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Effects of pear tree physiology on fire blight progression in perennial branches and on expression of pathogenicity genes in *Erwinia amylovora*

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Abstract

The interaction between *Erwinia amylovora* (the causal agent of fire blight) and the physiological status of pear trees was examined under orchard conditions. The physiological status of the trees was defined qualitatively, using host phenology and vigour as measures, and quantitatively, using the sorbitol content in annual shoots as a measure. Qualitatively, tree response to fire blight was governed by phenological stage at the time of infection and vigour: low vigour trees inoculated in the autumn (just before entering dormancy) and high vigour trees inoculated in the spring (soon after bloom) were more susceptible than high vigour trees inoculated in the autumn and low vigour trees inoculated in the spring. Quantitatively, the rate of symptom progression in perennial branches (*SPR*) was significantly ($P \leq 0.001$) correlated to the absolute value of the rate of sorbitol content change ($|SCR|$). The relationship between *hrp* genes expression of transformed *E. amylovora* (estimated according to *hrpE* and *hrpJ* expression) and $|SCR|$ was determined on 1 year-old trees. Expression of *hrp* genes was significantly correlated with $|SCR|$ ($P = 0.004$) and 63.5% of the variability in the *hrp* genes expression was attributed to $|SCR|$ values. The expression of *hrp* genes increased gradually and asymptotically with increasing $|SCR|$ values; further increase in $|SCR|$ did not affect the expression.

Abbreviations: SPR – the rate of symptom progression in perennial branches; $|SCR|$ – the absolute value of the rate of sorbitol content change.

Introduction

Fire blight, caused by the bacterium *Erwinia amylovora* is the most destructive disease of pears and other pome fruit trees worldwide (van der Zwet et al., 1988; van der Zwet and Beer, 1995). In Israel, the pathogen primarily infects the flowers but it may develop on all plant parts, including the fruits, leaves, and branches. After colonizing the flowers, *Erwinia amylovora* cells invade the intercellular spaces of the cortical parenchyma and reach the vascular system (van der Zwet and Keil, 1979). The pathogen may then progress to the supporting branches and advance further down to the main limbs and the trunk of the tree. Infected tissues often become necrotic and may eventually die (Vanneste and Eden-Green, 2000). The wilting associated with fire blight is due to the collapse of the parenchyma. *Erwinia amylovora* does not produce cell-wall degrading enzymes (Seemüller and Beer, 1976); its pathogenicity is dependent on a functional type III secretion system, and these systems enable the secretion of effector proteins into the extracellular medium. *In vivo*, upon contact

with eukaryotic host cells, pathogenicity-related proteins are directly injected into the eukaryotic cells, and others are secreted into the intercellular medium. Harpin is one of the proteins shown to pass through the type III secretion system in *E. amylovora* (Wei et al., 1992), and is crucial for disease development, as *E. amylovora* harpin-mutants are severely affected in their pathogenicity (Barny, 1995). *HrpE* is one of the genes on the *hrpA* operon required for secretion of harpin (Kim et al., 1997).

The extent of fire blight progression in perennial branches influences the damage inflicted upon the trees. When main limbs are infected, not only is the current-year yield impaired, but also the normal development of the tree and its future growth and production. If the pathogen invades the trunk, the entire tree may die (Vanneste and Eden-Green, 2000). The rate and extent of fire blight progression in perennial tissues may vary among trees within the same orchard, among branches on the same tree, and over time on an individual branch: in highly susceptible tissues fire blight symptoms may progress at rates exceeding 2.5 cm day^{-1}, but in resistant tissues the progression is markedly slower (Momol et al., 1999; Blachinsky et al., 2003; Nosarszewski et al., 2004). Furthermore, the pathogen has been occasionally isolated from non-symptomatic but, nevertheless infected tissues (van der Zwet, 1969). The reasons for the diverse responses of perennial branches to *E. amylovora* progression are not fully understood, but various reports have implicated factors related directly or indirectly to host growth and development, such as the age of the tissue (Rosen, 1929; Tullis, 1929; van der Zwet and Keil, 1979; van der Zwet and Beer, 1995; Momol et al., 1999), tree vigour (Blachinsky et al., 2003) and the phenological stage of the host (Crosse et al., 1972; Beer and Norelli, 1977; Blachinsky et al., 2003). It was recently reported that temperature did not affect the rate of fire blight progression under Israeli conditions (Blachinsky et al., 2003).

