orchard 1: Streptomycin 20WP, 0.5 kg/ha on May 4 and 1.0 kg/ha on May 8
Orchard 2: May 3
Orchard 3: May 2
Orchard 4: May 2
Orchard 5: May 2 and May 6 with 800 l/ha
Orchard 6: May 2 and May 5
Orchard 7: Kasumin 2L on May 26 and June 3
Orchard 8: Kasumin 2L on May 16 and June 2
Orchard 9: No treatment with antibiotics
Orchard 10: No treatment with antibiotics
Orchard 11: May 2
Orchard 12: Streptomycin 20WP, May 8
Orchard 13: May 3

Risk assessment models

Maryblyt™ 4.3 (*Steiner* 1990a, *Steiner and Lightner* 1996)

Maryblyt is a comprehensive fire blight model applied to identify apple blossom infection periods which can be confirmed by symptom appearance at predicted intervals (*Steiner*, 1990b). It has been widely used in different countries and under different climatic conditions. This model generally provides the optimum timing of protective spray applications, especially if field risks are also considered (*Billing*, 2000). The model lists four requirements for blossom blight, which are needed to occur in sequence: 1) blossom present = B; 2) 110 degree hours; DH>18.3 °C accumulated since the time of the first bloom = H; 3) a wetting event from dew, rain, or spray, or >2.5 mm rain on the previous day = W, and 4) an average temperature of 15.6 °C on the day of wetting = T. If all criteria are met, i.e. all 4 parameters of BHWT have a plus (+) display in the data output window, blossom "infection" is likely, and R within the item BHWTR is denoted by I. The model predicts a "high" risk (H) when blossom is present and any 2 of the remaining 3 required factors are satisfied.

Billing's integrated system (*BIS; Billing* 1996, 1999, 2000)

As concerns a high incidence of blossom blight, the BIS stresses the importance of a warm period prior to the wetting of open flowers. The critical sum of degree days (DD) above a maximum temperature of 18 °C (DDmax18) is taken as >17 when open flowers are wet on a day with a mean temperature of >15 °C; it is recorded as Bwet (i.e. blossom infection risk of wetted flowers, denoted as Bw). Rainfall of >2.5 mm, or a leaf wetness period of >3 h is considered to be an adequate wetting event. Flower wetting appears to be unnecessary when DDmax18 is 17 and the maximum temperature on such a day is >27 °C, or the mean temperature is >20 °C (recorded as Bdry and denoted as Bd). Counting of the DDmax18 sums starts on the first day of blooming. The DD sum is restarted when the maximum temperature falls to 16–17 °C for 2 days, or to 15 °C for 1 day. The disease development rate can also be assessed. Starting on the day after each day with a predicted infection risk, the DD13mean value is calculated with 0.5 °C increments. The critical value of DD13mean >47 forecasts the early signs of apple blossom blight. The BIS model is intended for use throughout the growing season. The main aim in the methodology is simplicity (*Billing*, 2000).

Mean temperature prediction line (MTL; *Thomson et al.*, 1982)

This method is based on the relation of temperature to the occurrence of the pathogen in the flowers. As a result of monitoring data from 132 orchards for a 4-year period, Thomson and his coworkers reported that the pathogen could not be found in the flowers before the daily temperature exceeded a value determined by a straight line drawn from 16.7 °C on the 1st of March to 14.4 °C on the 1st of May (later, it was proposed to use the line drawn horizontally at 15.6 °C, Thomson, unpublished data). This line was used as a simple guide to the timing of the first protective spraying in California. It is still used not only in California, but also in Washington, Oregon and Utah (*Billing*, 2000).

During this study in Hungary, the prediction line was drawn horizontally and the accuracy of prediction was improved by taking into consideration wetting events, too. An actual infection risk (denoted by I) means that the mean temperature was above the prediction line and there was a wetting event (>1 mm rainfall, or >1 h of leaf wetness) on the same day, or the day before. A high infection risk (H) means that the mean temperature is above the line without a contemporary wetting event. A moderate infection risk (M) means that the mean temperature is >15.0 °C, but <15.6 °C.

Smith's Cougarblight 98C Model (*Smith* 1993, 2000)

This is a situation-specific fire blight risk assessment model developed by analysing experience from 17 of the 20 years before 1992. The distinctive feature is the use of a 4-day heat sum (hours over 15.5 °C) prior to a wetting event.

The risk of infection increases with increase in the sum of degree hours. On the basis of the guideline of *Smith* (1993, 2000), we considered a '4-day degree hour total' above 270 preceding wetting events (rainfall of >2.5 mm or a leaf wetness period >3 h) as a predicted infection risk.

The model can be used in conjunction with regional weather data and single tabulated guides. During the years of our study (1999–2003), we checked the accuracy of prediction on use of the Smith degree hour estimation look-up chart by comparing it with the DH total values calculated from temperatures measured automatically every 12 min by a weather station in the orchard. There was always excellent accord between the results of these approaches, i.e. the differences (if any) were not large enough to influence the actual prediction (data not shown).

Estimating the size of the epiphytic population of Erwinia amylovora

Flower sampling

The flower samples should represent the entirety of the flowers. To estimate the *E. amylovora* population in open blossoms in the orchard with acceptable accuracy (±5%), 20 blossoms collected from each of 15 trees are needed (*Shtienberg* et al., 2002). We usually sampled 15 flowers from each of 5 trees of 4 cultivars, i.e. altogether 300 flowers in an orchard (see *Table 7*).

The *sampling procedure termed "traditional"* was used in 1999, 2000 and 2001. Five representative trees were selected for each cultivar in a given orchard, and 5 apparently symptomless inflorescences were taken from each tree. Flower samples were collected on the 4th day after the opening of the first flower. The inflorescences were wrapped in moist filter paper, placed in plastic bags and mailed overnight to the laboratory where the samples were processed further as follows. Open flowers were taken from the inflorescences, and the corolla and calyx, the stamina and the lower third of the receptacle were removed with razor blades. The remaining parts of the flowers were placed into 5 ml sterile PBS solution in a 15 ml capped tube, and incubated at room temperature for 4–6 h with gentle agitation on a rocking table. The PBS solution was then decanted off and the epiphytic microbial population was collected by centrifugation (3000 rpm, 15 min), resuspended in 1 ml PBS, transferred into an Eppendorf tube, centrifuged (10 000 rpm, 1 min) and resuspended in 100 µl PBS. 10 µl of this suspension was applied to the surface of solid nutrient medium, dried under a sterile airflow and incubated at room temperature. The microbial population grown on these plates were used for a new round of PCR when ambiguous results were obtained by PCR during the routine detection process. The remainder of the suspension was lysed by heating at 95 °C for 10 min in the presence of Triton X-100 (1% final concentration).

The most labour-intensive and time-consuming part of the detection process is the processing of flower samples as outlined above. This step, however, does not need special skills: the growers could do it themselves if they are provided with the necessary information about how to do it, including proper instructions concerning the precautionary measures to be taken to prevent cross-contamination of the samples.

During 2001, 2002 and 2003, we used a new *sampling protocol, termed "mail extraction"* (*Dorgai and Bubán,* 2002), which involved the co-operation of our partners as follows: The king flower of each flower cluster was sampled (5 trees/cultivar, 15 inflorescences/tree), trimmed as described above and the remaining parts of the flowers (15 for each sample) were placed into 5 ml sterile PBS solution in a 15 ml capped tube which was shipped to the co-operating orchards before blooming. The tubes containing the samples were resealed and mailed to the laboratory by Express Mail Service. This arrangement had the advantage of the epiphytic population being extracted during the shipment. The PCR analysis could therefore be completed much more quickly, usually within the day on which the samples arrived in the laboratory. In 2003, the only modification was that the 15 king flowers for one sample were picked from 15 trees.

