Development of strategies for fire blight control 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 Germany, many potential control agents have been considered and more than 40 different preparations have been tested systematically in laboratory trials *in vitro* and on detached blossoms to select the products with highest efficacy. In eleven field trials conducted since 2004 according to EPPO guideline PP1/166(3) BlossomProtect™ and Myco-Sin® had the highest efficiencies (79% and 65%, respectively). In 2010 LX4630 was tested the first time and showed comparable efficiency to BlossomProtect. BlossomProtect contains blastospores of the yeast *Aureobasidium pullulans.* The blastospores are sensitive to fungicides including sulphur and lime sulphur that are used for apple scab control. In some cases, *A. pullulans* enhanced fruit russet depending on the number of applications and of the apple variety treated. Spray strategies in which BlossomProtect was used alternating with sulphur fungicides did not hamper the efficacy of BlossomProtect against fire blight. However, up to 8 applications had to be made during bloom to achieve fire blight and scab control. Applying BlossomProtect and wettable sulphur in a tank mixture reduced the total number of applications to four without reducing significantly the efficiency against fire blight. With a strategy to alternate BlossomProtect applications with sprays of a mixture of wettable sulphur and Myco-Sin, the total number of applications during bloom could be reduced to four, with only a slight decline in fire blight control. In addition in this strategy the reduction of the number of applications of BlossomProtect from four to two reduced the risk of 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. 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 favorable 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 efficient control agents are needed to prevent blossom infections. In the USA several bacterial antagonists were tested and three commercial products are available (BlightBan® A506, Bloomtime Biological FD™ Biopesticide, Serenade®), which were inconsistently effective in reducing blossom infections in field trials conducted between 2001-2007, when examined individually (Sundin et al., 2009).

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 *in vitro* laboratory tests, *in vivo* assays 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 44 control agents tested, 31 suppressed *E. amylovora in vitro*, illustrating their potential for a bacteriostatic mode of action (Kunz et al., 2009). The *in vitro* activity was indicative of 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 compounds, five contained *Aureobasidium pullulans*, two contained *Bacillus amyloliquefaciens* and one was an acidic stone dust (Kunz et al., 2009). In addition, products based on calcium formiate showed high efficacy in recent tests *in vitro* and on detached blossoms.

In field trials carried out from 2004 to 2009 using these control agents, BlossomProtect™ had the highest efficiency (Kunz et al., 2008; Kunz et al., 2010; Kunz et al., 2004; Kunz et al., 2006). However, *A. pullulans,* the active microorganism in BlossomProtect, could be sensitive to fungicides including sulphur and lime sulphur, and at high concentrations can cause fruit russet (Spotts and Cervantes, 2002). Therefore field trials were carried out in which (i) further preparations, which showed high efficiency in the laboratory and (ii), different spray strategies with BlossomProtect, which aimed at fire blight as well as scab control without enhancing fruit russet, were tested.

Material and Methods

Field trials to test the efficiency against fire blight were carried out in accordance with the EPPO guideline PP1/166(3). One tree per orchard 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. 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., 2008; Kunz et al., 2006) and from the trials in Darmstadt in 2008 and 2009 (Kunz et al., 2010) have already been published.

In **Darmstadt in 2010** a field trial in a randomized block design with four replicates was carried out in an orchard of apple \`Idared\` planted in 1999. When approximately 20% of the flowers were open (April 25th) the first application of the test products (Table 1) was made and afterwards one tree per plot was inoculated with a suspension containing $1x10^8$ cells/ml of *E. amylovora* (strains Ea797, Ea839 and Ea851). When approximately 40% (April 27th), 70% (April 29th) and 90% (May 1st) of the flowers were open, the test products were applied again. Total numbers of blossom clusters were counted on May 4th, and blossom clusters showing fire blight symptoms were counted on June 8th. From these data, the fire blight incidence was calculated for each plot. Statistical analyses of square root transformed data were done using one-way analysis of variance, and mean separation was accomplished using Tukey\`s Multiple Comparison Test ($P \leq 0.05$).

Field trials on the influence of treatments on fruit russet: Experiments were conducted in organic apple orchards in a randomised block design with four replicates per treatment. In 2008 and 2010 trials were carried out on the apple variety ‘Santana’ in Mainau, where the influence of the number of applications of BlossomProtect on fruit russet was evaluated, by spraying plots once, twice, three or four times with 12g/L BlossomProtect with a backpack sprayer. Application dates were April 28th, April 30th, May 3rd and May 6th in 2008 and April 28th, April 30th, May 4th, and May 7th in 2010. All the fruit from four trees per plot were classified into four classes according to the russeted area of the fruit surface (class 1: no russet; class 2: 1-10%, class 3: 11-30% and class 4: more than 30% of the fruit skin russeted) on August 18th in 2008 and August 24th in 2010. For each plot the russet index was calculated (Haug and Kunz, 2005). Statistical analyses were done using one-way analysis of variance, and mean separation was accomplished using Tukey`s Multiple Comparison Test ($P \leq 0.05$).