All the above suggest that the physiological status of pear trees is the predominating factor in determining their response to *E. amylovora* and governing the rate and severity of fire blight progression in the perennial branches. The physiological status of the trees may be defined qualitatively according to visual measures such as host phenological stage and vigour. A qualitative coincidence between tree vigour and fire blight progression in pear branches, in cases of infections occurring at different phenological stages, was recently reported (Blachinsky et al., 2003). However, quantifying this relationship is a more difficult task, as the physiological status of a tree cannot be defined in terms of discrete variables. Sugar content is often used as a robust indirect indicator of the physiological status of plants in studies dealing with plant physiology, growth and development. For trees of the Rosaceae family, sorbitol (D-glucitol) is the preferred sugar (e.g., Bieleski, 1969; Loescher, 1987; Cui et al., 2003; Veberic et al., 2003; Chen and Cheng, 2004; Nosarszewski et al., 2004); it is the principal transport and storage carbohydrate compound of numerous plant species of the Rosaceae family, including pears (Lewis and Smith, 1967; Loescher, 1987). The sorbitol content in the phloem sap fluctuates in accordance with the phenological stage of the tree and in line with its growth and activity: sorbitol content in young, actively growing tissues is markedly lower than in old, non-growing tissues ($<15\%$ as compared with 60–80% of the soluble carbohydrate, respectively); in the former sorbitol is converted to fructose by NAD^+-dependent sorbitol dehydrogenase (NAD-SDH) (Sakai, 1966; Bieleski, 1969; Li and Li, 2005). Suleman and Steiner (1994) determined the relationships between sorbitol content in apple leaves and the length of fire blight symptoms in the corresponding shoots: an increase in sorbitol content was associated with a decrease in the length of *E. amylovora* symptoms, and the authors concluded that the osmotic potential of the host cells, regulated by sorbitol, governed the susceptibility of the tissue to fire blight. Suleman and Steiner (1994) conducted their study on detached apple shoots, and, to the best of our knowledge, no such studies have been carried out on mature orchard trees, and the seasonal fluctuations in sorbitol contents have not been taken into account.

Major progress has been made over the years in understanding the interactions between *E. amylovora* and plants of the Rosaceae family at the population (epidemiology) and at the molecular (pathogenicity) levels, but there are still gaps in our knowledge of the interaction between the pathogen and its host at the organism level, specifically, about the role of host physiology in

determining *E. amylovora* pathogenicity to host tissues (Eastgate, 2000). Several lines of evidence suggest that the physiological status of the host governs the response of individual tissues and of the tree as a whole, to *E. amylovora*. Studying the role of host physiology in *E. amylovora* pathogenicity is important not only for satisfying scientific curiosity; it is crucial for the development of effective fire blight management strategies. In the present study tree vigour and phenological stages were used as qualitative indicators and sorbitol content was used as a quantitative indicator of the physiological status of the trees. We have used these indicators to determine the role of host physiology in (i) host response to fire blight, and (ii) *hrpE* and *hrpJ* expression by *E. amylovora*. Experiments were carried out with mature, orchard-grown trees and with one year-old potted trees.

Materials and methods

Experimentation in orchard-grown trees

During 2002–2004, trials were conducted in a pear orchard at the Khula Experimental Station located in the Khula Valley, northern Israel. The orchard was planted in 1992 and consisted of 500 trees. The trees in the trials were cv. Spadona, which is highly susceptible to *E. amylovora*. The trees were cultivated (i.e., irrigated, fertilized, pruned, etc.) and maintained (i.e., weed and pest control, etc.) in the same way as most commercial pear orchards in Israel. In October 2002 and October 2003, all trees in the orchard were inspected visually and classified according to vigour, as follows (Blachinsky et al., 2003). Trees bearing few or no succulent annual shoots on most terminal branches or with short (< 10 cm) and thin (< 5 mm) succulent annual shoots, were classified as having low vigour. Those that had numerous, long (> 30 cm) and thick (> 10 mm) succulent annual shoots on most terminal branches, were classified as having high vigour. In each year, 20 low vigour trees and 20 high vigour trees were selected for further experimentation; those in each group were similar in height, branching capacity and productivity potential. Trees in each group were then randomly assigned to two sub-groups: the first sub-group comprised 10 trees to be artificially inoculated with *E. amylovora*; those assigned to the second sub-group (10 trees) were not inoculated and were used for determining changes in sorbitol content over time. The sorbitol content in the inoculated trees was not recorded, as it was assumed that fire blight development would alter the normal growth of the trees. Artificial inoculations were carried out in the autumn (five trees of each group) or in the spring (the remaining five trees) as described below.

Individual branches were artificially inoculated as follows. During each season, 40 individual branches were selected; half of them were on trees with low vigour and the rest on trees with high vigour. One year-old branches (resulting from the growth of the previous season and supporting succulent annual shoots) were selected for inoculation. Four branches were inoculated on each tree, none of them being on the same main limb. Each succulent shoot was superficially cut about 1–2 cm above its growth base where it emerged from the one year-old branch, and a drop of a suspension containing about 10^8 *E. amylovora* cells ml^{-1} was placed on the wound. A mixture of *E. amylovora* strains *Ea*238 and *Ea*249 was used in all inoculations. Inoculations were performed in November and October for the corresponding 2002/2003 and 2003/2004 autumn seasons and in April and March for the corresponding 2003 and 2004 spring seasons. The former two inoculations were done when the autumn leaf fall had commenced, and fire blight subsequently progressed while the trees were dormant in the winter. The latter two inoculations were done towards the end of bloom and fire blight subsequently progressed during early shoot growth in the spring and early summer. Disease incidence (i.e., the percentage of inoculated shoots that exhibited the typical fire blight symptoms) was determined in each season, for each group of vigourous trees.