As concerns a comparison of the traditional and mail-extraction protocols, a general tendency towards a rate of detection of *Erwinia amylovora* that was 1 or 2 orders of magnitude less by the mail-extraction protocol was observed (*Table 1*), though similar numbers were also measured (*Dorgai and Bubán,* 2002). Since *Erwinia amylovora* survives 24 h of incubation in PBS, this tendency can not be attributed to cell death. It is more likely that the cells multiply on the surface of the intact flowers in the moist environment, when shipped in the "traditional" way. The observed phenomenon could also possibly be attributed to statistical fluctuation, since different samples taken from a given tree could naturally harbour different sizes of the epiphytic population.

PCR and product analysis

Erwinia amylovora was detected by PCR as described earlier (*Dorgai and Bubán,* 2002), with primers specific for the plasmid pEA29 (*McManus and Jones,* 1995). For the first round of PCR, primers A and B and 2 µl of the 10-fold diluted Triton-lysate were used. Nested PCR was performed

Table 1 Size of the epiphytic population estimated by PCR in samples mailed as intact flowers (traditional) or in the extraction buffer after processing (mail extraction)

Procedure	Cultivar and treatments	Flower samples				
		1	2	3	4	5
mail extr.	Sampion, control*	10^2–10^3	10^4–10^5	–	–	–
mail extr.	Sampion, Bion**	10^4–10^5	10^4–10^5	10^3–10^4	10^3–10^4	10^2–10^3
traditional	Sampion, control*	10^4–10^5	–	10^3–10^4	–	–
traditional	Sampion, Bion**	–	–	10^4–10^5	10^4–10^5	10^4–10^5

Samples were collected in Orchard 5 on May 2, 2001.
* = Trees were sprayed with streptomycin (Erwin 25WP, 0.5 kg/hectare) on May 1.
** = Trees were treated with Bion 50WG (20 g/100 l) on April 17 and April 25.
– = no bacteria were detected.

with the primer pair AJ75 and AJ76, using 2 µl of the first PCR reaction for template. The reaction was carried out in a 30 µl volume containing 50 pmol of each primer; 2 U Taq polymerase; 2 µl template; 250 µM dNTP, each; 10 mM Tris-HCl, pH: 9.0; 0.1% Triton X-100; 2.5 mM $MgCl_2$ and 50 mM KCl. Amplification was performed in a PTC 200 thermal cycler (MJ Research), using 30 cycles of denaturation at 95°C, annealing at 52 °C and synthesis at 72 °C, for 1 min each.

The products of the PCR reactions were separated by gel electrophoresis on 1% agarose gels. Images of the ethidium bromide-stained gels were digitized with a GDS 7500 Gel Documentation System and analysed with the GelBase/GelBlot™ Pro software (UVP).

The population size (in terms of genome-equivalent, GE) was estimated within orders of magnitude, by measuring and comparing the strengths of the PCR signals with those given by known amounts of bacteria mixed with healthy extracts. The detection threshold was 10–100 GE/flower, and a good correlation was found up to 10^5 GE/flower.

Estimation of the incidence of fire blight

All trees of 8 cultivars within the experimental plot in Orchard 5 were thoroughly supervised for the incidence of fire blight *(Table 9)*. The 'quotient of infection' was calculated as (the number of symptomatic inflorescences or shoots/the number of trees investigated) ×100. Practically, a quotient of <10 indicates a rather moderate incidence of fire blight. An estimation of the incidence of fire blight in this accurate way could not be expected from the co-operating farmers in large, commercial orchards. In 2002, they provided us with verbal information, describing the disease severity in their orchard. In 2003, Orchards 1, 3 and 6 used the rating scales below referring to the whole orchards, in general, while in Orchards 2, 7 and 8 the rating was based on the observations on 10 selected trees/cultivar.

The rating scales used to score disease severity were as follows:

for flowers: 0 = no infection in flowers,
1 = slight infection with no risk of a loss of yield,
3 = a yield loss is probable,
5 = a serious yield loss is expected,

for shoots: 0 = no shoot blight was observed
1 = <5 cuts/tree,
3 = 5–10 cuts/tree,
5 = >10 cuts/tree were needed to cut out the infected parts of the canopy.

Results

1999–2001

The start of a massive infection period was predicted by 3 of the 4 models on the same day, April 28 in 1999 and April 25 in 2000, and by 2 models in 2001, on May 2 *(Table 2)*. The Cougarblight predictions agreed with the Maryblyt and BIS predictions in 1999 and 2000, but had a delay of 1 day in 2001. The MTL model forecast the infection risk 1 day late in 1999 and 2000, and 2 days earlier in 2001 than did BIS and Maryblyt. Weather-related infection risks just outside the blooming time (at petal fall), predicted by MTL in 2000, or by both MTL and Cougarblight in 1999 and 2001, were confirmed by BIS.

The period of 20 days including the blooming time in 1999 was rainy (on 12 of the 20 days), during which an average temperature of >18.3 °C was measured on 2 days only. The epiphytic population of *Erwinia amylovora* was below the detection limit (10^1 GE/flower) in 60% of the flowers investigated, and was about 10^2 in the remaining 40%, as estimated by PCR. In the next season (2000), the same period included only 1 day with a rainfall of 1.4 mm, while the daily mean temperature was >18.3 °C on 11 of the 20 days and, due to the heavy dew formation, leaf-wetness periods of >3 h were frequently recorded. An epiphytic population of 10^2–10^4 GE/flower was detected in 68% of the samples. In 2001, periods of weather-related infection risk occurred rather late during the blooming time, with a bacterial population of 10^3–10^5 GE/flower in 33% of the samples. There were no convincing differences in the size of the epiphytic population in the flowers of cultivars with a high (e.g. Sampion) or a low (e.g. Freedom) susceptibility to fire blight.

Because of the considerable overlap between the blooming time and periods with a persistent infection risk, the highest weather-related infection risk occurred in 2000 *(Table 2)*. This coincided with a characteristic epiphytic population of bacteria of 10^2–10^4 GE/flower. The infection periods in 1999 and 2001 were predicted rather late within the blooming time, when the bacterial population had only a limited, or hardly any chance to increase in flowers older than 3 days (*Gouk and Thomson,* 1999). Not surprisingly, the incidence of fire blight was sporadic in 1999 and 2001, but epidemic in 2000. The differences in weather-related risks *(Table 2)* were well reflected by the percentage of trees with fire blight in the two years investigated, and especially by the 3 most sensitive cultivars of the 6 apple cultivars tested *(Table 3)*. The considerable variability in susceptibility of the 3 different types of 'Jonagold' is noteworthy.

2002

Prediction of risk of E. amylovora infection by various models

The cultivars listed in Tables 4 to 6 are the most representative as regards the bloom phenology and the blooming time in the given orchard.

