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Abstract

In organic fruit growing effective control strategies are needed to prevent blossom infections by the fire blight pathogen Erwinia amylovora. Many potential control agents are under discussion and have been tested in vitro and in vivo. 19 out of 27 tested preparations showed a high efficacy against E. amylovora in vitro. Nevertheless, on detached apple blossoms only 7 of them led to a symptom reduction by more than 50%. In six field trials conducted according to the EPPO guideline PP1/166(3) BlossomProtect (82%), Myco-sin (65%) and Funguran (58%) had the highest efficiency. In 2006 and 2007, strategies to integrate BlossomProtect in spray schedules of organic apple production have been tested. The use of sulphur or lime-sulphur before or after BlossomProtect did not influence the efficiency of BlossomProtect, which showed that fire blight control is possible without compromising apple scab control. The addition of Cutisan to BlossomProtect reduced fruit russet. An alternating use of BlossomProtect and Myco-sin was shown to be possible.

Keywords: Fire blight, Erwinia amylovora, BlossomProtect, Myco-sin, Fruit russet

Introduction

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. Since pruning of diseased material and other sanitation methods could not stop the spread of the disease, efficient control agents are needed to prevent blossom infections. Many potential control agents were under discussion, but seldom reliable data on the efficacy were available. Therefore a three step evaluation procedure was established including laboratory tests *in vitro* and *in vivo* as well as field trials. The laboratory tests in shaken cultures and on detached blossoms gave information on the mode of action of the control agents (Kunz 2006). Many control agents suppressed *E. amylovora in vitro*, illustrating their potential for bacteriostatic behaviour. The *in vitro* activity was indicative for activity but not sufficient to predict a high effectiveness on detached blossoms, or in the field (Kunz et al. 2006, Kunz et al., 2004).

On detached blossoms and in field experiments BlossomProtect had the highest efficiency (Kunz et al. 2004, Kunz et al., 2006). Therefore it was included in fire blight control strategies in organic apple orchards. BlossomProtect consists of the yeast *Aureobasidium pullulans* which is sensitive to fungicides including sulphur and lime sulphur, which are used for apple scab control. Since BlossomProtect is not miscible with these fungicides, strategies for alternating BlossomProtect with sulphur or lime sulphur applications have been tested in field trials.

Material and Methods

Measurements of growth rates of *E. amylovora* in liquid cultures and the reduction of fire blight symptoms on detached blossoms was measured as described (Kunz et al. 2006).

Field trials were done in accordance with the EPPO guideline PP1/166 (Kunz et al., 2004).

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In the test orchards one tree per plot was inoculated with the pathogen, from this tree *E. amylovora* 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. The field trials conducted in the year 2004 in Groß-Umstadt and Karsee (Kunz et al., 2004) as well as the trials from 2006 in Darmstadt and Karsee (Kunz et al., 2006) are already described.

In 2007 two trials were done. In Darmstadt in an orchard of the variety `Idared` planted in 1999, copper preparations and BlossomProtect were tested. When approximately 25% of the flowers were opened (April 17) the first application of the test preparations was done and afterwards one tree per plot was inoculated with a suspension containing $2x10^7$ cells/ml of *E. amylovora* (Ea702, Ea725 and Ea743). When approximately 40% (April 19), 70% (April 21) or 90% (April 23) of the flowers were opened the test preparations were applied again. Total numbers of blossom clusters were counted the 16^{th} of April. The blossom clusters showing fire blight symptoms were counted the 23^{rd} of May. From these data the fire blight incidence was calculated for each plot. Mean incidence for the different treatments were statistically compared using Tukey`s Multiple Comparison test (p≤0.05).

Table1: Application dates (X) in the field trial in Karsee 2007, treatments against fire blight and apple scab, progress in flower opening and risk according to the fire blight warning system Maryblyt (M=moderate; HT- = High risk, average temperature is to low for infections).