Disease severity on the branches of the inoculated trees was measured and recorded; the measurements of symptom progression were initiated soon after the appearance of visible symptoms and continued, at 1- to 2-week intervals, until the following spring experiment (autumn inoculations) or when symptom progression ceased (spring inoculations). At each assessment, the length (in cm) of the fire blight symptom in each branch was measured. As infections progressed from branch to branch measurements were taken separately on each branch, and the cumulative length of fire

blight symptoms on the various perennial branches was calculated for each infection. Symptom lengths were then averaged among the trees of each vigour group (i.e., low or high) and the standard error was calculated for each group. The rate of symptom progression (SPR_i, cm day^{-1}) between two successive sampling dates (t_i) was calculated from the records of the symptom lengths at these dates (CL_i, cm) and the time intervals between them ($\Delta t = t_i - t_{i-1}$): $SPR_i = (CL_i - CL_{i-1})/\Delta t$.

Determination of sorbitol content

Each season, non-inoculated trees were sampled to determine the sorbitol content in the branches. Sampling was initiated on November 2002 and October 2003 and continued at 14- to 21-day intervals (28 days on one occasion) for about a year. At each sampling date, one year-old branches, two year-old branches and main limbs were sampled from five low- and five high-vigour trees (replicates). For sampling the one year-old branches and the two year-old branches two or three branches of up to 10 cm in length were cut; for sampling the main limbs, peeled pieces of bark about 5 cm^2 in area (about 10 g) were removed. The samples were immediately placed in an ice box in the orchard and later stored at −80 °C pending processing. The samples were processed as follows: First, the xylem was removed and the bark taken from each tree was lyophilized for 2–3 days. Then, 0.5–1 g of dry matter was ground with a coffee grinder. The samples were weighed and placed in glass tubes, 5 ml of 80% ethanol was added to each tube, boiled, and the liquid was transferred to new glass tubes. The procedure was repeated twice and all the liquid was placed in a warm bath (60 °C) under a hood until dry. One ml of distilled de-ionized water was added to the residue and centrifuged at 10,000 rpm for 1 min. The supernatant was filtered through a 0.45 μm membrane (Whatman, Clifton, NJ, USA), and stored at 20 °C. Soluble sugars were separated with an analytical HPLC system (Kontron 320, Zurich, Switzerland) with auto-sampler (Kontron 360), fitted with an Aminex HPX–87C column (BIO-RAD 250 × 4.0 mm), with distilled dionized water flowing at 0.5 ml min^{-1} as the mobile phase. The sorbitol content of the sample (SC_i, mg g dw^{-1}) was determined by comparing the refractive index of each sample with an external standard with a refractive-index detector (Model 475, Lumitron,). Data were analyzed on a Chromatopac integrator. The rate of change of sorbitol content between successive sampling dates (SCR_i, mg g dw^{-1} day^{-1}) was calculated from the records of the sorbitol contents at these dates and the time intervals (Δt) between the samplings: $SCR_i = (SC_i - SC_{i-1})/\Delta t$. The absolute values of SCR_i, $|SCR_i|$, were calculated and used in the analyses.

Experimentation in one year-old potted trees

The relationship between sorbitol content or $|SCR_i|$ and *E. amylovora* pathogenicity was studied in one year-old potted trees. Pear trees (cvs Spadona and Spadochina on quince rootstock, both highly susceptible to *E. amylovora*) were grown in a net house at the Agricultural Research Organization, the Volcani Center. The Spadona trees were uniform and their height above the rootstock was about 1.5 m. The Spadochina trees were more variable; their heights ranged from 0.5 to 2 m above the rootstock and, whereas some exhibited high vigour, others were less vigourous. Trees did not have wooden branches except the main stem. Two experiments were conducted separately using 'Spadona' or 'Spadochina' trees. The trees were artificially inoculated in 2005, in January (Spadona) or April (Spadochina). A superficial cut was made with a sharp knife at the top of the main branch and a 20 μl drop of bacterial suspension, containing 10^9 cells ml^{-1}, was placed on the wound. To increase the variation in $|SCR_i|$ between trees, about half of the trees in each experiment were pruned prior to inoculation by cutting off the upper 10 cm of the main shoot. It was assumed that pruning would enhance growth and alter the sorbitol content in the cut area. Sorbitol content was determined on the day of inoculation and 2 days later. Soluble sugars were extracted and sorbitol content and $|SCR_i|$ were determined as described above.

