Fire blight control strategies in organic fruit growing

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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*. In a research project, funded by the "Bundesprogramm Ökologischer Landbau", 44 different preparations have been tested. In nine field trials conducted since 2004 according to EPPO guideline PP1/166(3) BlossomProtect and MycoSin had the highest efficiencies. BlossomProtect contains blastospores of* Aureobasidium pullulan*s, which, as a fungus, is sensitive to fungicides including sulphur that are used for apple scab control. In some cases, it enhanced fruit russet.*

Spray strategies, in which BlossomProtect was alternated with sulphur fungicides did not hamper the efficiency of BlossomProtect against fire blight. However, up to 8 applications had to be done during bloom to achieve fire blight and scab control. As MycoSin is known to enhance the efficacy of sulphur against apple scab and there is a proven effect against fire blight, a strategy to alternate BlossomProtect applications with sprays of a mixture of Netzschwefel Stulln and MycoSin was tested in field trials 2008 and 2009. With this strategy the total number of applications during bloom could be reduced to four, with only a slight decline in fire blight control. In addition, the number of applications of BlossomProtect was reduced from four to two in this strategy and thus, the risk of fruit russet declined.

Keywords: Fire blight control strategy, *Erwinia amylovora*, BlossomProtect, MycoSin

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. Sanitation methods like pruning of infected shoots and uprooting of infected trees are necessary to reduce infection pressure in the orchards. However, it is not possible to eliminate all fire blight bacteria due to their epiphytic and endophytic abundance on and in trees free of symptoms (Vögele et al, in this Conference proceeding). Under favourable weather conditions *E. amylovora* multiplies on blossom surfaces (e.g. stigma) and invades the plant tissue by the nectarthodes in the hypanthium (Pusey and Smith, 2008). Each blossom is a potential infection site and therefore efficient control agents are needed to prevent blossom infections. Many potential control agents were under discussion, but seldom reliable data on the efficiency were available. 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 et al., 2009). Out of 44 tested control agents 31 suppressed *E. amylovora in vitro*, illustrating their potential for bacteriostatic mode of action (Kunz et al., 2009). The in vitro activity was indicative for activity in general, but not sufficient to predict a high effectiveness on detached blossoms. On detached blossoms 13 control agents reduced fire blight symptoms by more than 60%. Five were copper com-

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pounds, five contained *Aureobasidium pullulans*, two contained *Bacillus amyloliquefaciens* and one was an acidic stone dust (Kunz et al., 2009).

In field trials done from 2004 to 2007 out of these control agents, BlossomProtect had the highest efficiency (Kunz et al., 2008, Kunz et al., 2006, Kunz et al., 2004). However, *A. pullulans*, the active microorganism in BlossomProtect, is sensitive to fungicides including sulphur and lime sulphur, and at high concentrations can cause fruit russet (Spotts and Cervantes, 2002). Therefore different spray strategies with BlossomProtect were tested in field trials, which aimed at fire blight as well as scab control without enhancing fruit russet.

Material and Methods

Field trials to test the efficiency against fire blight were done in accordance with the EPPO guideline PP1/166(3). In the 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), the trials from 2006 in Darmstadt and Karsee (Kunz et al., 2006) as well as the trials from Darmstadt and Karsee 2007 are already published (Kunz et al., 2008).

In **Karsee 2008** different fire blight control strategies were tested on potted apple trees of the variety \`Pinova\`. Five new control agents (Folanx Ca29; Cueva, Kupferprotein 08, BoniProtect, Hydrocal super) and a mixture of BlossomProtect and Netzschwefel Stulln were tested by applying them according to the phenological stage of the flowers (Ph) in comparison to BlossomProtect. In addition three control strategies were tested according to the warning system Maryblyt (Mb), which indicated that three applications against fire blight were necessary, which were supplemented with two applications against apple scab (sc). (application rates and details see in table 3). Weather data were recorded in the orchard, from which forecasts were calculated by the Maryblyt model. Fungicides against apple scab were applied alternating to the fire blight applications (tab. 1). When approximately 30% of the flowers were opened (May 14th) the first application against fire blight was done and afterwards one tree per plot was inoculated with a suspension containing 1×10^7 cells/ml of *E. amylovora (*Ea385, Ea610 and Ea725). All treatments, the progress of flowering and the risk according to Maryblyt are listed in table 1. Total numbers of blossom clusters were counted on May 23rd. Blossom clusters showing fire blight symptoms were counted on June 19th. 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 ($p \leq 0.05$).

