Field Results for the Efficacy of Fire Blight Control Agents in the Last Fifteen Years in Germany

S. Kunz
Bio-Protect GmbH
Konstanz
Germany

C. Donat
Bio-ferm GmbH
Tulln
Austria

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Abstract

Fire blight caused by *Erwinia amylovora* is the most serious bacterial disease in apple and pear. During the last four decades, it has spread throughout Europe. Under favorable weather conditions *E. amylovora* multiplies on blossom surfaces and invades the plant tissue by the nectarthodes in the hypanthium routed by chemotaxis. Each blossom is a potential infection site and therefore efficient control agents are needed to prevent blossom infections. Streptomycin, although banned in the EU, is sometimes used in emergency situations in a few countries (e.g., Germany and Austria). In USA and Israel, *E. amylovora* developed resistance against this antibiotic. Since 1997, field trials were conducted in Germany to find alternative control agents or strategies to replace streptomycin. About 100 different materials were tested during the last 15 years. Some of them are now registered for fire blight control in Germany. From the products available, Blossom Protect, which is composed of two strains of *Aureobasidium pullulans* and an acidic buffer, showed the highest efficacy, followed by LMA (potassium-aluminum sulfate), calcium-formate, Myco-Sin and Serenade. Blossom Protect alone or in combinations with other preparates provided a level of control similar to streptomycin in trials in Germany as well as in the USA; it is now is registered in many European countries, Morocco, Turkey, USA and Canada.

INTRODUCTION

The fire blight pathogen *Erwinia amylovora* spread throughout Europe during the last four decades. The first detection in Germany was on the Island of Sylt in the North of the country in 1971. The pathogen was spread to the southern parts of Germany and reached Baden-Württemberg in 1981 and the apple growing region around the Lake Constance in 1993. Sanitation measures such as pruning infected shoots and uprooting infected trees were the only control measures. After 1993, it was realized that eradication of the disease would not be possible owing to epiphytic and endophytic establishment of *E. amylovora* on and in trees free of symptoms (Voegele et al., 2010). Under favorable weather conditions, *E. amylovora* multiplies on blossom surfaces (e.g., stigma) and invades the plant tissue through the nectarthodes in the hypanthium (Pusey and Smith, 2008) routed by chemotaxis (Raymundo and Ries, 1980). Each blossom is a potential infection site and therefore efficient control agents are needed to prevent blossom infections.

In 1994 streptomycin was used for the first time in Germany to control fire blight (Meinert, 1997). The plant protection services started field trials under natural infection conditions in 1994 to prove the efficacy of streptomycin. In 1997, Fried established a system to test products under nearly natural conditions with high infection pressure, by inoculating one tree per plot in field trials (Fried, 1997). Although antibiotics are in general not allowed for plant production in Germany, streptomycin was permitted in emergency situations. The detection of residues of streptomycin on treated apples and in honey from bee hives nearby treated orchards illustrated the need for alternative products in fire blight control.

Since 1997 field trials were conducted in Germany to find alternative control agents or strategies to replace streptomycin. Up to seven trial sites in southern Germany were used. Fungicides, plant strengtheners, fertilizers and disinfectants were tested. In addition research was initiated to develop new control agents for fire blight control resulting in products like Blossom Protect (*A. pullulans* + citric acid buffer) and LMA (potassium-aluminum sulfate). A summary of the published results from this field trials are given here.

MATERIAL AND METHODS

Field trials. Experiments to test the efficacy against fire blight were carried out in Germany in accordance with the EPPO guideline PP1/166 (3). One tree per orchard plot was inoculated with *E. amylovora* ($1 \times 10^6 - 5 \times 10^8$ CFU/ml). From this tree the pathogen was spread over the entire orchard by natural vectors (Fried, 1997). Only the results from trees which had not been inoculated were taken into account for the figures and the discussion in this paper. In two cases, trials were done under natural infection conditions without artificial inoculation (Knaus and Joseph, 2012; Renner, 2001). In the USA field testing was done with artificial inoculation ($5 \times 10^5 - 1 \times 10^6$ CFU/ml) and the inoculated blossom clusters were evaluated for fire blight symptoms.