Erwinia amylovora cells used for inoculation (strain *Ea*238) contained the promoter of the *hrpE* gene and the reporter gene *inaZ*. *HrpE* (accession number U56662) promoter was amplified by using primers *hrp*E5Bam (5-AAAGGATCCAACGGG TTACCCAGCGATGA-3) and *hrp*E3Hind (5-AA AAAGCTTCGCTGGACCCCGCTGATTGAT-GACAT-3). The PCR product was cloned into

pPROBEGI' containing the promoter-less ice nucleation (*inaZ*) reporter gene (Miller et al., 2000) to yield the plasmid *hrpE*p-*inaZ*. *HrpE*p-*inaZ* was mobilized into *Ea*238 and resulted in *Ea*238 (*hrpE*p-*inaZ*). Two days after inoculation, bark samples (0.1–0.2 g) were taken a few mm below the inoculation point with a sharp knife. Each sample was placed in a plastic bag and 1 ml of distilled water was added. The samples were then crushed with a hammer; the resulting extract was subjected to a series of 10-fold dilutions and used for determining the ice nucleation activity and *E. amylovora* population size. Ice nucleation activity was determined with a freezing-droplet assay according to Loper and Lindow (1987), for 40 drops, each of 10 μl, of each dilution. The ice nucleation activity was expressed as Log (ice nuclei per cell). The *E. amylovora* population size in the sample was determined by plating three 10 μl drops of each dilution of the extract on LB agar containing gentamycin (the antibiotic on the vector). Calculation of *inaZ* was based on the method described by Vali (1989) using the formula: $N = V_t \times \ln[1/(1 - P_f)/V_d \times D]$; where N is the concentration of ice nuclei in the sample; V_t and V_d are the volumes of the dilution tube and the droplet, respectively; D is the serial dilution (10^0–10^{-6}), and P_f is the proportion of frozen drops at the selected dilution. The *inaZ* activity was calculated by dividing N by the *E. amylovora* population size in the sample and transforming to Log (ice nuclei cell^{-1}). In addition, *inaZ* activity was determined under the same conditions, with *E. amylovora* cells (strain *Ea*238) containing the promoter of the *hrpJ* gene and the reporter gene *inaZ*, as a positive control. *HrpJ* promoter was amplified by using primers *hrp*J5hind (5-CCGGATCCCCGCCAAAA TTTGC AATAA-3) and *hrp*J3Hind (5-GGGAA GCTTG GGTAAAACGGGAGCAATT-3).

Data analysis

Differences in disease incidence between trees of the two physiological groups (high vs. low vigour) and the two seasons of inoculation (autumn vs. spring) were determined by a χ^2-test. Differences in symptom length and in sorbitol content between high and low vigour trees and at each phenological stage were determined using a non-paired *t*-test (at $P \leq 0.05$). The relationships between $|SCR|$ values and the rate of symptom progression or *hrpE* expression were determined by means of regression analysis. $|SCR|$ was the dependent variable and rate of symptom progression or *hrpE* expression was the independent variable in the analysis.

Results

Disease incidence was higher in spring-inoculated than in autumn-inoculated trees (60–87.5 and 35–47.5%, respectively). In three of the four experiments, differences in disease incidence between high and low vigour trees were not significant (determined by the χ^2-test at $P = 0.05$). In the 2003 spring experiment, the disease incidence on high vigour trees (87.5%) was significantly higher ($P = 0.03$) than that on low vigour trees (66.7%). The season of infection and the vigour status of the trees governed the response to *E. amylovora* (Figure 1). Following autumn infections, fire blight symptoms progressed more rapidly in low vigour trees than in high vigour ones (Figure 1a and c; $P < 0.05$). Symptoms continued to progress (in most cases) until the subsequent spring, 4 months after inoculation (Figure 1a and c). However, the response was different when infections were applied in the spring: in 2003, symptoms progressed significantly more rapidly in high vigour trees than in low vigour ones (Figure 1b; $P < 0.05$). In 2004, there was no significant difference in symptom progression between low and high vigour trees. In both springs, symptom progressions halted within 1–2 months after inoculation.

The sorbitol content in pear branches fluctuated markedly over time, in line with the phenological stages of the trees and in accordance with their vigour group. The actual sorbitol levels varied among the various tissues, and also between one year-old branches, two year-old branches, and main limbs but they all exhibited similar fluctuations over time (Table 1). Since there were no significant differences in the fluctuations of the sorbitol content in branches from different ages, measurements made in one year-old branches were used for further analyses. The sorbitol contents in such shoots during 2002–2003 are presented in Figure 2. Fluctuations in sorbitol content were more pronounced in late summer, autumn and winter, when the trees were dormant, than in the spring and early summer during bloom, fruit set and fruit growth. During autumn

