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Development of strategies for fire blight control in organic fruit growing

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Abstract: Fire blight (*Erwinia amylovora*) is the most serious bacterial disease in pome fruit. Effective control strategies are needed to prevent blossom infections by *E. amylovora*. In Germany, many potential control agents have been considered and 64 different preparations were tested in laboratory trials to select the products with highest efficacy. In thirteen field trials conducted since 2004 Blossom Protect™ and Myco-Sin® had the highest efficiencies.

Blossom Protect contains blastospores of the yeast *Aureobasidium pullulans.* The blastospores are sensitive to fungicides that are used for apple scab control and, in some cases *A. pullulans* enhanced fruit russet when applied during bloom. The increase in fruit russetting depends on the number of applications and on the variety treated. Therefore spray strategies are needed controlling both, fire blight and apple scab, without increasing fruit russetting. Fire blight and apple scab control could be achieved using tank mixtures of Blossom Protect + wettable sulphur. With a strategy to alternate Blossom Protect applications with sprays of a mixture of wettable sulphur + Myco-Sin fire blight and apple scab was controlled significantly. In addition, this strategy reduced the number of Blossom Protect applications to two, and by this, reduced the risk for fruit russetting.

Key words: *Erwinia amylovora*, fire blight, Blossom Protect™, Myco-Sin®, control strategies

Introduction

Fire blight caused by *Erwinia amylovora* is the most serious bacterial disease in apple and pear. Sanitation methods such as 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 because of their epiphytic and endophytic abundance on and in trees free of symptoms (Voegele *et al*., 2010). Under favourable weather conditions *E. amylovora* multiplies on blossom surfaces (i.e. stigma) and invades the plant tissue through the nectarthodes in the hypanthium (Pusey and Smith, 2008). Each blossom is a potential infection site and therefore effective control agents are needed to prevent blossom infections.

In Germany, many potential control agents have been considered, but seldom have reliable data on their efficacy been available. At the University of Konstanz a three-step evaluation procedure was established including laboratory tests and field trials. The laboratory tests in shaken cultures and on detached blossoms gave information on the modes of action of the control agents (Kunz *et al*., 2009). Of 64 products tested in the laboratory, 26 were chosen for field trials. Blossom Protect™ had the highest efficiency in all these trials (Kunz *et al.*, 2011; Kunz *et al.*, 2009). However, *A. pullulans,* the active microorganism in Blossom Protect, is sensitive to fungicides and at high concentrations applied to blossoms can cause fruit russetting (Spotts and Cervantes, 2002). Indeed, in 2007 three to four applications of

Blossom Protect caused a significant increase in fruit russetting on the apple varieties ´Santana´, ´Goldrush` and `Jonagold`, but not on the varieties ´Sansa` and ´Braeburn` or on the pear variety `Williams` (Kunz *et al.*, 2008). Several trials in the consecutive years showed that the increase in fruit russetting caused by Blossom Protect depends on the variety treated and on the number of treatments. We concluded, that on varieties not susceptible to fruit russet (e.g. „Topaz", „Gala", „Braeburn", „Goldrush"), fire blight control with up to four applications of Blossom Protect during bloom is possible, while on susceptible varieties (e.g. „Golden Delicious", „Jonagold", „Elstar", „Idared", „Santana", „Sansa`,) the number of applications should be reduced to two (Kunz *et al.*, 2011). The second point which should be addressed using Blossom Protect for fire blight control is its embedding into spray schedules for apple scab control.

Therefore further field trials were carried out in 2011 to test (i) further preparations, which showed high efficiency in the laboratory and (ii), different spray strategies with Blossom Protect, which aimed at fire blight as well as scab control without enhancing fruit russetting.

Material and methods

Products tested in field trials

The products tested in the field trials 2011 have been supplied by the companies (Table 1).

Table 1: Supplier, dose rate, active ingredients and abbreviations (abbr.) of the products used in field trials 2011.