Orchard 1 (Table 4): 2 of the 4 acute infection risks (I) prognosed by the MTL during the blooming time were confirmed by Maryblyt. As for the other 2 days (April 19 and April 29), "merely" high infection risks were predicted by Maryblyt due to the lack of an epiphytic infection potential

Table 2 Probable fire blight infection days (Újfehértó, 1999, 2000, 2001)

Date	1999					2000					2001				
	Bloom time *	MTL	Cougar-blight	BIS	Mary-blyt	Bloom time *	MTL	Cougar-blight	BIS	Mary-blyt	Bloom time *	MTL	Cougar-blight	BIS	Mary-blyt
April 15	PB										PB				
16	PB	H									PB				
17	B1		I		M						PB				
18	B1		I		M	PB	I	I			PB				
19	B1		H		M	PB	H	I			PB				
20	BB				M	B1	H			M	PB				
21	BB					B1	H			H	PB				
22	BB				M	BB	H			H	PB				
23	BB	M	M		H	BB	H			H	B1				M
24	BB		M		M	BB	H			H	B1				M
25	BB		H		M	BB	H	I	Bw	I	B1				M
26	BB		H		M	BB	I	I	Bw	H	B1				M
27	BB		H		H	BB	I	H	Bw	H	BB				M
28	BB	M	I	Bw	I	BB	I	H		H	BB				M
29	BB	I	I	Bw	I	BB	H	H	Bd	H	BB	I			M
30	BB	H	I	Bd	H	B2	I	I	Bw	I	BB	H			H
May 1	BB	I	H	Bd	H	B2	I	I	Bw	I	B2	H		Bw	I
2	PF	I	I	Bw		B2	H	I		M	B2	I	I		I
3	PF	H	H			B2		H		M	B2	I	I	Bd	I
4	PF					PF		H			B2	I			I
5						PF	I	H			B2	I			H
6							I	H	Bw		B2	H			M
7											PF	I	I	Bw	
8									Bd		PF	H	I		

* dates of 'Jonathan' bloom,
Note: M = medium -, H = high fire blight risk, I = infection predicted,
Bw = Bwet = blossom infection risk of wetted flowers, Bd = Bdry = blossom infection risk without wetting

Phenology: PB = pink bud
BB = full bloom
PF = petal fall

(H in BHWTR). The high infection risk (H of MTL) on April 21 and April 22 was predicted as a real infection risk by the BIS model (Bd), and a similar situation was observed at about petal fall (May 2 to 4). As for the Cougarblight prediction, on April 20 the DH total was 239, i.e. close to the threshold value of 270. The infection risk forecast by Cougarblight for April 21 was confirmed by BIS (Bd).

Orchard 2 (Table 4): the single day of a probable infection risk was April 20, because of the average temperature of 15.4 °C and a period with leaf wetness of 600 min that day, followed by an average temperature of 15.9 °C on April 21. It is noteworthy, however, that the infection factor of temperature (T in BHWTR) was consequently missing throughout the whole period of blooming.

Table 3 Fire blight incidence* in trees of apple cultivars, Újfehértó, 2000, 2001

Cultivars	May 18, 2000		June 5, 2001	
	No. of trees investigated	Percentage of blighted trees	No. of trees investigated	Percentage of blighted trees
Freedom	113	0.9	70	1.4
Jonica	113	10.6	70	5.7
Jonagold deCosta	113	15.0	70	7.1
King Jonagold	113	0.9	70	1.4
Pinova	226	9.3	210	7.1
Sampion	226	21.2	140	5.0

High density planting established in 1998 (autumn)
*blossom and/or shoot blight

Orchard 3 (Table 5): an acute infection risk (I) was predicted on the same day (April 26) by MTL, Maryblyt and BIS as Bw. Another day of risk indicated by BIS (Bw) was April 28, with an average temperature of 15.1 °C plus rainfall of 4.3 mm, and with a high (145) epiphytic infection potential (H in BHWTR). As concerns the prediction of Maryblyt, there was a risk period involving the days April 25, 28, 29 and 30: when the only factor missing for infection is an average temperature of 15.6 °C on the day of wetting, "we still consider it a very high risk of infection and treatment is recommended" (*Breth and Aldwinckle,* 2002). The high risk (H of Cougarblight) on April 25, 26, 27 and 28 was based on the DH total values ranging from 234 to 263 on those days. Finally, an especially dangerous situation evolved by the end of the blooming time, starting with May 3; there was a continuously high risk by MTL, and an acute infection risk by BIS (Bd).

Orchard 4 (Table 5): during the blooming time, there were 1 and 2 days of acute infection risk (I) by MTL and BIS (Bw), respectively. The prognosis of Maryblyt for the same days was a high infection risk only, because of the lack of a proper epiphytic infection potential (H in BHWTR). No infection risk was predicted by the Cougarblight.

Orchard 5 (Table 6) is a fire blight-prone apple orchard, due to the closeness of a pear gene bank. A high (H) infection risk was forecast by 3 of the 4 models on April 21. There was an acute infection risk (I, Bw) on April 28, forecast by both

Table 4 Predicting *Erwinia amylovora* infection risk by using various methods

	'Jonathan', in Orchard 1 (2002)					'Mutsu', in Orchard 2 (2002)					
Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR	Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR
April 15	B1				+ – + – M	April 15	PB				
April 16	B1				+ – + – M	April 16	B1				+ – – – L
April 17	B1				+ – – – L	April 17	B1				+ – + – M
April 18	BB	H			+ – – + M	April 18	BB				+ – + – M
April 19	BB	I	H	Bw	+ – + + H	April 19	BB	M	M		+ – + – M
April 20	BB	I	H	Bd	+ + + + I	April 20	BB	I	H		+ – + – M
April 21	BB	H	I	Bd	+ + – + H	April 21	BB	M	H		+ – – – L
April 22	BB	H		Bd	+ + – + H	April 22	BB	M			+ – – – L
April 23	BB	I		Bd	+ + + + I	April 23	BB				+ – – – L
April 24	BB				+ + – – M	April 24	BB				+ – – – L
April 25	BB				+ + – – M	April 25	BB		L		+ – + – M
April 26	BB				+ – – – L	April 26	BB				+ – + – M
April 27	BB		L		+ – + – M	April 27	PF				
April 28	BB				+ – – – L	April 28	PF		L		
April 29	BB	I			+ – + + H	April 29	PF				
April 30	BB	H			+ – – + M	April 30	PF		L		
May 1	PF	M				May 1					
May 2	PF	H		Bd		May 2					
May 3	PF	H		Bd		May 3					
May 4		I		Bd		May 4					
May 5		M				May 5					

Erwinia amylovora colonization of flowers: see Tab. 7

Note: PB= pink bud, B1= first flowers open, BB= full bloom, B2= secondary bloom,
PF=petal fall L, M and H= risk for infection is low, moderate and high, respectively
I = acute infection risk, Bw= blossom infection risk of wetted flowers, Bd= blossom infection risk without wetting events

Table 5 Predicting *Erwinia amylovora* infection risk by using various methods

	'Pinova', in Orchard 3 (2002)					'Jonathan', in Orchard 4 (2002)					
Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR	Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR
April 15	PB					April 15	PB				
April 16	PB					April 16	PB				
April 17	PB					April 17	B1				+ – – – L
April 18	PB				+ – – – L	April 18	B1				+ – – – L
April 19	PB				+ – – – L	April 19	B1				+ – – – L
April 20	B1		M		+ – + – M	April 20	B1	I			+ – + + H
April 21	B1	M	H		+ – + – M	April 21	BB	H			+ – – + M
April 22	B1	M	H		+ + – –M	April 22	BB				+ – – – L
April 23	BB				+ + – – M	April 23	BB	M			+ – – – L
April 24	BB				+ + – – M	April 24	BB	I		Bw	+ – + – H
April 25	BB		H		+ + + – H	April 25	BB	M	L		+ – + – M
April 26	BB	I	H	Bw	+ + + + I	April 26	BB				+ – + – M
April 27	BB		H		+ + – – M	April 27	BB		L		+ – + – M
April 28	BB	M	H	Bw	+ + + – H	April 28	PF		L		
April 29	BB				+ + + – H	April 29	PF				
April 30	BB				+ + + – H	April 30	PF		L		
May 1	BB	H				May 1	PF				
May 2	BB					May 2		H		Bd	
May 3	BB	H		Bd		May 3		H		Bd	
May 4	BB	H		Bd		May 4		H		Bd	
May 5	BB	H		Bd		May 5		H		Bd	