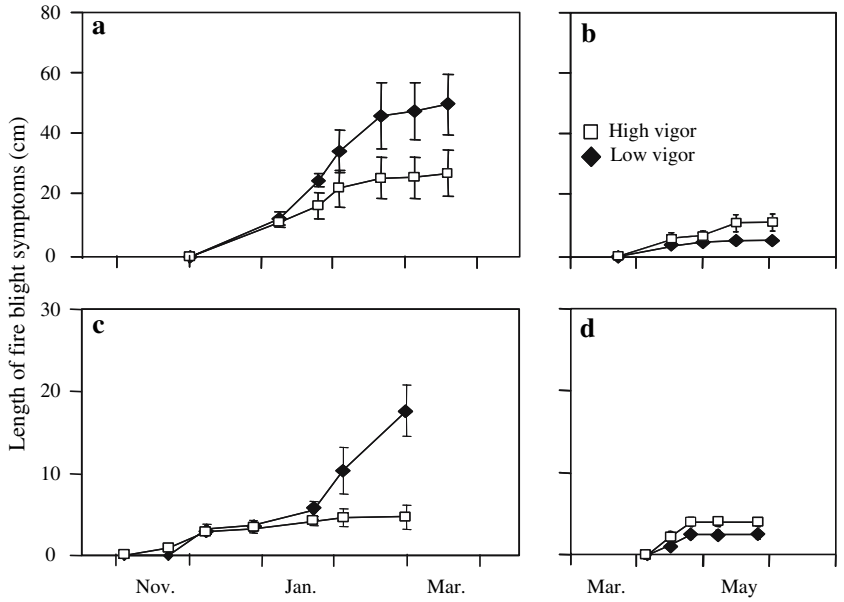

Figure 1. Fire blight (caused by *E. amylovora*) progression in perennial pear branches as affected by the vigour status of the trees and the season of infection. Trees with low vigour (LV): succulent annual shoots did not develop at most terminal branches and the annual shoots that did develop were short (< 10 cm) and thin (< 5 mm). Trees with high vigour (HV): numerous, long (> 30 cm) and thick (> 10 mm) succulent sprouting annual shoots at most terminal branches. Vertical bars represent the standard error of the mean for the following number of replicates: (a) Autumn 2002/3. HV = 6, LV = 15; (b) Spring 2003. HV = 15, LV = 20; (c) Autumn 2003/2004. HV = 29, LV = 35and (d) Spring 2004 HV = 24, LV = 12. D.

and winter 2002/2003, when the trees were dormant, the sorbitol content in one year-old branches sampled from high vigour trees was significantly higher (in January and March) than that in trees with low vigour. In both autumns (2002 and 2003) there was a significant difference in the sorbitol content ($P < 0.05$) between high and low vigour trees. Similar trends in sorbitol

Table 1. Sorbitol content in the sap of annual and perennial tissues of low and high vigour pear trees, at different phenological stages

Tree vigour[a]	Sampled tissue	Dormancy[b]				Bloom and early shoot growth				Fruit set to harvest			
		Min. SC[c]	Max. SC	Mean SC	CV SC[d]	Min. SC	Max. SC	Mean SC	CV SC	Min. SC	Max. SC	Mean SC	CV SC
Low	Annual shoots	9.4	24.0	17.7	32.8	13.3	19.2	17.0	19.1	23.0	27.9	26.1	10.1
	Two year-old branches	8.6	24.7	17.5	32.2	13.3	19.8	16.0	17.1	21.6	26.8	23.2	11.5
	Main limbs	13.1	29.6	22.4	27.5	20.4	25.1	22.8	10.3	22.8	33.9	26.6	19.1
High	Annual shoots	18.5	28.6	24.8	25.2	20.2	25.3	22.3	11.8	18.4	29.1	23.9	18.8
	Two year-old branches	16.2	32.6	21.8	27.4	17.8	20.5	19.0	7.3	20.0	25.7	21.9	12.2
	Main limbs	19.3	45.4	27.8	35.0	19.7	23.3	21.3	8.7	15.8	24.6	21.1	18.8

[a]Trees with low vigour: succulent annual shoots did not develop at most terminal branches and the annual shoots that did develop were short (< 10 cm) and thin (< 5 mm). Trees with high vigour: numerous, long (> 30 cm) and thick (> 10 mm) succulent sprouting annual shoots at most terminal branches.
[b]Dormancy: from November 2002 to February 2003; Blooming and sprouting: from March to mid-April 2003. Fruit set to harvest: from late April to mid-August.
[c]SC: Sorbitol content (mg g dw^{-1}).
[d]CV: Coefficient of variation (%). CV = SD/Mean × 100.

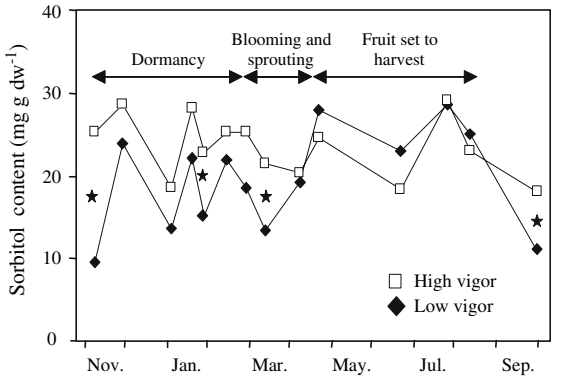

Figure 2. Changes in sorbitol content in annual shoots of high and low vigour pear trees from November 2002 to October 2003. Sorbitol content was determined by HPLC after ethanol extraction of the bark. Asterisks represent significant differences ($P \leq 0.05$) between high and low vigour trees at a certain sampling date, as determined by non-paired *t*-test. The phenological stage of the trees is indicated in the upper part of the graphs. Trees with low vigour: succulent annual shoots did not develop at most terminal branches and the annual shoots that did develop were short (< 10 cm) and thin (< 5 mm). Trees with high vigour: numerous, long (> 30 cm) and thick (> 10 mm) succulent sprouting annual shoots at most terminal branches.

content fluctuations among high and low vigour trees were observed in samples taken in the 2003/2004 season, although the fluctuations were more moderate (results not shown).

