Epiphytic fitness of a biological control agent of fire blight in apple and pear orchards under Mediterranean weather conditions

Marta Pujol, Esther Badosa & Emilio Montesinos
Institute of Food and Agricultural Technology-CIDSAV-CeRTA, University of Girona, Girona, Spain

Abstract
The behaviour of Pseudomonas fluorescens EPS62e was investigated in apple and pear orchards under Mediterranean climatic conditions. The trials studied the influence of weather conditions, plant host species, presence of indigenous microbial community and spread from treated to nontreated trees on colonization and survival. Population dynamics were assessed by real-time PCR and CFU-counting methods. With inoculated flowers, weather conditions were optimal for colonization, and EPS62e established high and stable population levels around 10^8 CFU per organ, according to both methods of analysis. The plant host species did not influence the colonization rate, and the biocontrol agent dominated the microbial communities of blossoms, representing up to 100% of the total cultivable population. With inoculated leaves, the EPS62e population decreased to undetectable levels 30 days after treatment according to both methods used. EPS62e spread moderately in the orchard, being detected in nontreated flowers of trees 15–35 m from the inoculation site. The combined use of real-time PCR and CFU-counting methods of analysis permitted the identification of three physiological states for EPS62e in the field, which consisted of active colonization, survival and entry into a viable but nonculturable state, and cell death.

Introduction
Fire blight is a serious disease caused by Erwinia amylovora that affects several plant species, mainly belonging to the rosaceous family, such as fruit trees (Pyrus spp., Malus spp.) and ornamentals (Cotoneaster spp., Crataegus spp., Pyracantha spp.). This disease has a worldwide distribution, causing important economic losses (Van der Zwet & Keil, 1979; Van der Zwet & Beer, 1995; Vanneste, 2000). Erwinia amylovora infections usually start in blossoms, where the pathogen multiplies rapidly and enters into plant host tissues, where the infection progresses, being able to kill trees within a single growing season (Vanneste & Eden-Green, 2000). The control of fire blight has been mainly based on interfering with multiplication of E. amylovora on blossoms by means of spray treatments with antibiotics (e.g. streptomycin, oxytetracycline) or copper compounds (e.g. copper hydroxide, copper oxychloride, copper sulphate) (Psallidas & Tsiantos, 2000). Nevertheless, the wide use of chemicals has important drawbacks, such as the appearance of antibiotic-resistant strains of E. amylovora (Moller et al., 1981; Loper et al., 1991; Jones & Schnabel, 2000; Vanneste & Voyle, 2002), which may cause the failure of disease control and the persistence of such chemicals in the environment and food. Therefore, new strategies of fire blight management are increasingly being used, such as biological control (Wilson & Lindow, 1993; Johnson & Stockwell, 2000; Stockwell et al., 2002).

Because the organs most susceptible to infection by E. amylovora are flowers, biological control of fire blight is based on the establishment of antagonist bacteria on blossoms prior to the arrival of the pathogen (Wilson et al., 1992; Johnson et al., 1993; Wilson & Lindow, 1993). Several biological control agents have been developed and are authorized or in the process of registration, such as Pseudomonas fluorescens A506 (Wilson & Lindow, 1993), Pantoea agglomerans C9-1 (Ishimaru et al., 1988), Pa. agglomerans D325 (Pusey, 1997), Pa. agglomerans Pc10 (Vanneste et al., 2002), Bacillus subtilis QST713 (Aldwinckle et al., 2002), and B. subtilis BD170 (Broggini et al., 2005). The mechanisms of action of the antagonist strains are mainly based on competition for growth-limiting resources, antibiotic action, or both. Research on new antagonists in our laboratory led to the selection of Ps. fluorescens EPS62e because of its high...
epiphytic growth of biocontrol agents in the host plant is determined by the physical environment (availability of nutrients, weather conditions) and the microbiological environment (indigenous microbial community). The ability of antagonists to colonize blossoms under field conditions is crucial to ensure effective biological control of fire blight (Johnson & Stockwell, 1998). Therefore, evaluation of the capacity of the introduced bacterial antagonist to dominate in different weather conditions is of great interest. Moreover, such knowledge will be useful for prediction of the population dynamics of the biocontrol agent in the host plant (Johnson et al., 2000).