Table1: Application dates (X) in the field trial in Karsee 2008, treatments against fire blight (FB) and apple scab, progress in flower opening and risk according to the fire blight warning system Maryblyt (M=moderate; HT- = High risk, but average temperature is too low for infections; I=infection).

Date, May 2008	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
FB acc. to phenology	X		X			X				X			X		
FB acc. to Maryblyt	X									X			X		
Apple scab			X			X									
Inoculation	X														
opened flowers (%)	30		65			83				90			95		99
risk acc. to Maryblyt	M	HT-	I	M	M	M	M	M	M	M	M	M	H	I	I

In **Darmstadt** field trials in randomized block design with 4 replicates were done in **2008** and 2009 in an orchard of the variety \`Idared\` planted in 1999. In 2008 Temauxin A as an inducer of resistance was first applied in the red bud stage (April 23rd), and a second and last time on April 28th. When approximately 15% of the flowers were open (April 26th) the first application of the other test preparations (tab. 4) was done and afterwards one tree per plot was inoculated with a suspension containing 2x10^{7} cells/ml of *E. amylovora* (Ea815, Ea797 and Ea743). When approximately 40% (April 28th), 70% (April 30th), 90% (May 2nd) or 100% (May 6th) of the flowers were open these test preparations were applied again. Total numbers of blossom clusters were counted April 18th, and blossom clusters showing fire blight symptoms were counted May 20th. 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 ($p<0.05$).

2009: Temauxin A was applied in the red bud stage (April 15th). When approximately 20% of the flowers were opened (April 19th) the first application of the other test preparations (tab. 4) was done and afterwards one tree per plot was inoculated with a suspension containing 8x10^{7} cells/ml of *E. amylovora* (Ea763, Ea839 and Ea840). When approximately 45% (April 21st) of the flowers were opened Temauxin A was applied a second and last time, and the other test preparations were applied a second time. In addition, a second inoculation took place on the same trees per plot as on April 19th (1.6x10^{7} cells/ml of *E. amylovora*), because the opening of flowers then was unevenly distributed in the different replicates. When 75% (April 23rd) and 90% (April 25th) of the flowers were opened the test preparations were applied again. Total numbers of blossom clusters were counted April 27th, and blossom clusters showing fire blight symptoms were counted June 2nd. 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 orchards in a randomised block design with four replications per treatment. Control agents (12 g/l BlossomProtect; 0,6g/l SPU2700) were applied with a backpack sprayer (tab. 2). All of the fruit from 4 trees per plot were classified into 4 classes according to the russeted area per apple. For each plot the russet index was calculated (Haug and Kunz, 2005).

Table 2: Variety and dates of treatments and evaluations in the field trials 2008 in Mainau and 2009 in 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 08		Lindau 2009					
Variety	Sansa	Santana	Gala	Idared	Gold-rush	Braeburn	Summerred	Topaz
No. of treatments	3	4	3	3	3	3	3	3
Application dates	04-30 05-03 05-06	04-28 04-30 05-03 05-06	04-24 04-26	04-22 04-24 04-26	04-22 04-24 04-26	04-22 04-24 04-26	04-22 04-24 04-26	04-22 04-24 04-26
Evaluation date	08-18	08-18	09-18	09-18	09-18	09-18	07-25	09-18
Russet index								
Control	1.74	1.56	1.02	1.26	1.17	1.11	1.45	1.02
BlossomProtect	1.96*	2.06*	1.04	1.47*	1.23	1.11	1.44	1.02
BlossomPr.+ SPU2700								1.02

Results

In the field trial in Karsee 2008, two days with high risk for fire blight infections and three additional infection days were detected by the Maryblyt model (tab. 1). A disease incidence of 4.4% was observed in the untreated control. The analysis of variance revealed no significant effect of the treatments and strategies on disease level. However, the application of BlossomProtect and the mixture of BlossomProtect and Netzschwefel Stulln when applied according to phenology as well as the tested strategies reduced fire blight incidence significantly compared to the control in a T-test (tab. 3).