Preparation	abbr.	active incredient	rate [%]	supplier
Blossom Protect™	BlosP	*Aureobasidium pullulans* and buffer pH 4	1.2	Bio-Protect GmbH
Boni Protect®	BP	*Aureobasidium pullulans*	0.15	Bio-Protect GmbH
Chitoplant®	Chito	Chitosan	0.1	ChiPro GmbH
LX4630	LX	Calciumformate	1.5	Lanxess Distribution GmbH
Myco-Sin®	MS	acidic rock powder	1.0	Biofa AG
Netzschwefel Stulln	NS	wettable sulphur	0.25	Biofa AG
OmniProtect	OP	potassium carbonate	0.5	Bio-Protect GmbH
Vacciplant®	Vac	Laminarin	0.075	Belchim Crop Protection

Field trials

Field trials to test the efficiency of products against fire blight were carried out in accordance with the EPPO guideline PP1/166(3). One to four trees per orchard plot were inoculated with the pathogen. From the inoculated trees *E. amylovora* was spread over the entire orchard by natural vectors. Only the results from trees which had not been inoculated were taken into account. Results from the field trials conducted in the year 2004 in Groß-Umstadt and Karsee (Kunz *et al.*, 2004), from the trials in 2006 and 2007 in Darmstadt and Karsee (Kunz *et al.*, 2006; Kunz *et al.*, 2008) and from the trials in Darmstadt in 2008, 2009 and 2010 (Kunz *et al.*, 2010; Kunz *et al.*, 2011) have already been published.

In **Darmstadt in 2011** a field trial in a randomized block design with four replicates was carried out in an orchard of apple cultivar `Idared` planted in 1999. When approximately 20% of the flowers were open (April 11) the first application of the test products (Table 1) was made and afterwards one tree per plot was inoculated with a suspension containing $2.2x10^7$ cells/ml of *E. amylovora.* In block 1 and block 2 the development of the blossoms was faster than in blocks 3 and 4. Therefore blocks 1 and 2 were sprayed again at 40% open blossoms (April 14). All blocks were sprayed again when 55% (April 17) and 80% (April 20) of the flowers were open. The 20^{iest} of April a second inoculation was done with a pathogen suspension containing $2.2x10^7$ cells/ml. In blocks 3 and 4 the products were applied the fourth time when 95% of the blossoms were opened (April 23). Total numbers of blossom clusters were counted on April 26, and blossom clusters showing fire blight symptoms were counted on May 23^{rd}. From these data, the fire blight incidence was calculated for each plot. Statistical analyses of the data were done using one-way analysis of variance, and mean separation was accomplished using Tukey`s Multiple Comparison Test ($P \leq 0.05$).

In **Mühlingen in 2011** a field trial in a randomized block design with four replicates was carried out on potted apple trees of the cultivar `Gala`. Six strategies were tested in comparison to an untreated control and the standard application, in which four applications of Blossom Protect were done according to the phenological stage of the blossoms. Products used, application dates and the phonological stages open (percent open blossoms) are shown in Figure 2. When approximately 20% of the flowers were open (May 5), four trees per plot were inoculated with a suspension containing $1.5x10^8$ cells/ml of *E. amylovora.* The same trees were inoculated a second time four days later (May 9^{th}; 81% open blossoms) with $7x10^7$ *E. amylovora* cells/ml. Total numbers of blossom clusters were counted on May 11^{th}, and blossom clusters showing fire blight symptoms were counted on June 3^{rd}. From these data, the fire blight incidence was calculated for each plot. Apple scab (*Venturia inaequalis*) incidence was evaluated on 250 leaves per plot on May 31^{st}. The percentages of infected area of leaves were averaged.

Results and discussion

In the field trial in Darmstadt 2011, four products were tested for their efficiency against fire blight (Table 2). The weather conditions were not favourable for fire blight infections until 80% of the blossoms were opened. Then four days with high risk of fire blight infections were detected by the Maryblyt model (http://www.caf.wvu.edu/kearneysville/maryblyt/, March, 23rd 2010). A disease incidence of 43.8% blighted blossom clusters was observed in the untreated control. Blossom Protect™ applied according to the phenological stage was used as the standard application and resulted in a significant reduction of fire blight symptoms by 47%. The other products tested in this trial had no significant effects (Table 2). The efficiency of 47% measured for Blossom Protect in Darmstadt 2011 was the lowest efficiency measured for Blossom Protect in all trials done in this project since 2004 (Kunz *et al*., 2009; Kunz *et al*., 2010; Kunz *et al*., 2011).