The dispersal of the biological control agent in the orchard is also important, because it contributes to secondary colonization of blossoms. In the case of this being significant, it may avoid the need to perform repeated applications or may decrease the frequency of applications. This is an advantage compared to chemical pesticides, especially in certain apple and pear cultivars that are more affected by secondary blooming (Lindow & Suslow, 2003).

The improvement of colonization and survival of EPS62e, as has been reported for biocontrol agents of postharvest diseases by means of osmoadaptation (Bonaterra et al., 2005), may help to achieve the required population level for the control of fire blight in different plant tissues, and may increase its efficacy under unfavourable conditions. However, it is necessary to have specific quantitative methods to track different states of activity of the EPS62e population in the field. Previously, we have developed a system to measure the environmental fate of strain ESP62e after field release by combining real-time PCR and culture-based methods (Pujol et al., 2006). Using these methods of analysis, we studied the behaviour of this strain in apple plants in controlled environment experiments and field trials under Atlantic climatic conditions (Pujol et al., 2006). The combined use of these methods revealed that, depending on experimental conditions, there was an active colonization or entry into a viable but nonculturable (VBNC) state. However, it remained unknown if the same behaviour occurs on a different host such as pear, or under different conditions such as a Mediterranean climate, where fire blight outbreaks have been reported in the past (Van der Zwet, 2002).

In the present work, we evaluated the ability of strain EPS62e to colonize pear in comparison to apple trees under Mediterranean field conditions using real-time PCR and CFU-counting methods. We also studied the relative yield of EPS62e in relation to the cultivable resident bacterial population, and the incidence of spread and secondary colonization of nontreated pear and apple trees.

**Materials and methods**

**Bacterial strain, growth conditions and DNA extraction**

*Pseudomonas fluorescens* EPS62e was isolated from a healthy fruit of Conference pear cultivar in Girona, Spain (Cabrefiga, 2004). For specific monitoring in field trials, a spontaneous nalidixic acid-resistant mutant was obtained in the laboratory. The bacterial strain was cultured in Luria–Bertani (LB) agar supplemented with 50 mg L\(^{-1}\) of nalidixic acid at 25 °C for 24 h. Inoculum suspensions were prepared in sterile water, and adjusted spectrophotometrically at 10\(^8\) CFU mL\(^{-1}\). Independent EPS62e cell suspensions were prepared for each trial performed. For real-time PCR, DNA was extracted as described by Llop et al. (1999), with minor modifications. One millilitre of plant homogenate was centrifuged at 10,000 g for 10 min. The pellet was suspended in 500 μL of extraction buffer [200 mM Tris–HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, 0.5% sodium dodecyl sulphate, 2% polyvinyl pyrrolidone (PVP)], and tubes were shaken for 1 h at room temperature. Then, tubes were centrifuged at 5000 g for 5 min, and 450 μL of the supernatant was transferred to a new tube, to which 450 μL of isopropanol was added. One hour later, tubes were centrifuged at 13,000 g for 10 min, and the DNA pellet was dried and suspended in 100 μL of sterile ultrapure water. Extracted DNA was stored at −20 °C.

**Field experiments**

Field trials were performed with trees of Golden Delicious apple cultivar and Doyenne du Comice pear cultivar in experimental orchards located at the Mas Badia Agricultural Experiment Station (Girona, Spain) during spring and summer of 2005. Blossom trials were performed in both apple and pear trees, whereas leaf trials were only performed in apple trees. Two independent trials were done for each type of plant material (flowers, leaves) and each species (apple, pear). The experimental design of each plot consisted of treated and nontreated trees. Each treatment was composed of three replicates of three trees per replicate that were separated by two untreated trees. Treated and nontreated trees within a plot were separated by 10 trees. Treated trees were used to study colonization and survival of EPS62e, and nontreated trees were used to evaluate its dispersal capacity in the orchard.