In the field trials in Darmstadt 2008 and 2009 high disease incidences of 42.7% and 20.5% were determined, respectively. BlossomProtect applied according the phenological stage was used as standard in both trials and resulted in an efficiency of 56% and 81%, respectively. The addition of 3g/l Cutisan to BlossomProtect tended to enhance the efficiency in 2008. Application of Baci M (*Bacillus amyloliquefaciens*) led to 41% efficiency. In both trials the resistance inducer Temauxin A was applied in the red bud stage and once during bloom, which resulted in a disease reduction of 35% to 38%. In 2009 a strategy, in which three BlossomProtect applications complemented the use of Temauxin A, showed fire blight control, comparable to that of four applications of BlossomProtect as stand alone treatment. Alternating BlossomProtect and a mixture of Netzschwefel Stulln with MycoSin reduced fire blight incidence by 74% (tab. 4).

Table 3: Treatments (Ph= phenology; Mb= according to Maryblyt; sc=used for apple scab control, #=tank mix), applied concentrations, number of treatments (No.), fire blight incidence on June 19 and efficiency (Eff.) in the field trial in Karsee 2008. Different letters behind the incidence indicate a significant difference in Tukey`s Multiple Comparison Test ($p \leq 0.05$). *Significant difference to the control in the T-test ($p<0.05$).

	Strategy	No.	Incidence fire blight (%)	Eff. (%)
Folanx CA29 (10 g/l)	**Ph**	4	**1.8 (a)**	**59**
BlossomProtect (12 g/l)	**Ph**	4	**0.9 (a)***	**80**
Hydrocal super (dusted)	**Ph**	4	**4.5 (a)**	**0**
Cueva (8.5 ml/l)	**Ph**	4	**2.6 (a)**	**41**
Kupferprotein (5.3 ml/l)	**Ph**	4	**3.0 (a)**	**33**
BoniProtect (1 g/l)	**Ph**	4	**3.6 (a)**	**19**
BlossomPr. (12 g/l)+Netzschw. Stulln (2.5 g/l)#	**Ph**	4	**1.0 (a)***	**77**
BlossomProtect (12 g/l) alternating with Cueva (8.5 ml/l)	**Mb sc**	3 2	**1.9 (a)**	**57**
BlossomProtect (12 g/l) alternating with Netzschwefel Stulln (2.5 g/l)+ MycoSin (10 g/l)	**Mb sc**	3 2	**1.3 (a)***	**87**
Cueva (8.5 ml/l) alternating with Netzschwefel Stulln (2.5 g/l)+ MycoSin (10 g/l)	**Mb sc**	3 2	**1.0 (a)***	**77**
control		-	**4.4 (a)**	**-**

Table 4: Treatments, applied concentrations, number of treatments (No.), fire blight incidence and efficiency (Eff.) in the field trials in Darmstadt 2008 and 2009. Different letters behind the incidence indicate a significant difference in Tukey`s Multiple Comparison test ($p \leq 0.05$).

	2008			2009		
	No.	Incidence (%)	Eff. (%)	No.	Incidence (%)	Eff. (%)
BlossomProtect (12 g/l)	5	**18.8 (b)**	**56**	4	**3.9 (c)**	**81**
BlossomPr. (12 g/l) + Cutisan (3 g/l)	5	**14.8 (b)**	**65**	-	**-**	**-**
Baci M (5 g/l)	5	**25.0 (ab)**	**41**	-	**-**	**-**
Temauxin A (2 g/l)	2	**27.8 (ab)**	**35**	2	**12.7 (ab)**	**38**
BlossomPr. (12 g/l) alternating with sulfur (2.5 g/l)+MycoSin (10 g/l)	-	-	-	2 2	**5.3 (bc)**	**74**
Temauxin A (2g/l) alternating with BlossomPr. (12 g/l)	-		-	2 3	**4.4 (c)**	**78**
Control	-	42.7 (a)	-	-	**20.5 (a)**	**-**

The influence of BlossomProtect on fruit russet was tested in organic apple orchards located on the Isle of Mainau and in Lindau. BlossomProtect (12g/l) was applied 1 to 4 times during bloom at different application dates on the variety `Santana`in 2008. One or two applications had no significant influence on fruit russet, independent of the date of application. Three or four treatments increased fruit russet significantly (fig. 1). In ´Sansa`, three treatments increased the fruit russet significantly. In 2009 'Idared` reacted with a significant increase in fruit russet after three applications with BlossomPortect, whereas two applications on 'Gala' or three applications on ´Braeburn`, ´Goldrush`, ´Summerred` or 'Topaz' did not significantly increase fruit russet (tab. 2). 'Topaz' showed no increase in fruit russet, even when in two of the three treatments SPU2700 was added to BlossomProtect.