In 13 field trials since 2004, 26 different preparations have been tested for efficacy against fire blight (Kunz *et al*., 2009; Kunz *et al*., 2010), from which 16 products are commercially available in Germany as plant protection agents, plant strengtheners or fertilizers. Blossom Protect on average reduced fire blight incidence by 78% and Myco-Sin® by 61%, when sprayed three to five times per season according to the phenological development of the blossoms (Figure 1). The both products containing calciumformate, FolanxCa29 and LX4630, reduced blossom blight by 60% and 59% in our trials, respectively.

Table 2: Treatments, applied concentrations, number of treatments (No.), fire blight incidence and efficiency in an apple field trial in Darmstadt 2011. Different letters beside the incidence indicate a significant difference according to Tukey`s Multiple Comparison test (P $\leq$ 0.05).

Treatments	No.	Incidence (%)	Efficiency (%)
Untreated control	-	43.8 (a)	-
BlossomProtect™ (12 g/L)	4	23.3 (b)	47
Chitoplant (1g/L)	4	38.8 (ab)	12
LX4630 (15 g/L)	4	27.2 (ab)	38
Myco-Sin® (10 g/L)	4	27.5 (ab)	37

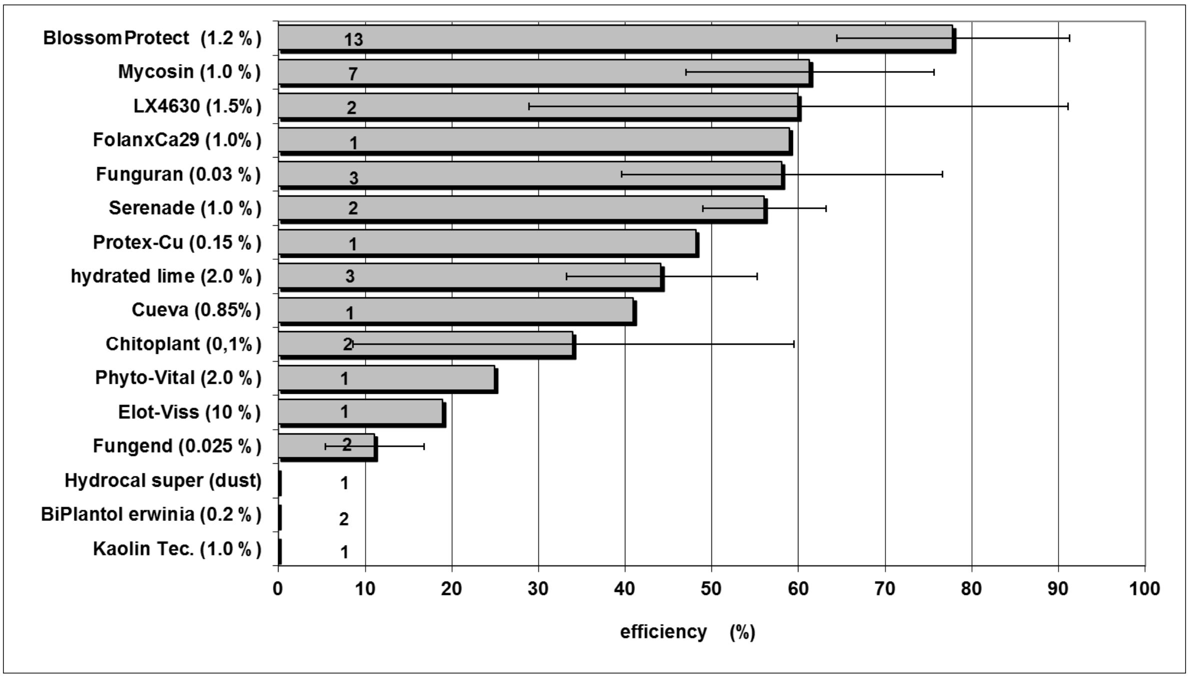


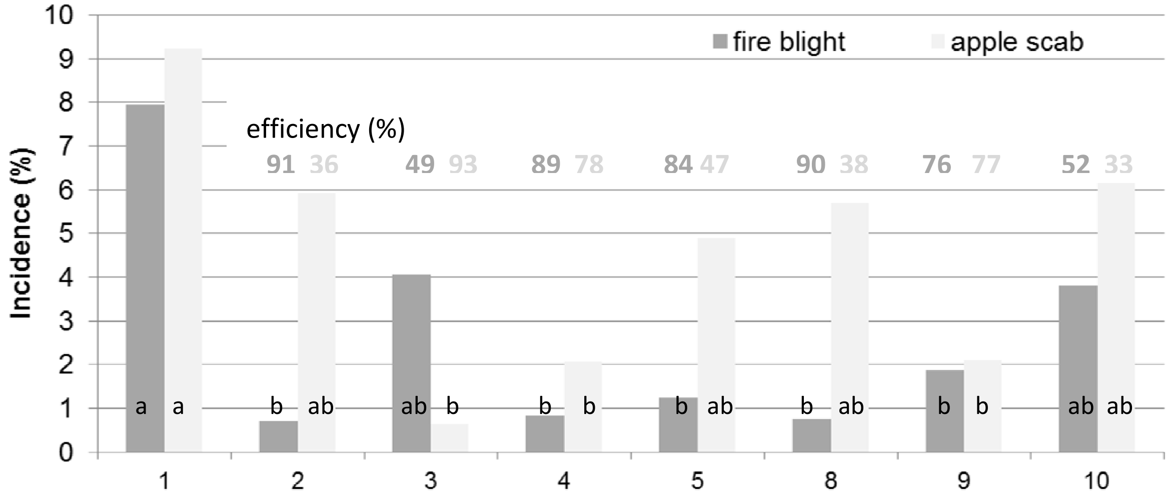
Figure 1: Efficiency of products commercially available in Germany against fire blight blossom infections in field trials 2004 to 2011. Efficiencies were calculated from evaluations of trees with secondary infections. The products were applied three to five times according to the phenological stage of the blossoms. The numbers in the column show the number of trials.

Scheer and Bantleon (2009) and Fried (2010) described inconsistent efficiencies for LX4630 and Folanx Ca29 against fire blight in field trials with efficiencies below 50% in average. The copper fungicide Funguran applied with 135g metallic copper per ha, showed 58% efficiency (Figure 1). Other copper formulations (Protex-Cu and Cueva) used with 100g metallic copper/ha showed lower effects against fire blight in field trials. For higher efficiencies higher copper concentrations should be used. However, applications of high amounts of copper increases the risk for fruit russetting in many apple and pear cultivars and the use of copper is under discussion in many countries because of ecological purposes. Therefore we did no trials with higher copper concentrations. Serenade (*Bacillus subtilis*) reduced fire blight symptoms in our trials by 56%. This corresponds to results from trials in Germany with different Serenade formulations since 2000, in which in average 54% efficiency in five trials (Fried, 2010) or 40% efficiency in seven trials (Scheer, 2009) were found. Sundin *et al*. reported in average 36% efficiency using Serenade in 11 trials in the USA (Sundin *et al*., 2009). All the other products having lower efficiencies than 50% (Figure 1) will not be recommended for use in fire blight control.