Blossom trials consisted of a single spray inoculation until the runoff point of an EPS62e suspension at 10\(^8\) CFU mL\(^{-1}\) at the maximum flowering peak using a motorized atomizer. Before the treatment, the bacterial suspensions were checked by CFU-counting and real-time PCR methods. Both methods gave the expected results, indicating that cells were in an optimal physiologic state. When this inoculation procedure was used in previous trials, with a hand-held sprayer, the
amount of bacterial suspension retained was estimated to be between 0.5 and 1 mL, which represented $5 \times 10^7$–$10^8$ CFU per blossom. The first sampling time after treatment was at 12 h, and blossoms were then periodically sampled over 54 days. Each sample was obtained by bulking six blossoms from the three trees of each replicate. Samples were transported to the laboratory and homogenized (Masticator, IUL Instruments, UK) with 60 mL of extraction buffer (0.14 M NaCl, 0.26 M NaH$_2$PO$_4$·H$_2$O, 0.75 mM Na$_2$HPO$_4$·12H$_2$O, 2% PVP-10, 1% mannitol, 10 mM ascorbic acid, 10 mM reduced l-glutathione) for 60 s. Twenty-two days after treatment, the fruit size necessitated a slight modification of the extraction procedure; orbital shaking of samples in the extraction buffer for 1 h was used instead of the homogenization procedure.

Leaf trials were performed by spraying EPS62e suspension at $10^8$ CFU mL$^{-1}$ onto foliar surfaces using a motorized atomizer. Samples were collected 12 h after treatment and periodically over the next 30 days. Each sample consisted of 12 leaves picked at random from the three trees of each replicate. Samples were transported to the laboratory, weighed, and homogenized in 30 mL of the extraction buffer for 60 s.

Weather parameters were monitored with an automatic weather station located at the experimental orchards of Mas Badia (Girona, Spain). The temperature, relative humidity and rainfall were monitored with a CR10X datalogger (Campbell Scientific Ltd, Leicester, UK) connected to combined temperature – relative humidity (model HMP35AC) and rainfall (model ARG100) electronic sensors.

**Assessment of population levels of *Ps. fluorescens* EPS62e and total cultivable bacteria**

Two monitoring methods were used to estimate population levels of the biocontrol agent and the total cultivable bacterial population. To assess the population size of EPS62e, real-time PCR was used for all sampling times, whereas the CFU-counting method was applied only for some selected sampling dates. The total cultivable population size was estimated by CFU counting.

For real-time PCR, 1 mL of each homogenized sample was processed following the DNA extraction procedure described above. Each DNA sample was evaluated in triplicate by PCR with a specific primer pair and probe design for EPS62e developed in a previous study (Pujol et al., 2006), using 450F and 450R primers, and a 450 TaqMan probe. PCR was performed in a final volume of 20 μL containing 1 × PCR TaqMan buffer A (PE Applied Biosystems), 6 mM MgCl$_2$, 0.2 mM dNTP, 0.3 μM each primer, 0.2 μM probe, 1 U of AmpliTaq Gold DNA Polymerase (PE Applied Biosystems), and 1 μL of the extracted DNA. The thermocycle conditions consisted of an initial denaturation step at 95 °C for 10 min followed by 50 cycles, each consisting of 95 °C for 15 s and 60 °C for 1 min. The quantification was obtained by means of a standard curve specifically developed for each trial (apple blossoms, apple leaves and pear blossoms), where a homogenate of each plant material, obtained before the biocontrol agent treatment, was mixed with several dilutions of known concentrations of an EPS62e cell suspension prior to DNA extraction. The dilutions used covered a 5 log range and followed the DNA extraction procedure described above. Three replicates of each dilution were used in the PCR to obtain the standard curve, with a threshold fixed at 0.03 and the baseline from the 3rd to the 12th cycle. The quantification of samples was obtained by plotting the threshold cycle ($C_t$), i.e. the cycle in which the fluorescence emitted by an amplified product crosses over a defined threshold, against the concentration of known samples.