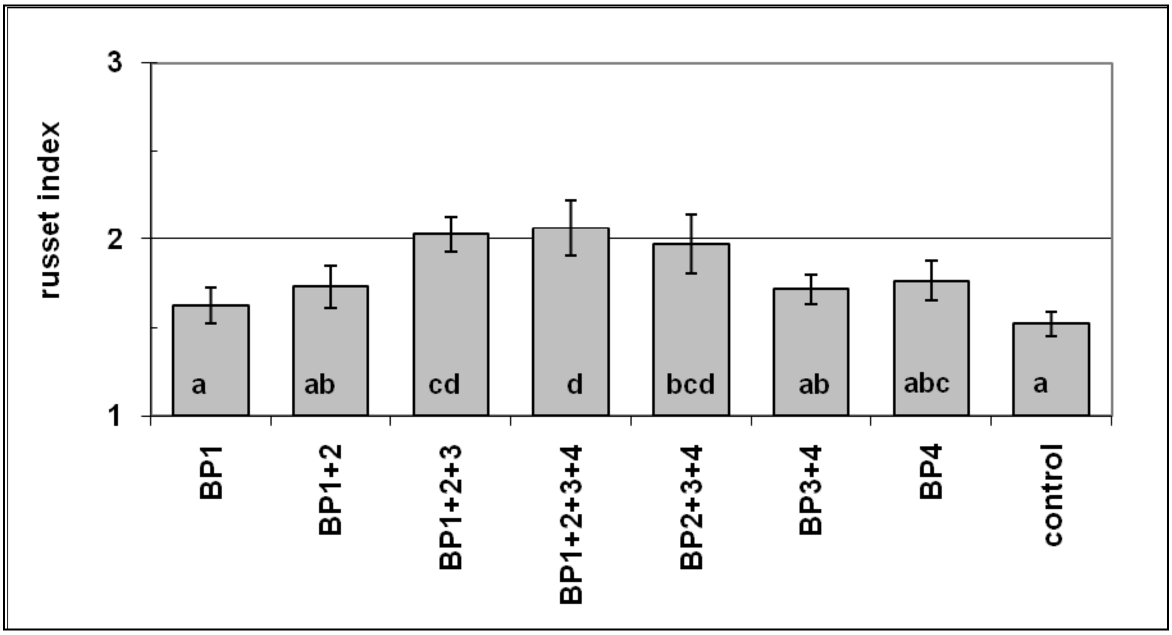

Figure 1: Russet index on fruits of the variety ´Santana´ in Mainau 2008 after varying numbers of treatments with BlossomProtect (12 g/l) (BP) in comparison to an untreated control**.** The numbers 1-4 represent the date of the treatment (1=4-28; 2=4-30; 3=5-03; 4=5-06). Different letters indicate a significant difference in Tukey´s Multiple Comparison test ($p \leq 0.05$).

Discussion

Since 2004, 44 preparations were tested for their efficiency against the fire blight pathogen *Erwinia amylovora in vitro* and on detached apple blossoms. Copper compounds, *A. pullulans, B. amyleloliquefaciens* and an acidic stone dust performed best in the detached blossom system and were studied further in field trials. In seven field trials done until 2007, BlossomProtect (*A. pullulans*) showed the highest efficiency, followed by MycoSin, Copper compounds and Serenade Max (*B. amyloliquefaciens*) (Kunz et al., 2009).