RESULTS AND DISCUSSION

Since 1997, field trials on fire blight control were conducted in Germany according to Fried (Fried, 1997) to find alternatives to the antibiotic streptomycin. Data on the efficacy of about 100 different products, product combinations and strategies were published. Out of this wide range of products only five were under discussion for further use in fire blight control.

Myco-Sin (acidic rock powder) was first tested in 1997 (Fried, 1997). It was listed as a plant strengthener in Germany and is registered as a plant protection agent in Switzerland. It is widely used in organic apple growing in both countries. Myco-Sin was tested in 9 field trials. Three to six applications were done and the efficiency ranged from 12 to 80% with an average of 52% (Fig. 1).

Serenade (*Bacillus subtilis*) was tested in different concentrations and formulations since 2000 (Fried, 2001b). Serenade formulations were tested in 15 field trials in Germany. There was no clear influence of the formulation on the performance of the product, so the results from different formulations are averaged. Three to seven applications were done and the efficiency ranged from 0 to 61% with an average of 43% (Fig. 1). Serenade is registered for fire blight control in USA and several European countries including Germany. However, up to now it has not been available on the market to purchase in Germany and therefore has not been used in the orchards. Comparable efficiencies were revealed in field trials done in Eastern United States between 2001 and 2006. Serenade formulations reduced fire blight incidence significantly in eight of eleven trials. Mean average percent reduction of blossom blight by the Serenade formulations and streptomycin was 36 and 66%, respectively (Sundin et al., 2009).

A test formulation of *A. pullulans* was used in four field trials in 2002 with an efficiency of 28% in average (Fried, 2002; Harzer, 2002; Moltmann et al., 2002). After these disappointing results, the formulation for *A. pullulans* was changed and in 2003 Blossom Protect (*A. pullulans* in combination with a citric acid buffer) was tested for the first time and reduced fire blight symptoms by 74% (Fried et al., 2004). In 17 field trials, Blossom Protect was applied two to four times and reduced fire blight incidence between 44 and 91% with an average of 78% (Fig. 1). It was listed as a plant strengthener in Germany since 2005 and is widely used in organic pome fruit production in strategies with Myco-Sin (Kunz et al., 2012). It also is registered as a plant protection agent in USA, Canada, Morocco, Turkey and several European countries. In a three year study in Oregon, a strategy using lime sulfur and fish oil for thinning in early bloom followed by two applications of Blossom Protect achieved 91% control, compared to 88% control achieved with streptomycin (Fig. 2) (Johnson and Temple, 2013). Blossom Protect contains living cells of *A. pullulans* and has to be combined with a citric-acid buffer (Buffer Protect) to adjust the spray suspension to pH 4. At this pH *A. pullulans* colonizes floral surfaces but growth of *E. amylovora* is inhibited. *A. pullulans* was detected on most of the treated blossoms on stigmas as well as in the hypanthium (Johnson and Temple, 2013) and by growing in the nectar, *A. pullulans* keeps the pH at low levels and disturbs the chemotaxis of *E. amylovora*, which requires a neutral pH (Raymundo and Ries, 1980). Blossoms Protect should be applied the day before infection conditions are fulfilled as *A. pullulans* needs few hours to establish in the blossom.

Fertilizers containing calcium-formate (Folanx Ca29 and LX4630) were tested in Germany since 2007 in different concentrations with one to four applications. In 12 trials efficiency ranged between 16 and 82% with an average of 55% (Fig. 1). These kinds of fertilizers are available in many countries and may be included in fire blight control strategies.

LMA (potassium-aluminum sulfate) was tested the first time in 2010 (Fried, 2011). Two to four applications and different concentrations were tested in recent years. Fire blight incidence was reduced between 54 and 81% with an average of 73% (Fig. 1). LMA received a registration for emergency use in 2013 in Germany. No data from other countries are available up to now.