Erwinia amylovora colonization of flowers: see Tab. 7

Note: PB= pink bud, B1= first flowers open, BB= full bloom, B2= secondary bloom,
PF=petal fall L, M and H= risk for infection is low, moderate and high, respectively
I = acute infection risk, Bw= blossom infection risk of wetted flowers, Bd= blossom infection risk without wetting events

Table 6 Predicting *Erwinia amylovora* infection risk by using various methods

	'Jonathan', in Orchard 5 (2002)					'Conference', in Orchard 6 (2002)					
Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR	Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR
April 15	B1				+−+−M	April 15	B1				+−+−M
April 16	B1				+−+−M	April 16	B1				+−−−L
April 17	B1				+−−−L	April 17	B1				+−−−L
April 18	B1		L		+−+−M	April 18	B1				+−−−L
April 19	B1	M	L		+−+−M	April 19	B1	H			+−−+M
April 20	B1	M	M		+−+−M	April 20	B1				+−−−L
April 21	BB	H	H		+−++H	April 21	B1	H			+−++H
April 22	BB				+−−−L	April 22	BB	H			+−−+M
April 23	BB				+−−−L	April 23	BB				+++−H
April 24	B2		M		+−+−M	April 24	BB				+−−−L
April 25	B2		L		+−+−M	April 25	BB				+−−−L
April 26	B2		L		+−+−M	April 26	BB				+−−−L
April 27	B2		L		+−+−M	April 27	BB		L		+−−−L
April 28	B2	I	M	Bw	+−++H	April 28	PF		L		
April 29	B2				+−+−M	April 29	PF				
April 30	B2				+−+−M	April 30	PF		L		
May 1	B2				+−+−M	May 1					
May 2	B2	H		Bd	+−++H	May 2		H		Bd	
May 3	B2	H		Bd	+−−+M	May 3		H		Bd	
May 4	B2	H		Bd	+−−+M	May 4					
May 5	B2	H		Bd	++−+H	May 5					

Erwinia amylovora colonization of flowers: see Tab. 7

Note: PB= pink bud, B1= first flowers open, BB= full bloom, B2= secondary bloom,
PF=petal fall L, M and H= risk for infection is low, moderate and high, respectively
I = acute infection risk, Bw= blossom infection risk of wetted flowers, Bd= blossom infection risk without wetting events

MTL and BIS, coinciding with a high infection risk (H) predicted by Maryblyt. During the last 4 days of the blooming time (May 2 to 5), the high infection risk (H) forecast by MTL was predicted as an acute infection risk by BIS (Bd).

Orchard 6 (Table 6): within the period of blooming, there was a certain risk of infection only on April 21 (H both by MTL and Maryblyt). A weather-related infection risk occurred again on May 2 and 3 (H by MTL and Bd by BIS), but it was at or after the petal fall, i.e. too late to be manifested as an actual infection risk as regards blossom blight.

Epiphytic population of E. amylovora

The size of the epiphytic population was estimated in apparently symptomless, open flowers by PCR. With the assistance of the growers, it was possible to provide them with correct information about the presence (if any) and the size of the bacterial population in their orchards within 48 h of sample collection.

The relationship between the age of the flowers and the chance of development of a high epiphytic population on the stigmas is well known (*Thomson et al.,* 1999; *Gouk and Thomson,* 1999). Hence, during the sampling of the flowers, their age must be known. Fortunately, the opening (anthesis) of the king flower within the flower cluster is the most easily observable stage of the bloom phenology. Accordingly, our partners in this study were asked to record the opening of the king flowers (it was the 1st day of blooming) which they had to sample on the 4th day of blooming. During this time interval of 4 days, bacteria (if any were present in the orchard) had a chance to grow and develop a population high enough for estimation by PCR.

The acute infection risks denoted by I, Bw or Bd indicate that there were appropriate weather conditions for bacterial growth. Therefore, the estimated size of the epiphytic population *(Table 7)* should be fair, because samples of flowers for PCR detection were collected some days later than the first prediction of infection risk by MTL, coinciding with H, or I by Maryblyt *(Tables 4, 5 and 6).* Conversely, the proportion of infested flowers and/or the size of the epiphytic population in Orchard 3 *(Table 5)* were certainly higher in the period of the actual infection risk (April 26 and 28) than at the sampling of the flowers (April 22). The time interval between flower sampling and the prediction of acute risk days was also similar in Orchard 5 (see *Table 6*).

A detectable epiphytic population was measured in 1 sample of 1 of the cultivars only in Orchards 1, 2 and 6, and it was below the detection limit in Orchard 4 *(Table 7).* Furthermore, the size of the epiphytic population in the positive samples was rather small, 10^2–10^3 GE/flower. These observations are in accordance with the lack of an epiphytic infection potential (H in BHWTR), which was recorded relatively frequently that year (see above). The EIP is based on the assumption that abundant inoculum is

Table 7 Estimation of the size of the *Erwinia amylovora* epiphytic population by PCR in flowers of apple and pear cultivars (2002)

Orchard, Date of sampling Cultivars	Presence of *Erwinia amylovora*	
	GE/flower	not detectable
Apples		
Orchard 1, April 24		
Red Rome van Well	100–1000	4
Lysgolden	–	5
Ozark Gold	–	5
Idared	100–1000	4
Jonathan	–	5
Orchard 2, April 23		
Granny Smith	–	5
Mutsu	100 – 1000	4
Golden Reinders	–	5
Gala Must	–	5
Orchard 3, April 22		
Idared	100–1000	4
Pinova	100–1000	4
Gala	100–1000	4
Golden Reinders	1000–10000	3
Orchard 4, April 24		
Idared	–	5
Jonathan	–	5
Red Elstar	–	5
Red Rome van Well	–	5
Orchard 5, April 25		
Sampion	100–1000	
Sampion	100–1000	
Sampion	100–1000	
Sampion	100–1000	16
Freedom	100–1000	
Freedom	1000–10000	18
Pears		
Orchard 6, April 20		
Conference	–	5
Pap körte	–	5
Beurré d'Hardenpont	100–1000	4
Clapp's Favourite	–	5
Beurré Bosc	–	5

One sample consisted of 15 flowers/tree, and 5 trees/cultivar were selected for sampling, except in Orchard 5 where 20 trees/cultivar were investigated.

available in and around the orchard (*Steiner and Lightner*, 1996).

The proportion of colonized flowers, however, was considerable in 2 other places. One of them was Orchard 3, where an epiphytic population was measured in at least 1 of the flower samples of all the cultivars tested *(Table 7)*, and it corresponded with the severe incidence of fire blight observed later. The second case was Orchard 5, providing flower samples of both cultivars colonized by the pathogen. More flower samples proved to be infested in trees of cv. Sampion (known to be susceptible to fire blight) than in trees of cv. Freedom, a rather tolerant one against the disease. This (not too large) difference between these cultivars was also found a year earlier *(Table 8)*.

Incidence of fire blight

The level of infection should be recorded exactly by counting the strikes in the canopies. We did this at our research station (Orchard 5) by checking the incidence of blossom and shoot blight in several hundreds of trees, repeated 4 times during the season *(Table 9)*. This detailed work, however, can not be expected from the farmers in the commercial orchards. We assume that the relationship between the data concerning the weather-related risk *(Table 6)*, the epiphytic population *(Table 7)* and the incidence of fire blight symptoms *(Table 9)* in Orchard 5 could be similar in the other orchards involved in this study.

In that year, the occurrence of blossom and shoot blight was below the threshold of economic importance in Orchards 1, 2, 4 and 6; this is consistent with the occurrence of *E. amylovora*-colonized flower samples *(Table 7)*. The

Table 8 Distribution of the *Erwinia amylovora* population size in 10 flower samples/cultivar

Cultivars	Size of the epiphytic population			
	negative	10^2–10^3	10^3–10^4	10^4–10^5
Sampion	3	2	2	3
Freedom	5	1	1	3

Samples were collected in Orchard 5 (2001) and processed by the mail extraction protocol.