Observations and measurements made in the orchard during the 2 years of the study were used to examine the relationship between the length of fire blight symptoms and the sorbitol content in the sampled branches. Initial analyses were applied to the measured records of both parameters. Although some significant relationships were obtained, none involved either the season of infection (autumn or spring) or the vigour group of the trees (high or low). However, a highly significant relationship was found between rate variables derived from these parameters: the rate of symptom progression between two assessment dates (SPR_i) was significantly related to the absolute value of the rate of sorbitol content change, for the same time interval ($|SCR|$). For $|SCR|$ values up to about 0.2 mg (g dw day^{-1}), SPR_i did not change much, but further increases in $|SCR|$ resulted in a gradual increase in SPR. This relationship, which encompassed both the seasons of infection and the vigour groups of the trees, was highly significant ($P \leq 0.001$), and 77.5% of the variability in SPR was attributed to $|SCR|$ (Figure 3).

The relationship between $|SCR|$ and *hrpE* and *hrpJ* expression was determined on one year-old trees; Hrp genes expression increased gradually as $|SCR|$ increased up to about 2 mg g dw^{-1} day^{-1}, but further increase in $|SCR|$ did not affect the expression. The correlation which encompassed both cultivars, was highly significant ($P = 0.004$), and 63.5% of the variability in *hrpE* and *hrpJ* expression was attributed to $|SCR|$ values (Figure 4).

Discussion

The interaction between *E. amylovora* and the physiological status of mature, orchard-grown, pear trees was examined in this study. The physiological status of the trees was defined qualitatively, with host phenology and vigour used as measures, and quantitatively, with the sorbitol content in annual shoots used as a measure. Qualitatively, the tree response to fire blight was governed by their vigour group and their phenological stage at the time of infection: low vigour trees inoculated in the autumn (just before they entered dormancy) and high vigour trees inoculated in the spring (soon after bloom)

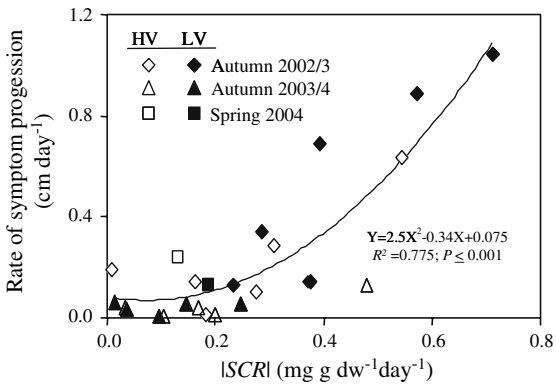

Figure 3. The relationships between the rate of fire blight progression (SPR) in perennial pear branches and the absolute value of the rate of sorbitol content change ($|SCR|$), for high and low vigour trees. Trees with low vigour (LV): succulent annual shoots did not develop at most terminal branches and the annual shoots that did develop were short (< 10 cm) and thin (< 5 mm). Trees with high vigour (HV): numerous, long (> 30 cm) and thick (> 10 mm) succulent sprouting annual shoots at most terminal branches.

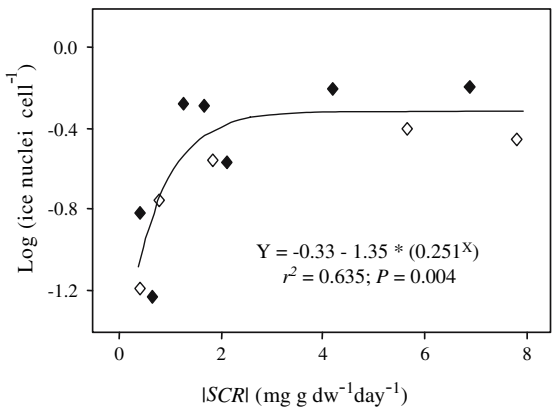

Figure 4. The relationship between the absolute value of the rate of sorbitol content change (|*SCR*|) and *hrpE* expression by *E. amylovora*. *HrpE* expression was estimated using ice nucleation activity; *Ea*238 containing *hrpE*p-*inaZ* was used for inoculation and *inaZ* activity was determined 2 days after inoculation. Full diamonds: trees of cv. Spadona. Empty diamonds: trees of cv. Spadochina.

were more susceptible than high vigour trees inoculated in the autumn and low vigour trees inoculated in the spring. In general, the present results corroborated those published recently for the same pathosystem (Blachinsky et al., 2003).