Streptomycin, which is used in most of the field trials in Germany as a chemical standard, was applied one to four times and reduced fire blight incidence by 83% on average in 34 trials (Fig. 1). Streptomycin was the most consistent product with the highest efficacy, followed by Blossom Protect and LMA (Fig. 1). In Schlachters Research Station, trials were conducted every year under natural infection conditions. Fire blight incidence in untreated plots exceeded 5% only in two seasons, which is the minimum level to get meaningful results. In 2012, 18% of the blossom clusters showed symptoms in untreated plots: streptomycin, Blossom Protect, calcium-formate and LMA reduced fire blight incidence significantly (Fig. 3). This trial, done under natural infection conditions, reflected the performance of the products determined in inoculated plots over the last 15 years.

In the USA, several bacterial antagonists were tested and three commercial products are available: BlightBan A506 (*Pseudomonas fluorescens*), Bloomtime Biological (*Pantoea agglomerans*), Serenade (*B. subtilis*). All were inconsistently effective in reducing blossom infections in field trials conducted between 2001-2007 when examined individually (Sundin et al., 2009). In contrast, Johnson and Temple (2013) reported consistent fire blight control over three years with a strategy using thinning agents (lime sulfur + fish oil) and Blossom Protect, comparable to streptomycin and better than oxytetracycline (Fig. 2). The results of Johnson and Temple confirmed the results from Germany that Blossom Protect is highly effective in fire blight control and should be implemented in control strategies to reduce or even replace antibiotics.

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Figures

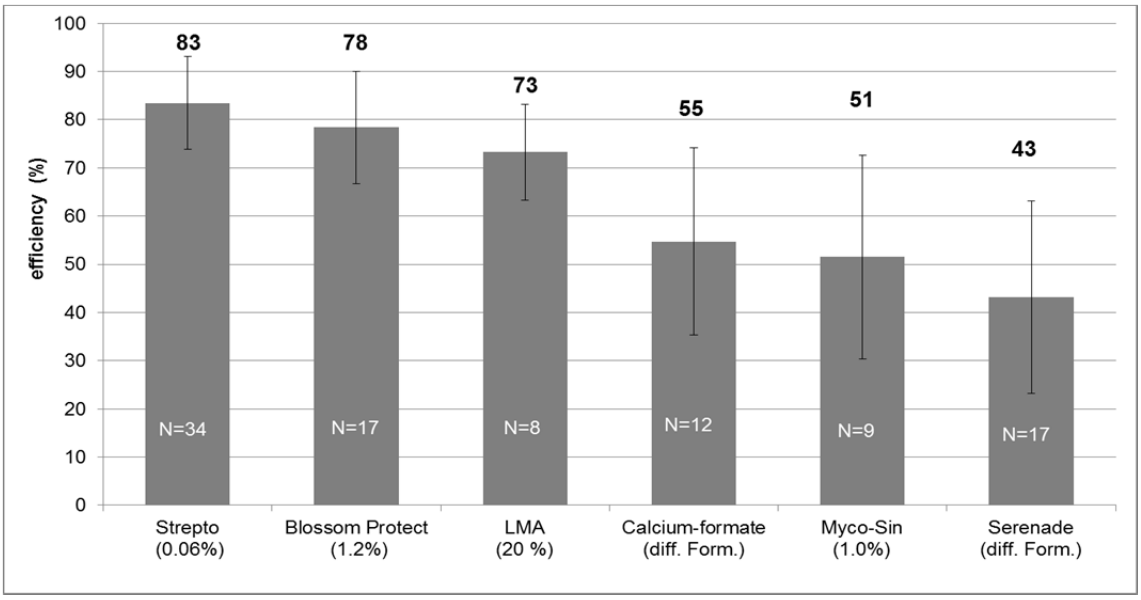
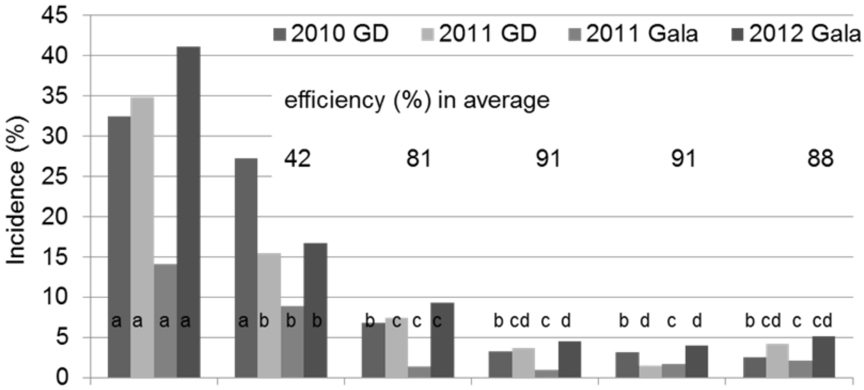
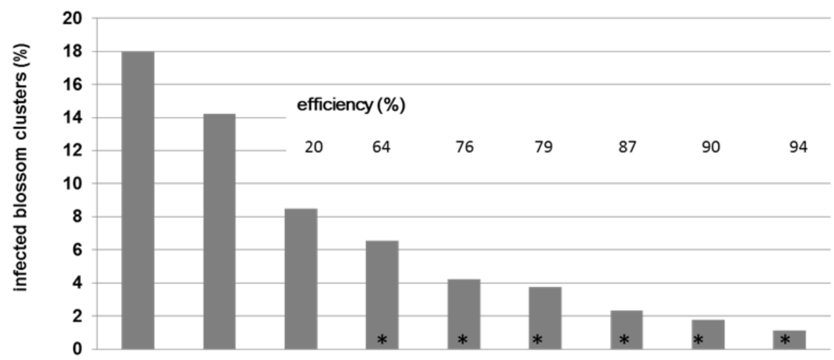