Table 9 Quotient of infection* by *Erwinia amylovora* in trees of apple cultivars**
Orchard 5 (Újfehértó), 2002

Cultivars	Number of trees investigated	Blossom blight May 23	Shoot blight			
			May 23	June 6	June 20	July 30
Sampion	280	67.8	37.1	82.1	30.3	5.3
Freedom	70	1.4	0	0	2.8	2.8
Jonica	70	22.8	1.4	2.8	5.7	0
Jonagold Decosta	70	0	0	1.4	2.8	1.4
King Jonagold	70	2.8	1.4	2.8	4.3	0
Elstar	140	0	1.4	0	10.7	0
Gala Must	129	0	0	10.0	10.0	0
Pinova	630	3.5	2.8	8.7	23.3	7.4

* (total number of infected inflorescens or shoots/number of trees investigated) × 100
** trees in their 4th leaves, planted on rootstock M.9, at a spacing of 3.5 by 1.4 m
Fire blight management: streptomycin as Erwin 25 WP 0.5 kg/ha, with a spray volume of 600 L/ha, on April 29 and May 3

fire blight story, however, proved to be more serious in Orchard 3, which is a large orchard, where the incidence of the disease in several plots (other than the one used for sampling) was epidemic. For the plot of the cultivars listed in *Table 7*, the owners had to cut out infected limbs twice, first on May 24 and again 3 weeks later. As concerns the cultivars grown in this plot, the removal of limbs with visible symptoms of fire blight was necessary mainly from trees of Gala and Pinova. Surprisingly, there was less infection in the case of cv. Idared, and no infection among trees of cv. Golden Reinders.

2003

Prediction of E. amylovora *infection risk by various models*

In advance, it is worth mentioning the particularities of the weather conditions during the critical periods of this season. The spring of 2003 was cold and warmed up slowly. For example, the average daily mean temperature for the last 10 days antedating the opening of the first flowers was to 11.6 °C (Orchard 4), 12.4 °C (Orchard 5), 11.4 °C (Orchard 6) and 13.0 °C (Orchard 1).

There was a sudden rise in temperature from the last few days of April, however, and this lasted well during May. This change resulted in an unusually high occurrence of daily maximum temperatures >27 °C and daily average temperatures >20 °C, known as threshold values for the prediction of an *E. amylovora* infection risk without wetting events (Bd by BIS). Furthermore, these rather hot days were followed by cool nights, leading to an increased risk of long-lasting dew formation. Because of the high temperatures, the blooming period was shorter than in other years (except Orchard 8, *Table 13*).

As concerns the start of the first massive infection risk, there was a perfectly consistent prediction in 9 of the 13 orchards. A coincidental forecast was provided by 3 of the 4 models in the other 4 orchards as follows:

Orchard 1 (April 30, *Table 10*): the infection risk by Maryblyt was only high (H), although, the epiphytic infection potential that day was high enough (133).

Orchard 4 (*Table 11*): Maryblyt and BIS predicted an infection risk (I and Bd, respectively) 2 days before it was forecast by MTL, Cougarblight and BIS (as Bw), on May 2.

Orchard 7 (*Table 13*): according to the Maryblyt model, there were 2 days of infection risk (I), on April 30 and May 2, 1 day before and 1 day after the date predicted by the other models (May 1).

Orchard 11 (May 2, *Table 15*): Maryblyt, MTL and Cougarblight predicted an acute infection risk, but no warning was given by the BIS model, though an infection risk was predicted 1 day before (Bd).

Overall, the first day on which there was an especially high infection risk was well estimated in 10 of the 13 orchards. Nevertheless, it was not easy to provide a usable prediction in Orchards 9, 10 and 13.

Epiphytic population of E. amylovora

A rather large (10^3–10^4 or higher) epiphytic population was found in 2 of the 5 flower samples/cultivar (except cv. Red Rome van Well in Orchard 1; *Table 17*). The epiphytic population of *E. amylovora* was below the detection limit in Orchards 3, 4, 5 and 6. Further, there was no, or hardly any bacterial colonization 1 year earlier in Orchard 4 and Orchard 6, respectively *(Table 7)*. In spite of the high occurrence of PCR-positive flower samples from Orchards 3 and 5 in 2002, no population of bacteria was found in 2003. year. The absence of *E. amylovora* population in 4 of the 5 orchards investigated may be attributed to the cool period before blooming (see above). The *E. amylovora* population was not investigated in the flowers of Orchard 2 and Orchards 7–13 involved first in our study that year. Nevertheless, on the basis of the incidence of fire blight *(Table 20)*, a detectable bacterial population should have been present in Orchards 2, 7 and 8.

Table 10 Predicting *Erwinia amylovora* infection risk by using various methods

	'Jonathan', in Orchard 1 (2003)					'Jonathan', in Orchard 2 (2003)					
Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR	Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR
April 25	PB					April 29	B1	I			+–++H
April 26	PB					April 30	B1	H		Bd	++–+H
April 27	B1	I			+–++H	May 01	BB	H	H	Bd	++–+H
April 28	BB	I			+–++H	May 02	BB	H	H	Bd	++–+H
April 29	BB	I	H		+–++H	May 03	BB	I	I	Bw	++++I
April 30	BB	I	I	Bw	++–+H	May 04	BB	M	I	Bw	+++–H
May 01	BB	H	H	Bd	++–+H	May 05	BB	I	I	Bw	++++I
May 02	BB	H	H	Bd	++–+H	May 06	B2	H	H	Bd	++–+H
May 03	BB	H	H	Bd	++–+H	May 07	B2	H	H	Bd	++–+H
May 04	B2	I	H	Bd	++–+H	May 08	PF	H	H	Bd	
May 05	B2	I	H	Bd	++–+H	May 09	PF	H	H	Bd	
May 06	PF	H	H	Bd							
May 07	PF	H	H	Bd							

Note: PB= pink bud, B1= first flowers open, BB= full bloom, B2= secondary bloom, PF= petal fall,
L, M and H= risk for infection is low, moderate and high, respectively
I= acute infection risk, Bw= blossom infection risk of wetted flowers, Bd= blossom infection risk without wetting eventes

Table 11 Predicting *Erwinia amylovora* infection risk by using various methods

	'Pinova', in Orchard 3 (2003)					'Jonathan', in Orchard 4 (2003)					
Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR	Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR
April 30	PB	H				April 26	PB				
May 01	PB	H				April 27	PB				
May 02	B1	H	H		++-+H	April 28	B1				+-+-M
May 03	B1	H	H	Bd	++-+H	April 29	B1	H	M		+--+M
May 04	BB	I	I	Bw	++++I	April 30	BB	H	H	Bd	++++I
May 05	BB	I	I	Bw	+-++H	May 01	BB	H	H		+-++H
May 06	BB	I	I	Bw	++++I	May 02	BB	I	I	Bw	+-++H
May 07	BB	H	M	Bd	++-+H	May 03	BB	H	H		+--+M
May 08	BB	I	I	Bw	++++I	May 04	BB		H		++--M
May 09	B2	I	I	Bw	+-++H	May 05	B2	H	H		++-+H
May 10	PF	H	H	Bd		May 06	B2	H	H	Bd	++-+H
May 11	PF	H	I	Bw		May 07	PF	H	H	Bd	
						May 08	PF	H	H	Bd	

Note: PB= pink bud, B1= first flowers open, BB= full bloom, B2= secondary bloom, PF= petal fall,
L, M and H= risk for infection is low, moderate and high, respectively
I= acute infection risk, Bw= blossom infection risk of wetted flowers, Bd= blossom infection risk without wetting eventes