Date, April-May 2007	23	24	25	26	27	28	29	30	01	02
Fire blight according to phenology (Ph)	Х		Х		Х			Х		
Fire blight according to Maryblyt (Mb)	Х				Х			Х		
Apple scab (sc)			Х		Х					Х
Inoculation	Х									
opened flowers (%)	10		50		80			95		99
risk according to Maryblyt	Μ	Μ	HT-	HT-	HT-	HT-	HT-	HT-	М	Μ

Table 2: Variety and dates of treatments and evaluations in the field trials 2007 in Mainau and Lindau. The means of the russet indices of BlossomProtect (12g/l) and the control were statistically compared with a two tailed T-test within one variety. Significant differences ($p \le 0.05$) are marked with an asterisk.

Location	Mainau		Lindau				
Variety	Sansa	Santana	Jonagold	Goldrush	Braeburn	Williams	
No. of treatments	2	3	4	3	4	3	
Application dates		04-15	04-16	04-16	04-16	04-14	
	04-20	04-17	04-18	04-18	04-18	04-16	
	04-23	04-20	04-21	04-21	04-21	04-18	
			04-22		04-22		
Evaluation date	08-09	08-09	08-23	08-23	08-23		
Russet index							
Control	1.18	1.17	1.18	1.05	1.03	2.11	
BlossomProtect	2.22	1.37*	1.64*	1.12*	1.03	2.30	

In Karsee different fire blight control strategies were tested on potted apple trees of the variety `Pinova`. Applications against fire blight were done either according to the phenological progress of the flowers (Ph) or according to the warning system Maryblyt (Mb).

Weather data were recorded in the test orchard and forecasts were calculated by P. Triloff, Friedrichshafen. Fungicides against apple scab (sc) were applied alternating to the fire blight applications (tab. 1). When approximately 10% of the flowers were opened (April 23) the first application against fire blight was done and afterwards one tree per plot was inoculated with a suspension containing 5×10^7 cells/ml of *E. amylovora* (Ea639, Ea705 and Ea763). The other treatments, the progress of flowering and the risk according to Maryblyt are listed in table 1. Total numbers of blossom clusters were counted the 2^{nd} of May. The blossom clusters showing fire blight symptoms were counted the first of June. From these data the fire blight incidence was calculated for each plot. Mean incidence for the different treatments were statistically compared using Tukey's Multiple Comparison test (p<0.05).

Field trials on the influence of treatments on fruit russet: Experiments were conducted in organic apple and pear orchards in a randomised block design with four replications per treatment. Control agents were applied with a backpack sprayer when fire blight infection periods were forecasted (tab. 2). All of the fruit from 4 trees per plot were classified into 4 classes according to the russeted area. For each plot the russet index was calculated (Haug et al., 2006).

Results

In addition to the 18 preparations already described in former publications (Kunz 2006, Kunz et al., 2006) nine preparations have been tested. The use of 10g/l Temprotect (Temmen GmbH) or 0.25g/l In-Wa Quarz (Kaus Hinrich Schulz) neither reduced the growth of *E. amylovora* in liquid cultures nor prevented symptom development on detached blossoms. With 40g/l Asepta Erwinia I (Asepta B.V.), 40g/l Asepta Erwinia II (Asepta B.V.), 20 g/l Temauxin A (Temmen GmbH), 20g/l Temauxin S (Temmen GmbH), 5g/l KHCO₃ or 5 g/l K₂CO₃ the growth of the pathogen in liquid cultures was inhibited completely, but the symptom reduction on detached blossoms was below 30%. BPGP07 (Bio-Protect GmbH) used in a concentration of 8.5 g/l was the only new preparation which had a high efficiency in both test systems and was therefore tested in the field.

In the field trial in Darmstadt 2007 a disease incidence of 33% infected blossom clusters was observed on the non treated trees. Four applications of 12 g/l BlossomProtect, 0.3 g/l Funguran or 10 g/l Kupferprotein reduced the fire blight incidence significantly with efficiencies of 82%, 62% or 71% respectively.