In Germany and other European countries Blossom Protect is recommended for fire blight control in organic orchards. Due to high efficiencies in field trials in the USA, which were comparable to that of antibiotics, the use of Blossom Protect will also be recommended in organic pome fruit production in the USA after registration will be passed (T. Smith and K. Johnson, personal communication). Blossom Protect contains blastospores of the fungus *Aureobasidium pullulans.* Reports on *A. pullulans* causing fruit russetting in apple and pear (Matteson-Heidenreich *et al*., 1997; Spotts and Cervantes, 2002) have been addressed in several field trials during our project. The results indicate that the enhancement of fruit russetting caused by Blossom Protect depends on the variety and on the number of treatments. On susceptible varieties the number of applications should be reduced to two.

In addition *A. pullulans* is sensitive to fungicides. Therefore spray strategies are needed, that give both fire blight and scab control during bloom. In the field trial in Mühlingen 2011 several strategies were tested and fire blight and apple scab were evaluated after the blossoming period (Figure 2). The weather conditions were not favourable for fire blight infections until 50% of the blossoms were opened. Then four days with a high epiphytic potential (EIP) were detected resulting in a risk for fire blight infections. A disease incidence of 7.9% blighted blossom clusters was observed in the untreated control. As a standard treatment Blossom Protect was applied four times according to the phenological stage of the blossoms and revealed a significant reduction of fire blight symptoms of 91% but no significant effect on apple scab. Three applications of Blossom Protect, in order to cover the days with high risk for fire blight infections according to Maryblyt, reduced blossom blight also significantly. Applying Blossom Protect and wettable sulphur in a tank mixture or alternating sprays of Blossom Protect with tank mixtures of Myco-Sin and wettable sulphur resulted in significant reductions of fire blight symptoms as well as apple scab symptoms (Figure 2).