Table 12 Predicting *Erwinia amylovora* infection risk by using various methods

	'Jonathan', in Orchard 5 (2003)					'Conference', in Orchard 6 (2003)					
Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR	Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR
April 27	PB					April 25	PB				
April 28	PB					April 26	PB				
April 29	B1	H			+--+M	April 27	B1				+-+-M
April 30	B1	H	H		+--+M	April 28	BB				+-+-M
May 01	BB	H	H		++-+H	April 29	BB	H			+--+M
May 02	BB	I	I	Bw	++++I	April 30	BB	H	H	Bd	++-+H
May 03	BB	H	H		++-+H	May 01	BB	H	H		++-+H
May 04	BB		H		+++-H	May 02	BB	M	I	Bw	+++-H
May 05	BB	H	H		++-+H	May 03	B2	I	I	Bw	++++I
May 06	B2	H	H	Bd	++-+H	May 04	PF		H		
May 07	PF	H	H	Bd		May 05	PF	H	H	Bd	
May 08	PF	H	H	Bd							

Note: PB= pink bud, B1= first flowers open, BB= full bloom, B2= secondary bloom, PF= petal fall,
L, M and H= risk for infection is low, moderate and high, respectively
I= acute infection risk, Bw= blossom infection risk of wetted flowers, Bd= blossom infection risk without wetting eventes

Incidence of fire blight

The well-detectable presence of *E. amylovora* in Orchard 1 was followed by an over-all blossom and shoot blight, especially in trees of cv. Jonathan *(Table 18)*. There is no doubt that the spraying of streptomycin in this orchard on May 4 and May 8 was a belated effort (see the predicted infection risk on April 30; *Table 10*). Moreover, it is difficult to form an opinion of the efficiency of the 4 sprayings with copper compounds carried out during the season in this orchard. In accordance with the result of the PCR detection, there was no blossom blight in Orchard 3. Taking into consideration the high incidence of fire blight in this orchard in 2002, the moderate occurrence of shoot blight *(Table 18)* may be caused by systemic infection. The same mode of infection may have resulted in blossom blight in trees of 2 cultivars in Orchard 6 *(Table 18)*. Streptomycin was applied 2 days (Orchard 3) or 1 day (Orchard 6) before the acute infection risk was forecast (*Tables 11* and *12*, respectively).

An epiphytic population was detected in flower samples from Orchard 5 in all years except 2003 during this study. Nevertheless, there was a moderate incidence of blossom and shoot blight, but a rather serious incidence among the trees of cv. Sampion *(Table 19)*. The first spraying of streptomycin took place on May 2, indicated as a highly risky day by all 4 prediction models *(Table 12)*; the second treatment was carried out on May 6. The differences between cultivars as concerns their sensitivity to *E. amylovora* are reflected clearly, though a high incidence of shoot blight in trees of cv. Freedom was never found in other years *(Table 19)*.

The rather epidemic incidence of fire blight in Orchard 7 *(Table 20)* is not surprising at all. There was a continuously high weather-related infection risk during the bloom period *(Table 13)*, followed by a heavy hailstorm on May 11. It

Table 13 Predicting *Erwinia amylovora* infection risk by using various methods

	'Jonathan', in Orchard 7 (2003)					'Jonathan', in Orchard 8 (2003)					
Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR	Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR
April 26	PB					April 27	B1				+-+-M
April 27	PB					April 28	B1	M			+-+-M
April 28	B1				+-+-M	April 29	BB	I	H		+-++H
April 29	BB	I	H		+-++H	April 30	BB	I	I	Bw	++++I
April 30	BB	I	H	Bw	++++I	May 01	BB	I	I	Bw	+-++H
May 01	BB	I	I	Bw	+-++H	May 02	BB	I	I	Bw	+-++H
May 02	BB	I	I	Bw	++++I	May 03	BB	I	I	Bw	++++I
May 03	B2	I	I	Bw	+-++H	May 04	BB	I	I	Bw	+-++H
May 04	B2	I	I	Bw	+-++H	May 05	BB	I	I	Bw	+-++H
May 05	PF	I	I	Bw		May 06	BB	I	I	Bw	++++I
May 06	PF	I	H	Bw		May 07	BB	I	I	Bw	++++I
						May 08	B2	I	I	Bw	++++I
						May 09	B2	I	I	Bw	++++I
						May 10	PF	I	I	Bw	
						May 11	PF	I	I	Bw	

Note: PB= pink bud, B1= first flowers open, BB= full bloom, B2= secondary bloom, PF= petal fall,
L, M and H= risk for infection is low, moderate and high, respectively
I= acute infection risk, Bw= blossom infection risk of wetted flowers, Bd= blossom infection risk without wetting eventes

seems that the repeated sprayings of Kasumin 2L provided hardly any protection against fire blight. The incidence of blossom blight was higher than that of shoot blight in Orchard 2 *(Table 20)*, in spite of the correct timing of streptomycin treatment on May 3. The opposite was true in Orchard 8 where there was an all-round occurrence of shoot blight *(Table 20)*, although Kasumin 2L was applied on May 16 and June 2. There was a high weather-related infection risk in Orchards 9–13, too *(Tables 14–16)*, but no incidence of fire blight was observed, presumable because of the absence of the pathogen. This assumption must have been especially true in Orchards 9 and 10, where no antibiotics were used.

Conclusions

The aim of this study was to answer the question of whether a reliable local forecast of a weather-related *Erwinia amylovora* infection risk could be made by easy-to-use prediction methods and by sampling flowers to estimate the presence and size of the pathogen population. During this work three simple methods (MTL, Cougarblight and BIS) were used, utilizing meteorological data recorded in a number of commercial orchards. Parallel forecasts were generated by the Maryblyt program for each participating orchard, the same data being used for the 4 methods, and the results were compared. We chose Maryblyt for reference

Table 14 Predicting *Erwinia amylovora* infection risk by using various methods

	'Idared', in Orchard 9 (2003)					'Idared', in Orchard 10 (2003)					
Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR	Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR
April 27	B1	H			+—+M	April 25	PB		M		
April 28	BB	H			+—+M	April 26	B1	H	M		+—++H
April 29	BB	H			++—+H	April 27	B1	I	M	Bd	++—+H
April 30	BB	H	H	Bd	++—+H	April 28	BB	H	H	Bd	++—+H
May 01	BB	H	H	Bd	++++I	April 29	BB	I	M	Bd	++—+H
May 02	BB	I	H	Bd	+—++H	April 30	BB	H	H	Bd	++—+H
May 03	B2	H	H	Bd	+—+M	May 01	BB	I	H	Bd	++—+H
May 04	B2	I	I	Bw	++++I	May 02	BB	I	I	Bw	++++I
May 05	PF	H	H	Bd		May 03	BB	H	M	Bd	+—++H
May 06	PF	H	H	Bd		May 04	B2	H	H	Bd	+—+M
						May 05	B2	H	H	Bd	++—+H
						May 06	PF	H	H	Bd	
						May 07	PF	H	H	Bd	

Note: PB= pink bud, B1= first flowers open, BB= full bloom, B2= secondary bloom, PF= petal fall,
L, M and H= risk for infection is low, moderate and high, respectively
I= acute infection risk, Bw= blossom infection risk of wetted flowers, Bd= blossom infection risk without wetting eventes

Table 15 Predicting *Erwinia amylovora* infection risk by using various methods

	'Idared', in Orchard 11 (2003)					'Idared', in Orchard 12 (2003)					
Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR	Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR
April 28	B1	I			+–++H	April 29	B1	H			+–+M
April 29	B1	H	M		+–+M	April 30	BB	H	H	Bd	++–+H
April 30	BB	H	M	Bd	++–+H	May 01	BB	I	I	Bw	++++I
May 01	BB	H	M	Bd	++–+H	May 02	BB	I	I	Bw	+–++H
May 02	BB	I	I		++++I	May 03	BB	I	I	Bw	+–++H
May 03	BB		L		++–M	May 04	BB	I	I	Bw	++++I
May 04	B2		L		++–M	May 05	B2	I	I	Bw	+–++H
May 05	B2	H	L		++–+H	May 06	PF	H	H	Bd	
May 06	PF	H	M	Bd		May 07	PF	I	I	Bw	
May 07	PF	H	H	Bd							

Note: PB= pink bud, B1= first flowers open, BB= full bloom, B2= secondary bloom, PF= petal fall,
L, M and H= risk for infection is low, moderate and high, respectively
I= acute infection risk, Bw= blossom infection risk of wetted flowers, Bd= blossom infection risk without wetting eventes

since this program is used in Hungary by the Plant and Soil Protection authority that issued permission and called for the use of streptomycin during the years of our study.