For CFU counting, 10-fold serial dilutions of sample homogenates were plated in either LB agar supplemented with 50 mg L$^{-1}$ of econazole nitrate for total cultivable bacteria, or LB agar supplemented with 50 mg L$^{-1}$ of econazole nitrate salt and 50 mg L$^{-1}$ of nalidixic acid for the specific quantification of EPS62e. Plates were incubated at 25 °C, and colonies were counted 48 h later.

**Statistical analysis**

Field experiments were performed twice for each fruit tree type (apple, pear) and placed in different locations. Each sample was analysed in triplicate for both real-time PCR and CFU-counting methods. Results were expressed as cells or CFU per blossom or fruit, or per gram of fresh weight of leaves. The population size obtained was transformed to log$_{10}$ CFU and was analysed using the General Linear Model procedure of spss v13.0 for Windows. Means were separated using Fisher’s least significant difference test ($P \leq 0.05$).

**Results**

**Colonization of pear and apple by *Ps. fluorescens* EPS62e under field conditions**

Real-time PCR showed good efficacy at monitoring EPS62e in blossoms and leaves. Linearity between $C_t$ values and EPS62e cells covered a 5–6 log range, and most values obtained from field samples were located within the concentration range of the standard curve.

The population dynamics of *Ps. fluorescens* EPS62e after its inoculation into pear and apple blossoms in relation to weather conditions are shown in Fig. 1. The biocontrol agent was established at high population levels in both pear (Fig. 1a and b) and apple (Fig. 1c and d) blossoms.

At the first sampling time, 12 h after inoculation, the population level assessed by real-time PCR was $c. 10^6$ cells per blossom, whereas the cultivable population level was about two orders of magnitude lower in pear and almost
three orders of magnitude lower in apple blossoms. This difference disappeared at the next CFU-counting sampling time, 7 days after treatment. By the end of the assay, 54 days after treatment, EPS62e reached a stable population level of $10^7–10^8$ CFU per fruit.

Weather conditions were characterized by a few isolated rainfalls in mid-May, mid-June and mid-July, with relative humidity between 50% and 85%. Mean daily temperatures were between 15 and 20 °C from April to May, and between 20 and 25 °C from June to July. Rainfall events caused a slight increase in population levels in apple trees but not in pear trees.

Population levels of EPS62e after inoculation onto apple leaves are also shown in Fig. 1. Population levels decreased significantly with time (Fig. 1e and f). Values assessed by real-time PCR started at $10^7$ CFU (g fresh weight)$^{-1}$ 12 h after treatment and decreased sharply to $10^5–10^6$ CFU (g fresh weight)$^{-1}$ over the next 10 days. However, population levels of cultivable EPS62e at the first sampling time were about 2 log lower than those assessed by real-time PCR. On the remaining sampling days, both measurements coincided. A population shoulder was observed 20 days after treatment in both experiments; this occurred 4 days after a rainfall event followed by an increase in mean daily

---

**Fig. 1.** Population dynamics of *Pseudomonas fluorescens* EPS62e determined by real-time PCR (white symbols) and CFU counting (black symbols) on pear flowers (a, b), apple flowers (c, d) and apple leaves (e, f) under field conditions at the Mas Badia Agricultural Experiment Station (Girona, Spain). Cells were sprayed once during bloom at the beginning of the experiment at $10^8$ CFU mL$^{-1}$. The SD of three replicates is represented by a vertical bar. Mean daily temperature (g), amount of rainfall (bars) and relative humidity (lines) (h) were monitored during trials.
temperatures. Then, the population levels decreased again, and at the last sampling time, 30 days after treatment, EPS62e was under the detection level of the methods. In contrast to values observed in blossom, those observed in leaf trials were highly variable.