Fig. 1. Efficiency of selected products in published trials on fire blight control in Germany 1997-2012 (Fried, 1997, 1999, 2001a,b, 2002, 2007, 2008, 2009, 2010, 2011, 2012a,b; Fried et al., 2004; Harzer, 2001, 2002; Harzer and Orth, 2008, 2012; Harzer and Krauthausen, 2011; Jelkmann, 2006; Kunz, 2012; Kunz et al., 2004, 2006, 2008, 2010, 2011; Laux et al., 2003; Scheer, 2009, 2012a,b; Scheer and Bantleon, 2009; Scheer et al., 2007). Only results from trees with secondary infections or natural infections were considered.



30% bloom	-	LSFO		LSFO	LSFO	
70% bloom	-	LSFO	Bloo	LSFO	LSFO	
Inoculation ($5x10^5$ -$1x10^6$ E. amylovora/ml)						
Full bloom	water	-	oxy	BlosP	BlosP+Bloo	Strep
Prior to petal fall	-	-	-	BlosP	BlosP	

Fig. 2. Fire blight incidence in four trials in Oregon and the average efficiency of the control strategies after artificial inoculation. Within one trial (year and cultivar) different letters indicate significant differences according to Fischer's LSD test ($p<0.05$). LSFO: Lime sulfur (2%) + fish oil (2%); Bloo: Bloomtime biological (*P. agglomerans*); oxy: FireLine (oxytetracycline); BlosP: Blossom Protect (*A. pullulans* + citric acid buffer); Strep: FireWall (streptomycin sulfate) (Johnson and Temple, 2013).



date	stage	EIP (%)									
10.4.	green bud		-	-	Vacc	-	-	-	-	-	-
17.4.			-	-	Vacc	-	-		-	-	-
22.4	5% open bl.		-	-	Vacc	-	-	-	-	-	-
27.4.		87	-	-	Vacc	-	-	-	BlosP	-	-
28.4.	80% open bl.	185	-	water	-	2 LMA	LX4630	1 LMA	-	Strep	Strep
29.4.		224	-	-	-	-	-	-	-	-	-
30.4	100% oben bl.	232	-	-	-	2LMA	-	1 LMA	BlosP	-	-

Fig. 3. Fire blight incidence in Schlachters 2012 and efficiency of the control agents under natural infection conditions. EIP was calculated with Maryblyt™ 7. The trial was done in a randomized block design with four replications. Means were compared with an LSD test. * marks treatments in which incidence was significantly reduced in comparison to the untreated control ($p<0.05$). Vacc: 0,75 L/ha Vacciplant; 2LMA: 20 kg/ha LMA; 1LMA: 10 kg/ha LMA; LX4630: 15 kg/ha LX4630; BlosP: 12 kg/ha Blossom Protect; Strep: 0,6 kg/ha streptomycin (Knaus and Joseph, 2012).