For all years and orchards tested, Maryblyt indicated 35 days on which there was an acute infection risk. The same days were indicated by all 3 methods in 23 cases, by 2 methods in 8 cases and by 1 method only in 4 cases. As regards those days that were "missed" by 1 or 2 of the 3 easy-to-use methods, an acute infection risk warning was given 1 day earlier in 3 cases, or 1 day later in 8 cases. There were only 5 events when one of the tested methods did not give a warning within ±1 day of the date indicated by Maryblyt; these were due to Cougarblight (4 cases) and MTL (1 case).

Besides the good correlation between the forecasts of the 4 methods compared, we observed a general tendency of the easy-to-use methods to give an acute infection risk warning more often than Maryblyt. For all the years and orchards involved in this study, BIS forecast the most days (65) in addition to those indicated by Maryblyt up to petal fall. However, on 38 of these days the infection risk warning was given as Bd, that is without wetting events, mostly (28 cases)

Table 16 Predicting *Erwinia amylovora* infection risk by using various methods

'Idared', in Orchard 13 (2003)					
Date	Stages of flowering	MTL	Cougar-blight	BIS	Maryblyt BHWTR
April 28	B1				+–+–M
April 29	B1				+–+–M
April 30	BB				+–+–M
May 01	BB	M		Bw	+++–H
May 02	BB	M	I		+++–H
May 03	BB	I	I	Bw	++++I
May 04	B2	I	I	Bw	+–++H
May 05	B2	I	I	Bw	+–++H
May 06	PF	I	I	Bw	
May 07	PF	I	I	Bw	

Note: PB= pink bud, B1= first flowers open, BB= full bloom, B2= secondary bloom, PF= petal fall,
L, M and H= risk for infection is low, moderate and high, respectively
I= acute infection risk
Bw= blossom infection risk of wetted flowers
Bd= blossom infection risk without wetting evente

Table 17 Size of the epiphytic population in flowers of apple cultivars

Cultivars	Serial number of flower samples				
	1	2	3	4	5
Charden	–	–	10^3–10^4	10^5–10^6	–
Jonathan	10^3–10^4	–	–	–	10^5–10^6
Idared	10^4–105	10^3–10^4	–	–	–
Red Rome van Well	10^2–10^3	–	–	–	–
Jonagold	–	10^4–10^5	–	10^4–10^5	–

Samples were collected in Orchard 1 (2003) and processed by the mail extraction protocol.
– : not detectable

Table 18 Severity of fire blight in apple and pear orchard 2003

Orchard	Cultivar	Disease severity*			
		in flowers		in shoots	
Orchard 1	Idared	May 17:	1	May 24:	1
				June 16:	1
				July 07:	1
	Jonathan	May 17:	3	May 24:	3
				June 16:	1
	Jonagold	May 17:	1	June 16:	1
				July 07:	1
	Red Rome van Well	May 17:	1	June 16:	1
				July 07:	1
Orchard 3	Gala	May 03:	0	June 15:	1
	Pinova	May 03:	0	June 15:	1
	Idared	May 03:	0	June 15:	0–1
	Golden Reinders	May 03:	0	June 15:	0–1
Orchard 6**	Beurré Bosc	May 30:	0	August 07:	0
	Conference	May 30:	0	August 07:	0
	Seress Olivér	May 30:	3	August 07:	0
	Clapp's Favourite	May 30:	3	August 07:	3

* estimated by using rating scales, see Material an methods in 2003
** orchard of pears

Table 19 Quotient of infection* by *Erwinia amylovora* in trees of apple cultivars**
Orchard 5 (Újfehértó), 2003

Cultivars	Number of trees investigated	Blossom blight May 28	Shoot blight		
			May 28	June 13	July 16
Sampion	385	17.8	8.0	31.4	6.0
Freedom	69	0.0	0.0	4.3	13.0
Jonica	68	0.0	0.0	4.4	5.8
Jonagold Decosta	70	2.8	0.0	4.3	15.7
King Jonagold	68	1.5	0.0	1.5	8.8
Elstar	140	9.3	2.1	4.2	13.6
Gala Must	39	2.6	0.0	0.0	2.6
Pinova	257	2.3	1.5	10.1	5.8

* (total number of infected inflorescens or shoots/number of trees investigated) × 100
** trees in their 5th leaves, planted on rootstock M.9, at a spacing of 3.5 by 1.4 m except 128 trees of Sampion and all the trees of Gala Must and Pinova on M. 26, 3.5 by 2.3 m
Fire blight management: streptomycin as Erwin 25 WP 0.5 kg/ha, with a spray volume of 800 L/ha, on May 2 and May 6

due to the unusually warm weather in 2003. On the remaining 27 days, the risk forecasts by BIS were based on wetting events (Bw), a number very similar to that provided by Cougarblight (26). The MTL method forecast an acute infection risk on 42 days more than Maryblyt, a number obviously higher than those of Cougarblight or BIS (Bw). It therefore seems to be more sensitive than the other models used in this study (see the occurrence of infection risks denoted by I in the Tables). This experience, however, should be approached from another aspect of investigation. Prediction by the BIS, Cougarblight and Maryblyt models is based (besides wetting events) on the accumulation of a critical heat sum above a given threshold temperature. In contrast with these models, the MTL method works with the daily average temperature of each individual day and with wetting events of lower limit values than the other 3 models. There can be no doubt that the conjunction of the factors of the weather-related infection risk can be achieved more easily within one day than in a succession of several days.

Table 20 Severity* of blossom blight and shoot blight in trees of apple cultivars
2003

Cultivars	Date	Serial number of trees selected for observation									
		1	2	3	4	5	6	7	8	9	10
Orchard 2, Blossom blight											
Idared	May 15	1	1	1	1	0	1	0	1	1	1
Gibson Golden D.		0	0	1	0	1	1	0	0	1	1
Jonagored		1	1	0	1	0	0	1	1	1	0
Orchard 2, Shoot blight											
Idared	June 15	0	1	0	1	0	1	0	0	1	1
Gibson Golden D.		0	0	0	0	1	1	0	0	0	1
Jonagored		1	1	0	0	1	0	0	1	0	0
Idared	July 15	0	0	0	1	0	0	0	0	1	0
Gibson Golden D.		0	0	0	0	0	0	0	0	0	1
Jonagored		1	0	0	0	0	0	0	1	0	0
Idared	August 15	1	1	0	0	1	0	0	0	1	1
Gibson Golden D.		0	0	0	0	0	1	0	0	1	0
Jonagored		1	0	1	0	0	1	1	0	0	1
Orchard 7, Blossom blight											
Jonathan	May 15	3	3	3	3	1	0	0	0	1	3
Orchard 7, Shoot blight											
Jonathan	May 25	5	5	5	5	3	0	0	0	3	5
	June 1	3	3	5	5	3	0	1	0	1	5
Orchard 8, Blossom blight											
Jonathan	May 10	0	0	0	0	0	0	0	0	0	0
Elstar		0	0	0	1	0	0	0	0	0	0
Idared		0	0	0	0	0	0	0	1	0	0
Orchard 8, Shoot blight											
Jonathan	June 21	1	1	1	1	1	1	1	1	1	1
Elstar		1	1	1	1	1	1	1	1	1	1
Idared		1	1	1	1	1	1	1	1	1	1

* estimated by using rating scales, see Material and Methods in 2003

Another of our observations in 2002 also emphasises the necessity of the local prediction. The infection risk in a given orchard was predicted by the MTL earlier than it was forecast by the same model running on the data recorded by a weather station situated to the north of the orchard. It was also true the other way around: the infection day predicted by MTL in the same orchard was later than the one forecasted on the basis of the environmental data from the regional weather station located to the south of the orchard (data not shown).