In the present study we have used the sorbitol content in the sap of one year-old branches as a quantitative indicator of the physiological status of the trees at a given time. One year-old branches are the strongest vegetative source tissues during plant growth and any change in the source/sink relationships of the trees immediately affects their sorbitol content. A significant relationship was observed between *SPR*, the measure used for determining host response to fire blight, and |*SCR*|, the measure used for quantifying the physiological status of the trees. Furthermore, a significant relationship was found between the expression of ice nucleation by the *hrpE* gene, and |*SCR*|. At |*SCR*| values up to 0.2 mg g dw^{-1} day^{-1} *SPR* did not change much, and the expression of ice nucleation by the *hrpE* gene was not determined at such low |*SCR*| values in our present experiments. Further increase in |*SCR*| resulted in gradual increases in both *SPR* and expression of ice nucleation by the *hrpE* gene. For the former relationship this increase was observed up to a value of 0.7 mg g dm^{-1} day^{-1} (the largest |*SCR*| value observed in mature trees) but for the latter relationship, expression of ice nucleation by the *hrpE* gene continued to increase asymptotically up to an |*SCR*| value of about 2 mg g dm^{-1} day^{-1}, and further increase in |*SCR*| did not affect the expression of ice nucleation by the *hrpE* gene. This was expected, as the enzymatic systems of the bacteria are likely to have a maximal capacity. Stresses such as osmotic or heat have been shown to activate responsive sigma factors and to enhance pathogenicity in a variety of pathogenic bacteria (Iriarte et al., 1995; Suh et al., 1999).

In light of the present results, we suggest that changes in the sorbitol content in the sap of pear trees may affect (directly or indirectly) stress-responsive sigma factors in *E. amylovora*, and that this phenomenon, in turn, activates the expression of *hrp* genes of the pathogen. As the changes in sorbitol content increased in magnitude and speed, the *hrp* gene expression of the bacteria and, complementarily, its pathogenicity to the host increased. It should be noted that *E. amylovora hrp* genes were activated when the sorbitol content changed over time, regardless of whether that change was an increase or a decrease. Furthermore, it should be noted that although a correlation between sorbitol content change over time and *E. amylovora hrp* genes was expressed, this does not necessarily imply that there is a direct cause-response relationship between the two; it could be that compound(s) other than sorbitol directly influence(s) *E. amylovora hrp* gene expression and this/these compound(s) is/are affected by changes in sorbitol content.

The quantitative analyses of the data may be used to explain the qualitative correlation between the phenology and the vigour of pear trees, and the response of this correlation to fire blight. The finding that the host response to *E. amylovora* was related to changes in the sorbitol content, rather than to its absolute value, implies that the source/sink relationships within the tree tissues are of importance. In general, trees were more resistant to fire blight in periods when the source/sink relationships were stable, such as during fruit set and fruit growth, than in periods when these relationships changed frequently, such as during the termination of dormancy. This interpretation may have direct implications for the development of strategies to manage fire blight. Apparently, cultural practices that readily disrupt the source/sink relationships, such as pruning or excess nitrogen fertilization, are expected to enhance host

susceptibility to *E. amylovora*, whereas cultural practices that stabilize these relationships, such as application of growth regulators, are expected to enhance host resistance to the pathogen. Effects of these practices on host response to *E. amylovora* have been demonstrated in numerous studies (Fernando and Jones, 1999; Momol et al., 1999; Costa et al., 2001; Toselli et al., 2002; Bubán et al., 2003; Shtienberg et al., 2003; Norelli and Miller, 2004) but, nevertheless, their direct effects on *E. amylovora* virulence await further experimental elucidation.

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References

Barny MA (1995) *Erwinia amylovora hrpN* mutants, blocked in harpin synthesis, express a reduced virulence on host plants and elicit variable hypersensitive reactions on tobacco. European Journal of Plant Pathology 101: 333–340.

Beer SV and Norelli JL (1977) Fire blight epidemiology – factors affecting release of *Erwinia amylovora* by cankers. Phytopathology 67: 1119–1125.

Bieleski RL (1969) Accumulation and translocation of sorbitol in apple phloem. Australian Journal of Biological Sciences 22: 611–620.

Blachinsky D, Shtienberg D, Oppenheim D, Zilberstaine M, Levi S, Zamski E and Shoseyov O (2003) The role of autumn infections in the progression of fire blight symptoms in perennial pear branches. Plant Disease 87: 1077–1082.

Bubán T, Foeldes L, Kormány A, Hautmann S, Stammler G and Rademacher W (2003) Prohexadione – Ca in apple trees: Control of shoot growth and reduction of fire blight incidence in blossoms and shoots. Journal of Applied Botany 77: 95–102.

ChenLS and ChengLL (2004) Photosynthetic enzymes and carbohydrate metabolism of apple leaves in response to nitrogen limitation. Journal of Horticultural Sciences and Biotechnology 79: 923–929.

Costa G, Andreotti C, Bucchi F, Sabatini E, Bazzi C, Malaguti S and Rademacher W (2001) Prohexadione – Ca (Apogee®): Growth regulation and reduced fire blight incidence in pear. HortScience 36: 931–932.

Crosse JE, Goodman RN and Shaffer WH Jr (1972) Leaf damage as predisposing factor in the infection of apple shoots by *Erwinia amylovora*. Phytopathology 62: 176–182.

CuiSM , ChenGL and NiiN (2003) Effects of water stress on sorbitol production and anatomical changes in the nuclei of leaf and root cells of young loquat trees. Journal of Japanese Society of Horticultural Science 72: 359–365.

Eastgate JA (2000) *Erwinia amylovora*: The molecular basis of fire blight disease. Molecular Plant Pathology 1: 325–329.

Fernando WGD and Jones AL (1999) Prohexodione calcium, a tool for reducing secondary fire blight infection. Acta Horticulturae 489: 597–599.