In Karsee 2007 a disease incidence of 11% was observed. During bloom, six days with high risk for fire blight infections were detected by the Maryblyt model (tab. 1). Following the control strategy of applying the control agents according to Maryblyt, three applications were necessary. This strategy was compared with the application of control agents according to the phenological progress of the flowers, in which 4 applications were done. In addition to the fire blight control according to Maryblyt in some treatments fungicides were applied for scab control (tab. 1). All treatments revealed a significant reduction of fire blight incidence (tab.3). BlossomProtect tended to have a higher efficiency than BPGP07, Myco-sin or the combination of BlossomProtect and Cutisan (used to reduce russet development). No difference was found between the application of BlossomProtect according to phenology or according to Maryblyt. The application of BlossomProtect. The scab control with lime sulphur, sulphur (Netzschwefel Stulln) and a mixture of sulphur and Myco-sin did not influence the efficiency of BlossomProtect.

Table 3: Preparations, applied concentrations, control strategy (Ph= phenology; Mb= according to Maryblyt; sc=used for apple scab control), number of treatments (No.; dates are given in tab. 1), fire blight incidence at the 1st of June and efficiency (Eff.) of the strategy in the field trial in Karsee 2007. Different letters behind the incidence indicate a significant difference in Tukey's Multiple Comparison test ($p \le 0.05$).

	Strategy	No.	Incidence fire blight (%)	Eff. (%)
Myco-Sin (10 g/l)	Ph	4	3.0 (c)	74
BlossomProtect (12 g/l)	Ph	4	1.3 (c)	89
BlossomProtect (12 g/l) + Cutisan (15 g/l)	Ph	4	2.9 (c)	74
BPGP07 (8.5 g/l)	Ph	4	2.4 (c)	78
Funguran (0.3 g/l)	Mb	3	6.9 (b)	38
BlossomProtect (12 g/l)	Mb	3	1.9 (c)	83
BlossomProtect (12 g/l) alternating with Lime sulphur(15 g/l)	Mb sc	3 3	2.5 (c)	77
BlossomProtect (12 g/l) alternating with Netzschwefel Stulln (2.5 g/l)+ Myco-Sin (10 g/l)	Mb sc	3 3	1.5 (c)	87
BlossomProtect (12 g/l) alternating with Netzschwefel Stulln (2.5 g/l)	Mb sc	3 3	1.8 (c)	84
control		-	11.2 (a)	-

The influence of control agents on fruit russet was tested in organic pear and apple orchards located in Lindau and on the Isle of Mainau. 12g/I BlossomProtect was applied 2 to 4 times during bloom to different varieties. The number of applications depended on the appearance of open flowers. On the pear variety 'Williams' and on the apple varieties 'Sansa' and 'Braeburn' no significant increase of fruit russet was detected. The apple varieties 'Santana', 'Goldrush' and 'Jonagold' reacted with a significant increase in fruit russet (tab. 2).

On the variety 'Jonagold' additional treatments were tested. The application of BlossomProtect led to the highest russet index, which was significantly reduced by the addition of Cutisan (15 g/l) (fig. 1). Funguran (0.3 g/l) increased the russet index significantly in comparison to the control. The addition of Cutisan tended to reduce the russet index of Funguran.

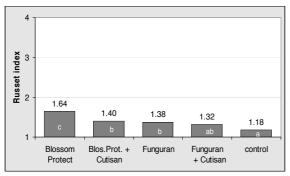


Figure 1: Russet index on fruits of the variety 'Jonagold' in Lindau after four treatments with BlossomProtect (12 g/l), Funguran (0.3 g/l) or each of both in mixture with Cutisan (15 g/l) in comparison to an untreated control. Different letters indicate a significant difference in Tukey's Multiple Comparison test ($p \le 0.05$).