Different Copper preparations were tested to determine the formulation, which gave best efficiency with the lowest copper input. However, in field trials the formulation of the product had no influence on the efficiency, which instead depended on the amount of copper used, which should be minimum 200g Cu/ha per treatment (Kunz et al., 2009). At this dose, however, there is a great risk of enhancing fruit russet and furthermore, the use of copper is under discussion for ecological reasons, anyhow. Therefore we focused on strategies using BlossomProtect and MycoSin, as these are already commercially available in Germany.

BlossomProtect contains blastospores of *A. pullulans*. Despite reports on *A. pullulans* being able to cause fruit russet (Matteson-Heidenreich et al., 1997, Spotts and Cervantes, 2002), no significant increase in russeted fruits after its application could be determined on the varieties ´Golden Delicious` or ´Jonagored` in former years (Kunz, 2006, Haug and Kunz, 2005) and in field trials carried out on almost 20 ha by organic farmers in 2004 and 2005 (Haug and Kunz, 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` (Kunz et al., 2008). Three treatments in ´Sansa` increased the fruit russet in our trials in 2008 and in ‘Idared`, whereas two applications on ‘Gala’ or three applications on ´Braeburn`, ´Goldrush`, ´Summerred` or ‘Topaz’ did not significantly increase fruit russet. ‘Topaz’ showed no increase in fruit russet, even when in two of the three treatments SPU2700 (150g Cu/ha) was added to BlossomProtect (tab. 2). In 2008 the influence of the number of BlossomProtect applications on the enhancement of fruit russet was tested on the variety ‘Santana’. Three or four applications of BlossomProtect revealed a significant increase in fruit russet, whereas one or two applications did not (fig.1). These results indicate that the enhancement of fruit russet caused by BlossomProtect depends on the variety and on the number of treatments. We conclude that on varieties not susceptible to fruit russet (e.g. ‘Topaz’, ‘Gala’, ‘Braeburn’) fire blight control with up to four applications of BlossomProtect during bloom is possible, while on susceptible varieties (e.g. ‘Golden Delicious’, ‘Jonagold’, ‘Elstar’, ‘Santana’, ‘Sansa`) the number of applications should be reduced to two.

Table 5: Efficiency (%) of BlossomProtect and spray strategies in field trials 2004-2009 (Kunz et al., 2006, Kunz et al., 2004, Kunz et al., 2008). Only the results from trees not inoculated with the pathogen were considered. The numbers in brackets indicate the number of applications of BlossomProtect or of the fungicides used in the described strategies.

	KA 04	**KA 06**	**DA 06**	**KA 07**	**KA 08**	**DA 09**
BlossomProtect (12 g/l)	85 (4)	86 (4)	85 (4)	83 (3)	80 (4)	81 (4)
BlossomProtect (12 g/l) altern. lime sulphur (15 ml/l)	68 (4) (4)		87 (3) (1)	77 (3) (3)		
BlossomProtect (12 g/l) altern. wettable sulphur (3 g/l)		88 (4) (3)	85 (4) (1)	84 (3) (3)		
BlossomProtect (12g/l) altern. wettable sulphur (3g/l) + MycoSin (10g/l)				87 (3) (3)	70 (3) (2)	74 (2) (2)
tank mixture: BlossomProtect (12 g/l)+ wettable sulphur (3 g/l)					77 (4) (4)	
BlossomProtect (12 g/l) altern. Temauxin A (20 ml/l)						78 (3) (2)

A. pullulans as a fungus is sensitive to fungicides including sulphur and lime sulphur, which are used for apple scab control. In practice, spray strategies are needed, which attain both, fire blight and scab control during bloom. Spray strategies, in which BlossomProtect was alternated with sulphur did not hamper the efficiency of BlossomProtect against fire blight. However, the use of lime sulphur tended to be more critical than the use of Netzschwefel Stulln and the total number of applications during bloom was very high (tab. 5). As MycoSin 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 MycoSin. This strategy was almost as effective against fire blight as BlossomProtect alone. The use of this strategy allowed the reduction of total applications during bloom from 6-8 to 4 and a reduction of the number of BlossomProtect treatments per year from 4 to 2, which will reduce costs and the risk of fruit russet. The tank mixture of BlossomProtect with Netzschwefel Stulln would be a further possibility on varieties not susceptible to fruit russet and showed significant control of both fire blight and apple scab in Karsee 2008. However, further testing is necessary before this tank mixture could be recommended for use in commercial orchards.

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