Iriarte M, Stainier I and Cornelis GR (1995) The rpoS gene from *Yersinia enterocolitica* and its influence on expression of virulence factors. Infection Immunology 63: 1840–1847.

Kim JF, Wei ZM and Beer SV (1997) The *hrpA* and *hrpC* operons of *Erwinia amylovora* encode components of a type III pathway that secretes harpin. Journal of Bacteriology 179: 1690–1697.

Lewis DH and Smith DC (1967) Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. New Phytology 66: 143–184.

Li TH and Li SH (2005) Leaf responses of micropropagated apple plants to water stress: Nonstructural carbohydrate composition and regulatory role of metabolic enzymes. Tree Physiology 25: 495–504.

Loescher WH (1987) Physiology and metabolism of sugar alcohols in higher plants. Physiology of Plantarum 70: 553–557.

Loper JE and Lindow SE (1987) Lack of evidence for *in situ* fluorescent pigment production by *Pseudomonas syringae* pv. *syringae* on bean leaf surfaces. Phytopathology 77: 1449–1454.

Miller WGJ, Leveau HJ and Lindow SE (2000) Improved gfp and *inaZ* broad-host-range promoter-host vectors. Molecular Plant–Microbe Interactions 13: 1243–1250.

Momol MT, Ugine JD, Norelli JL and Aldwinckle HS (1999) The effect of prohexadione calcium, SAR inducers and calcium, on the control of shoot blight caused by *Erwinia amylovora* on apple. Acta Horticulturae 489: 601–605.

Norelli JL and Miller SS (2004) Effect of prohexadione-calcium dose level on shoot growth and fire blight in young apple trees. Plant Disease 87: 756–765.

Nosarszewski M, Clements AM, Downie AB and Archbold D (2004) Sorbitol dehydrogenase expression and activity during apple fruit set and early development. Physiology of Plantarum 121: 391–398.

Rosen HR (1929) The life history of the fire blight pathogen, *Bacillus amylovorus*, as related to the means of over wintering and dissemination. Arkansas Agricultural Experimental Station Bulletin No. 283, Fayetteville, Arkansas.

Sakai A (1966) Seasonal variations in the amount of polyhydric alcohol and sugar in fruit trees. Journal of Horticultural Science 41: 207–213.

Seemüller EA and Beer SV (1976) Absence of cell wall polysaccharide degradation of *Erwinia amylovora*. Phytopathology 66: 433–436.

Shtienberg D, Zilberstaine M, Oppenheim D, Levi S, Schwartz H and Kritzman G (2003) New considerations for pruning in management of fire blight in pears. Plant Disease 87: 1083–1088.

Suh SJ, Silo-Suh L, Woods DE, Hassett DJ, West SE and Ohman DE (1999) Effect of *rpoS* mutation on the stress response and expression of virulence factors in *Pseudomonas aeruginosa*. Journal of Bacteriology 181: 3890–3897.

Suleman P and Steiner PW (1994) Relationship between sorbitol and solute potential in apple shoots relative to fire blight symptom development after infection by *Erwinia amylovora*. Phytopathology 84: 1244–1250.

Toselli M, Malaguti D, Maragoni B, Cabrini L, Bazzi C, Sponza G and Scudellari D (2002) Reduction of fire blight infection by manipulation of pear tree water status. Acta Horticulturae 590: 193–199.

Tullis EC (1929) Studies on the over wintering and mode of infection of the fire blight organism. Michigan Agricultural Experimental Station Bulletin No. 97.

Vali G (1989) Principles of ice nucleation. In: Lee RE, Warren GJ Jr. and Gusta LV (eds.) Biological Ice Nucleation and its Application (pp. 1–28) American Phytopathology Society, St. Paul, MN.

vander Zwet TB (1969) Study of fire blight cankers and associated bacteria in pear. Phytopathology 59: 607–613.

van der Zwet TB and Beer SV (1995) Fire Blight – Its Nature, Prevention and Control; Practical Guide to Integrated Disease Management. U.S. Department of Agriculture, Information Bulletin No. 631, Washington DC.

van der Zwet TB and Keil HL (1979) Fire Blight: A Bacterial Disease of Rosaceous Plants. USDA Handbook 510, Washington DC.

vander Zwet TB, Zoller BG and Thomson SV (1988) Controlling fire blight of pears and apple by accurate prediction of blossom blight phase. Plant Disease 72: 464–451.

Vanneste JL and Eden-Green S (2000) Migration of *Erwinia amylovora* in host plant. In: Vanneste JL (ed.) Fire Blight: The Disease and its Causative Agent, *Erwinia amylovora* (pp. 73–81) CABI Publishing, Oxon, UK.

Veberic R, Vodnik D and Stampar F (2003) Carbon partitioning and seasonal dynamics of carbohydrates in the bark leaves and fruits of apple (*Malus domestica* Borkh.) cv. 'Golden delicious'. European Journal of Horticultural Science 68: 222–226.

Wei ZM, Laby RH, Zumoff CH, Bauer DW, He SY and Collmer A (1992) Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. Science 257: 85–88.