**Impact of introduced* Ps. fluorescens* EPS62e on the indigenous microbial community**

Total cultivable bacteria and EPS62e reached similar population levels, between $10^7$ and $10^8$ CFU per blossom or fruit, in inoculated pear and apple trees. Therefore, the biocontrol agent accounted for 100% of the total cultivable bacteria a few days after inoculation, thus dominating the bacterial population of treated flowers in both fruit trees (Fig. 2a–d).

The total number of cultivable bacteria in apple leaves remained stable at between $5 \times 10^4$ and $2 \times 10^5$ CFU (g fresh weight)$^{-1}$. In contrast, the cultivable population of EPS62e showed a progressive decrease with time. EPS62e represented only 27.1% (trial 1) and 54.7% (trial 2) of the total cultivable bacteria upon inoculation, and then decreased to 0.5% (trial 1) and 26.1% (trial 2) at the end of the assay, being superseded by the indigenous microbiota (Fig. 2e and f).

**Spread from treated to nontreated trees**

EPS62e was detected at very low levels, c. $10^3$ CFU per blossom, 12 h after treatment in three of the four noninoculated blossom trials (Fig. 3). This basal or even nondetectable population level increased significantly 7 days later, reaching values between $10^5$ and $10^6$ CFU per blossom in all trials. In one trial, the EPS62e population showed a significant decrease until the end of the assay, representing 0.1% of the total cultivable bacterial population (Figs 2c and 3c). Nevertheless, in the remaining trials, EPS62e was stable in nontreated blossoms at high population levels until the end of the assay (Fig. 2a, b and d, and Fig. 3a, b and d). Values in nontreated trees were highly variable compared to those in treated trees.

In the case of apple leaves, the biocontrol strain was only detected at two sampling dates at very low levels close to the detection limit of the method (data not shown).

**Discussion**

EPS62e was an efficient colonizer of apple blossoms in the field in the northwestern France with Atlantic climatic conditions (Pujol et al., 2006). However, it was uncertain whether it would follow the same population dynamics when used in a different region, such as northeastern Spain, where the climatic conditions are Mediterranean, and in a different host plant species, such as pear. The trials performed in the present work showed that *Ps. fluorescens* EPS62e was able to reach very high population levels on both apple and pear blossoms, between $10^7$ and $10^8$ CFU per blossom after a single treatment during bloom. These values are higher than those reported for other biocontrol agents of fire blight, such as *Pa. agglomerans* C9-1 (Johnson et al., 1993; Nuclo et al., 1998; Stockwell et al., 1998) and *Pa. agglomerans* Eh252 (Johnson et al., 2000). Another important property of EPS62e is that the colonization pattern on blossoms was not greatly influenced by the host plant species. This is in agreement with the results reported for the biocontrol agent of fire blight *Pa. agglomerans* C9-1S (Johnson et al., 2000).

Previous studies have shown the influence of temperature, relative humidity and rain on the ability to colonize blossoms by biological control agents of fire blight (Nuclo et al., 1998; Johnson et al., 2000; Pusey, 2002; Lindow & Suslow, 2003; Thomson & Gouk, 2003; Pusey & Curry, 2004). However, climatic conditions during spring did not cause strong changes in mean population sizes of EPS62e in apple and pear flowers. Therefore, weather conditions during spring 2005 in Girona (Spain) were optimal for EPS62e, as population levels remained stable at high values for a long period of time, and the values assessed by real-time PCR and CFU counting did not significantly differ, indicating that
most cells detected by PCR were cultivable. Nonetheless, the Maine-et-Loire climate was even more favourable than in Girona, because population levels were slightly higher (Pujol et al., 2006).

It has been reported that E. amylovora generally grows to higher population levels than other antagonists at temperatures from 20 to 32 °C (Pusey & Curry, 2004). ESP62e seems to be well adapted to this temperature range, because any influence on its population in blossoms was observed after mean daily temperatures between 20 and 25 °C.