The prognoses indicated as I, Bw or Bd during or after petal fall at all locations had no importance, of course, as concerns the risk of blossom blight. Nevertheless, they call attention to those weather conditions which hasten the development of the symptoms, the doubling of the bacteria and the production of bacterial ooze; in this way, the preconditions for infection of the succulent, growing shoot tips will be established.

Any of the risk assessment models used could provide an increased accuracy in the prediction of the actual infection risk if they are combined with an estimation of the incidence of *E. amylovora* colonization in the open flowers. The data indicate that the proportion of flower samples with a detectable epiphytic population is more informative than the size of the epiphytic population itself. As a consequence, there is a real dangerous situation in an orchard when numerous flowers are colonized by *E. amylovora*. Accordingly, we have already tested the stigma imprint technique proposed by *Thomson et al.* (2002), and plan to use it in an extended scale in the coming years.

Our experience suggests that there are no convincing differences in the size of the epiphytic population in flowers of cultivars possessing high or low susceptibility to *E. amylovora*. How the pathogenicity of these bacteria can be manifested in flowers of cultivars with various sensitivities to fire blight is another matter, of course.

It was not the aim of this study to compare the models in terms of the differences in the control of fire blight. The reliability of methods for the prediction of an infection risk can not be judged merely from the aspect of the severity of fire blight (the incidence of blighted blossoms and shoots) that ensues later. Furthermore, fire blight is too sporadic to allow good comparisons of the validity and precision of different risk assessment models (*Billing*, 2000). The weather-related infection risk will be realized only in the presence of *E. amylovora*. If a very poor or even no bacterial population exists during the critical phenological stages (e.g. flowering), there is no real chance for the development of widespread symptoms. Nevertheless, current models utilize environmental parameters, but do not take into account the inoculum potential within the orchard (*Thomson et al.*, 2002).

We conclude that the easy-to-use methods tested could be used by the farmers to recognize weather-related risks, especially when coupled with estimations of the proportion of pathogen-infested flowers. This local prediction would provide immediate information (faster than the regional forecast systems) specifically for the given orchard. The local knowledge of an infection risk becomes even more important in view of the very recent (2004) regulation of the Plant and Soil Protection authority, strictly prohibiting the usage of streptomycin in apple orchards.

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References

Benedettini G., Bugiani R., Calzorali A., Finelli F. & Govoni P. (2002): Fire blight in Emilia-Romagna (Italy): searching possible relationships between epidemic spread, climate and territory using the regional database and GIS technology. *Acta Hort.* 590: 207–214.

Berger F., Zeller W., Gutsche V. & Rossberg D. (1996): A new fire blight forecasting system with results in southwest Germany. *Acta Hort.* 411: 155–161.

Billing E. (1996): BIS95, an improved approach to fire blight risk assessment. *Acta Hort.* 411: 121–126.

Billing E. (1999): Fire blight risk assessment: Billing's Integrated System (BIS) and its evaluation. *Acta Hort.* 489: 399–405.

Billing E. (2000): Fire blight risk assessment systems and models. In: Vanneste J. L. (ed.): Fire blight. The disease and its causative agent, *Erwinia amylovora*. 293–318. CABI Publishing, Wallingford, UK

Bonn W. G. & van der Zwet T. (2000): Distribution and economic importance of fire blight. In: Vanneste J. L. (ed.): Fire blight. The disease and its causative agent, *Erwinia amylovora*. 37–53. CABI Publishing, Wallingford, UK

Breth D. I. & Aldwinckle H. S. (2002): Comparison of models for blossom blight prediction in New York. *Acta Hort.* 590: 147–151.

Bubán T., Sallai P., Varga A. & Dorgai L. (2002): Investigation of the reliability of easy-to-use methods to predict *Erwinia amylovora* infection risk in apple orchards. *Acta Hort.* 590: 119–125.

Dorgai L. & Bubán T. (2002): Rapid estimation of the epiphytic population size of *Erwinia amylovora* by PCR. *Acta Hort.* 590: 175–179.

Gouk S.C. and Thomson S.V. (1999): Influence of age of apple flowers on growth of *Erwinia amylovora*. *Acta Hort.* 489: 525–528.

Gouk S. C., Spink M. & Laurenson M. R. (1999): FireWorks – a Windows-based computer program for prediction of fire blight on apples. *Acta Hort.* 489: 407–412.

Holtz B. A., Hoffman E. W. & Teviotdale B. L. (2002): Prediction of occurrence of fire blight in San Joaquin Valley of California. *Acta Hort.* 590: 167–174.

McManus P.S. & Jones A.L. (1995): Detection of Erwinia amylovora by nested PCR and PCR-dot-blot and reverse-blot hybridizations. *Phytopathology* 85 (5): 618–623.

Németh J. (1999): Occurrence and spread of fire blight (*Erwinia amylovora*) in Hungary. Management of the disease. *Acta Hort.* 489: 177–185.

Shtienberg D., Schwartz H., Manulis S., Kritzman G., Zilberstaine M., Oppenheim D. & Herzog Z. (2002): Coping with fire blight in pears: experience gained in Israel in the fire blight management (Fire.Man) project. *Acta Hort.* 590: 253–262.

Smith T. J. (1993): A predictive model for forecasting fire blight of pear and apple in Washington State. *Acta Hort.* 338: 153–157.

Smith T. J. (2000): Fire blight daily risk estimation model: Version 2000C(b) (Celsius). Internet site: http://www.wsu.edu/fbmdl98c.htm

Steiner P. W. (1990a): Predicting apple blossom infections by *Erwinia amylovora* using the MARYBLYT model. *Acta Hort.* 273: 139–148.

Steiner P. W. (1990b): Predicting canker, shoot and trauma blight phases of apple fire blight epidemics using the MARYBLYT model. *Acta Hort.* 273: 149–158.

Steiner P. W. & Lightner G. W. (1996): MARYBLYT^{TM} 4.3 – a predictive program for forecasting fire blight disease in apples and pears. University of Maryland at College Park, Maryland.

Thomson S. V., Schrott M. N., Moller W. J. & Reil W. O. (1982): A forecasting model for fire blight of pear. *Plant Disease* 66: 576–579.

Thomson S.V., Wagner A.C. & Gouk S.C. (1999): Rapid epiphytic colonization of apple flowers and the role of insects and rain. *Acta Hort.* 489: 459–464.

Thomson S. V., Ockey S. C. & Hansen D. R. (2002): Using stigma imprints to determine fire blight risks in pear and apple orchards. Oral pres., 10th Int. Congr. Plant Pathology, Prince Edward Island, Canada, 28–31 May, 2002.

Tsiantos J. & Psallidas P. G. (1996): First evaluation of 'Firescreens' for predicting fire blight epidemics in Greece. *Acta Hort.* 411: 145–149.