Vacciplant® was described as a resistance inducer able to reduce fire blight incidence when applied before bloom (Kelly and Bernard, 2002). In Mühlingen 2011, Vacciplant was tested in a strategy applying it twice before bloom, and then using Blossom Protect at days with high risk for fire blight. This strategy resulted in a high efficiency against fire blight but not against apple scab. However, the additional effect of Vacciplant compared to Blossom Protect as stand alone treatment was low (Figure 2). So this strategy should be tested further before it could be recommended to the growers. Tank mixtures of Boni Protect + Omni Protect + wettable sulphur controlled apple scab very well but not fire blight. Chitoplant did not reduce fire blight or apple scab significantly (Figure 2).



26.4. 1%	-	-	-	-	-	Vacc	-	-
29.4. 2%	-	-	NS+OP	NS	-	-	NS+MS	-
2.5. 10%	-	-	-	-	-	Vacc	-	-
5.5. 20%	-	BlosP	BP+NS+OP	BlosP+NS	-	-	NS+MS	Chito
7.5. 45%	-	BlosP	BP+NS+OP	BlosP+NS	BlosP	BlosP	BlosP	Chito
9.5. 81%	-	BlosP	BP+NS+OP	BlosP+NS	BlosP	BlosP	NS+MS	Chito
11.5. 97%	-	BlosP	BP+NS+OP	BlosP+NS	BlosP	BlosP	BlosP	Chito

Figure 2: Incidence of fire blight and apple scab in the field trial in Mühlingen 2011 and efficiency of different spray strategies. Treatment dates, percentage of open flowers and products applied in the different strategies are mentioned.

Different letters in the columns indicate a significant difference according to Tukey`s Multiple Comparison test ($P \leq 0.05$). BlosP = blossom protect(BP), NS = wettable sulphur, MS = Myco-Sin, Vacc = vacciplant, BP = Boni Protect, OP = Omni Protect, Chito = Chitoplant.

In former trials spray strategies, in which Blossom Protect was alternated with lime sulphur or wettable sulphur did not hamper the efficacy of Blossom Protect against fire blight (Table 2), but the sum of applications during bloom was high. Trials in Karsee 2008 and Darmstadt 2010 showed that the tank mixture of Blossom Protect with wettable sulphur had comparable efficiencies as the stand alone treatment with Blossom Protect. This was confirmed in Mühlingen 2011. The tank mixture reduced also apple scab in these trials. On varieties susceptible to fruit russetting the number of applications with Blossom Protect should be reduced to two. In this case, Blossom Protect should be applied twice in periods with high risk for fire blight infections and these applications should be supplemented by applications of a tank mixture of Myco-Sin + wettable sulphur, when additional risk days occur or applications for apple scab control are necessary.

Table 2: Efficiency (%) of BlossomProtect™ and spray strategies in field trials in Karsee (KA), Darmstadt (DA) and Mühlingen (MU) in the years 2004-2011. Only results from trees not inoculated with the pathogen were considered.

Treatments	**KA 04**	**KA 06**	**DA 06**	**KA 07**	**KA 08**	**DA 09**	**DA 10**	**MU 11**
BlossomProtect (12g/l)	85 (4)	86 (4)	85 (4)	89 (4)	80 (4)	81 (4)	82 (4)	91(4)
				83 (3)				84 (3)
BlossomProtect (12g/l) altern.	68 (4)		87 (3)	77 (3)				
lime sulphur (15ml/l)	(4)		(1)	(3)				
BlossomProtect (12g/l) altern.		88 (4)	85 (4)	84 (3)				
wettable sulphur (2.5g/l)		(3)	(1)	(3)				
BlossomProtect (12g/l) altern.				87 (3)	70 (3)	74 (2)		76 (2)
wettable sulphur (2.5g/l) +				(3)	(2)	(2)		(2)
Myco-Sin ® (10g/l)								
tank mixture: BlossomProtect					77 (4)		74 (4)	89 (4)
(12g/l)+ wet. sulphur (2.5g/l)								(4)
Vacciplant (0.375ml/l) before								90 (2)
BlossomProtect (12g/l)								(3)

The numbers in brackets indicate the number of applications of BlossomProtect or of the fungicides used in the described strategies. + = tank mix; altern. = alternating treatments.

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