The simultaneous use of real-time PCR and CFU counting was useful to assess EPS62e fitness after field release. Both methods differed in the estimation of EPS62e population level at the first sampling time, 12 h after inoculation, in all field trials performed in the present work. This phenomenon has been previously observed in Golden apple blossoms (Pujol et al., 2006). In this former study, the lack of coincidence in estimations of population levels by both methods was attributed to the entry into a VBNC state of part of the EPS62e population or to the presence of nondegraded DNA after cell death. As was observed in the present study, it is probable that the biocontrol agent was stressed upon inoculation, due to the sharp change in the biological and physical environment from laboratory culture conditions to the tree surface, which may cause a transient noncultivability or even a high cell death rate. The situation changed a few days after inoculation in blossom trials, because cultivable population levels were not significantly different from those estimated by real-time PCR. The slight difference observed between the methods in pear blossoms by the end of the assay was attributed to a loss of DNA from samples, probably due to DNA degradation during the extraction procedure.

The behaviour of EPS62e in leaves was completely different from that in flowers. Clearly, the phyllosphere was not an optimal habitat for this biocontrol agent. Under Atlantic climatic conditions, we have observed entry into a VBNC state because of the difference between values obtained by real-time PCR and CFU counting, where both estimates of EPS62e population levels were stable over time, but real-time PCR values were 100 times higher than those estimated by CFU counting (Pujol et al., 2006). However, in the present study, we did not detect this situation; rather, we observed cell death, indicated by a decrease in values with time and a positive correlation between values obtained by both methods of analysis. Probably, the weather conditions during July in Spain contributed to a significant death rate of EPS62e in the phyllosphere, because of the high temperatures and solar radiation. This is in agreement with studies showing that the phyllosphere is characterized by low nutrient availability and direct exposure to dry and hot weather (Lindow & Brandl, 2003). These harsh conditions probably caused a stress in EPS62e, forcing cells to enter into a VBNC state upon inoculation, followed by cell death and DNA degradation.

The different behaviour of EPS62e on blossoms and leaves confirmed that this biocontrol agent is less appropriate for foliar application than for blossom treatment in the management of fire blight. However, its capacity to colonize and survive in the phyllosphere could be improved by physiological means such as osmoadaptation during inoculum preparation (Bonaterra et al., 2005).

On the basis of the results obtained in France and Spain using simultaneous analysis of population levels of EPS62e by means of real-time PCR and CFU counting, three patterns of population change have been identified that are related to fitness. During active colonization, both methods gave similar population levels that increased with time. Upon survival under suboptimal conditions, population levels decreased but were stabilized over time, with higher values of real-time PCR than CFU counting, indicating possible entry into a VBNC state (Pujol et al., 2006). Under unfavourable conditions, cell death was detected, because real-time PCR and CFU counting gave similar and decreasing population levels over time.

Strain EPS62e completely dominated the cultivable bacterial communities of apple and pear flowers, indicating that this biocontrol agent is not significantly affected by indigenous competitors in flowers. These results are supported by the fact that the main mechanism of action of fire blight control is based on pre-emptive competitive exclusion of E. amylovora (Cabrefiga, 2004). The ability of EPS62e to
supersede the indigenous microbial community will proba-
bly determine its potential to exclude *E. amylovora* from its
epiphytic niche under field conditions, as long as it arrives
first at the sites of infection in the host.

The total cultivable bacterial population was different in
trees treated and not treated with EPS62e. In treated trees,
the population level observed was 10-fold higher than in
nontreated trees. These results are in agreement with those
of other studies performed on pear and apple flowers, where
the total population level was 10–100-fold higher in trees
inoculated with biocontrol agents than in noninoculated
trees (Johnson et al., 2000; Lindow & Suslow, 2003). This
difference was explained because some biocontrol agents
were more efficient in colonizing flowers than the indigenous
microbial community.