Discussion

Many preparations are under discussion for use in fire blight control. In a systematic evaluation 27 control agents have been tested in different test systems during the last four years. 20 control agents suppressed E. amylovora in vitro, illustrating their potential for bacteriostatic behaviour. However, on detached apple blossoms only six preparations were able to reduce symptom development by more than 50%. The efficacy of a preparation on detached blossoms corresponded to the efficacy in field trials (Kunz et al., 2006). Therefore only preparations with a high efficacy on detached blossoms were further tested in the field. BlossomProtect (A. pullulans), Myco-sin (stone meal) and Funguran (copper) showed the highest efficiencies in field trials 2004 to 2006 (tab. 4). In 2007 we concentrated on these active ingredients and tested copper compounds in Darmstadt and strategies to integrate BlossomProtect in spray schedules in Karsee. The field trials 2007 confirmed the good performance of BlossomProtect and Myco-sin in fire blight control found in former years (tab. 4). In Darmstadt the new copper compound Kupferprotein tended to have a higher efficiency than Funguran although the amount of copper which was applied was reduced from 135 g/ha using Funguran to 44 g/ha using Kupferprotein. As Funguran was not effective in Karsee and as there is a risk of enhancing fruit russet when using copper during bloom, the usefulness of copper for fire blight control is still questionable.

BlossomProtect contains blastospores of *A. pullulans*. Despite reports on *A. pullulans* being able to cause fruit russet (Matteson-Heidenreich et al., 1997, Spotts et al., 2002), no significant increase in russeted fruits after the application of BlossomProtect could be determined on the varieties 'Golden Delicious Reinders' or 'Jonagored'' in former years (Kunz, 2006, Haug et al., 2005) or in field trials carried out over nearly 20 ha by organic farmers in 2004 and 2005 (Haug et al., 2005). However, in 2007 three to four applications of BlossomProtect caused a significant increase in fruit russet on the varieties 'Santana', 'Goldrush' and 'Jonagold', but not on the varieties 'Sansa' and 'Braeburn or on the pear variety 'Williams'. The addition of Cutisan to BlossomProtect reduced the fruit russet on 'Jonagold' significantly but it tended also to slightly reduce the efficacy of BlossomProtect (tab. 4).

A. pullulans as a fungus is sensitive to fungicides including sulphur and lime sulphur which are used for apple scab control. BlossomProtect can not be mixed with these fungicides.

But spray strategies in which BlossomProtect was alternated with sulphur fungicides did not hamper the efficacy of BlossomProtect against fire blight (tab. 4). The use of lime sulphur tended to be more critical than the use of Netzschwefel Stulln. As Myco-sin is known to enhance the efficacy of sulphur against apple scab and there is a proven effect against fire blight, we tested a strategy to alternate BlossomProtect applications with sprays of a mixture from Netzschwefel Stulln and Myco-sin. This strategy was as effective against fire blight as BlossomProtect alone. The use of this strategy could allow the reduction of the number of BlossomProtect treatments from 4 to 2 applications per year, which will reduce costs and the risk for fruit russet. This hypothesis will be tested in field trials next year.

Table 4: Efficieny (%) of test preparations and spray strategies in field experiments 2004-2007. Data from field trials performed in Groß-Umstadt (GU), Darmstadt (DA) and Karsee (KA) in the years 2004 (Kunz et al., 2004) and 2006 (Kunz et al., 2006) were taken from the cited literature. Only the results from trees not inoculated with the pathogen were considered. In all trials the preparations have been applied four times during bloom unless marked with an asterisk (three applications). The numbers in brackets indicate the number of applications of the fungicides used alternating to BlossomProtect in the described strategies.

	Average	GU 04	KA 04	DA 06	KA 06	DA 07	KA 07
BlossomProtect (12 g/l)	82	66	85	85	86	82	89
Mycosin (10 g/l)	65	54	56	80	60		74
Funguran (0.3 g/l)	58				74	62	38*
Kupferprotein (10 g/l)	71					71	
BlossomProtect (12 g/l)	76						
+ Cutisan (15 g/l)					77		74
BlossomProtect (12g/l)			68	87*			77*
altern. Lime Sulphur (15 g/l)			(4)	(1)			(3)
BlossomProtect (12g/l)				85	88		84*
altern. Netzschwefel Stulln (2.5 g/l)				(1)	(3)		(3)

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