Our experimental approach consisted of a single treat-
ment at the flowering peak and a sampling design based on
bulk samples of several flowers or leaves. As flowering
takes several days and may be asynchronous, inoculated
flowers coexist with noninoculated ones at a given time.
Therefore, this sampling design may cause an underestima-
tion of the EPS62e population level determined from bulked
samples. However, we observed a very high population level
that was maintained consistently, not only in flowers but
also later in immature fruits (calyx end) for almost 2 months
after the single inoculation. Therefore, in any case, these
results showed that the biocontrol agent spread from treated
to nontreated flowers within the tree; otherwise, we would
have observed a decrease in the population level. To avoid
this problem of underestimation, other authors analysed
population levels in single flowers (Pusey, 2002; Pusey &
Curry, 2004). However, in the present study, due to the
combined use of real-time PCR and CFU-counting meth-
ods, the analysis of individual flowers or leaves as sample
units would generate an enormous number of samples that
would be difficult to manage.

The asynchronous flowering in some pear and apple
cultivars increases the difficulties of fire blight manage-
ment. The flowers that emerge last are highly susceptible to
*E. amylovora* infection, because they are not open at the
moment of treatment, and a seasonal increase in mean daily
temperatures favours epiphytic growth of the pathogen on
the stigma surfaces (Lindow & Suslow, 2003). One advan-
tage of biological control of fire blight over chemical control
is that some antagonists are able to spread in the orchard,
whereas chemical pesticides work by direct contact with
organs at the moment of application (Nucló et al., 1998;
Pusey, 2002). In the present study, we evaluated for the first
time the dispersal of EPS62e from treated to nontreated
trees to estimate secondary colonization. Strain EPS62e was
inoculated in a single application at the bloom peak stage,
and it was detected and multiplied in nontreated apple and
pear blossoms situated 15–35 m from the inoculation site,
although with high variability from trial to trial. One
possible explanation for the moderate dispersal is that the
favourable conditions for secondary colonization, such as
wind, rainfall and bees, were limited. Therefore, the low
amount of initial secondary inoculum deposited in bloss-
oms of nontreated trees could result in high variability
between trials, and also EPS62e could not reach the mini-
mum population size necessary for it to be able to dominate
the epiphytic niche. Although variability was observed in the
dispersal assays, the experiment demonstrated that EPS62e
was able to spread from treated to nontreated trees, reaching
the same population level reported for *Ps. fluorescens* A506
during secondary colonization (Lindow & Suslow, 2003).

In conclusion, we have defined the range of hosts, the
plant organ and the climatic conditions for optimal EPS62e
fire blight management. The physical and biological envi-
ronment of pear and apple blossoms was so optimal for
EPS62e that weather conditions did not greatly affect its
population level, encouraging a wide geographical range of
application. The competition of EPS62e for colonization
sites and nutrients allowed the biocontrol agent to dominate
completely the microbial community of flowers, leaving
poor options for *E. amylovora* for tree colonization and
infection. Finally, the dispersal and moderate secondary
colonization of noninoculated blossoms by EPS62e repre-
sents added value for further commercialization of this
biological control agent of fire blight.

**Acknowledgements**

This work was supported by the Spanish Ministry of
Education and Science (AGL2001-2349, AGL2004-07799
and research grant FP-2001-2130) and the Comissió Inter-
departamental de Recerca i Tecnologia of the Catalonian
Government (GRC-2001SGR00293). We thank P. Vilardell
for helpful assistance and Mas Badia Agricultural Experi-
ment Station for providing access to fruit tree experimental
orchards.

**References**

Evaluation of control of fire blight infection of apple blossoms
and shoots with SAR inducers, biological agents, a growth
regulator, copper compounds, and other materials. *Acta Hortic*
**590**: 325–331.

induced trehalose and glycine betaine accumulation improves
tolerance to desiccation, survival and efficacy of the
postharvest biocontrol agent *Pantoea agglomerans* EPS125.

Broggini GAL, Duffy B, Holliger E, Scharer HJ, Gessler C &
Patocchi A (2005) Detection of the fire blight biocontrol agent
Epiphytic fitness of a biocontrol agent of fire blight


Van der Zwart T & Keil HL (1979) *Fire Blight: A Bacterial Disease of Rosaceous Plants*. Department of Agriculture, Science and Education Administration, Washington, DC.