Results and Discussion

In 11 field trials since 2004, 24 different preparations have been tested for efficacy against fire blight (Kunz et al., 2010; Kunz et al., 2009), from which 15 preparations are commercially available in Germany as plant protection agents, plant strengtheners or fertilizers. BlossomProtect (*Aureobasidium pullulans*) on average reduced fire blight incidence by 79% and Myco-Sin® (acidic stone dust) by 65%, when sprayed 4 times per season according to the phenological development of the blossoms (Fig. 1).

In the field trial in Darmstadt 2010, one day with high risk of fire blight infections and one additional infection day were detected by the Maryblyt model (http://www.caf.wvu.edu/kearneysville/maryblyt/, 25.3.2010). A disease incidence of 23% blighted blossom clusters was observed in the untreated control. BlossomProtect applied according to the phenological stage was used as the standard application and resulted in a significant reduction of fire blight symptoms by 82%. The addition of 2.5 g/L Netzschwefel Stulln (sulphur) to BlossomProtect in a tank mixture slightly reduced the efficiency, to 74%. Application of LX4630 (calcium formiate) resulted in comparable efficiency to that of BlossomProtect. The addition of Myco-Sin to LX4630 enhanced the efficiency to 90% (Table 1). However, Scheer and Bantleon (2009) and Fried (2010) described inconsistent efficiencies for LX4630 with an average efficiency below 50%. Therefore further trials should be carried out with the mixture LX4630 and Myco-Sin. Furthermore, registration for LX4630 is needed for use in organic fruit growing.

In Germany and other European countries BlossomProtect is recommended for fire blight control in organic orchards. BlossomProtect contains blastospores of *A. pullulans*. Despite reports that *A. pullulans* can cause fruit russet (Matteson-Heidenreich et al., 1997; Spotts and Cervantes, 2002), no significant increase in russeted fruit after application of BlossomProtect could be determined on the varieties ´Golden Delicious` or ´Jonagored` in 2005 and 2006 (Haug and Kunz, 2005) or in field trials carried out on ca. 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 2008 and in ‘Idared` in 2010, 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 (150 g Cu/ha) was added to BlossomProtect (Kunz et al., 2010). However, the combination of BlossomProtect and copper increased fruit russet on ‘Jonagold’ after two applications in 2010, whereas BlossomProtect as a stand-alone treatment, as well as the combination of Myco-Sin and LX4630, did not (data not shown). In addition, the influence of BlossomProtect on fruit russet was tested in an organic apple orchard located on the Isle of Mainau in 2008 (Kunz et al., 2010) and 2010. BlossomProtect (12g/L) was applied one to four times during bloom on different application dates to the apple 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. 2). In 2010, although the differences between the treatments were not statistically significant, the increased russet indices after three or four applications supported the results from 2008 (Fig. 2). 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’, ‘Goldrush’), fire blight control with up to four applications of BlossomProtect 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.

A. pullulans is a fungus and sensitive to fungicides including sulphur and lime sulphur, which are used for apple scab control. In organic orchards, spray strategies are needed, that give both fire blight and scab control during bloom. Spray strategies, in which BlossomProtect was alternated with sulphur did not hamper the efficacy of BlossomProtect against fire blight (Table 2), but the sum of applications during bloom was very high. It would be easier if the application of BlossomProtect and wettable sulphur could be tank mixed. Trials in Karsee 2008 and Darmstadt 2010 showed that the efficiency of this tank mixture was comparable to that of BlossomProtect applications as a stand-alone treatment (Table 2). As Myco-Sin is known to enhance the efficacy of sulphur against apple scab and has a proven effect against fire blight, we tested a strategy to alternate BlossomProtect applications with sprays of a mixture of Netzschwefel Stulln and Myco-Sin. This strategy was almost as effective against fire blight as BlossomProtect alone. The use of this strategy allowed a reduction in the total number of applications during bloom from six to eight down to four and a reduction of the number of BlossomProtect treatments per year from four to two (Table 2), which will reduce costs and the risk of fruit russet.

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Literature cited

Fried, A. 1997. Feuerbrand - Bekämpfungsversuche von 1994-1997. Obstbau 22:598-602.

Fried, A. 2010. Feuerbrand-Bekämpfungsversuch 2009. Obstbau 35:75-79.

Haug, P. and Kunz, S. 2005. Erfahrungen aus zwei Jahren Feuerbrandbekämpfung mit dem Hefepräparat Blossom-Protect. Ökoobstbau:(3) 13-16.

Kunz, S., Schmitt, A. and Haug P. 2008. Field testing of strategies for fire blight control in organic fruit growing, p. 299-305 in: FÖKO e.V. (Ed.), 13th International Conference on cultivation technique and phytopathological problems in organic fruit-growing, FÖKO e.V., Weinsberg.

Kunz, S., Schmitt, A. and Haug P. 2010. Fire blight control strategies in organic fruit growing, p. 118-125 in: FÖKO e.V. (Ed.), 14th International Conference on cultivation technique and phytopathological problems in organic fruit-growing, FÖKO e.V., Weinsberg.

Kunz, S., von Eitzen-Ritter, M., Schmitt A. and Haug P. 2004. Feuerbrandbekämpfung im ökologischen Obstbau. Ökoobstbau: (4) 2-7.

Kunz, S., von Eitzen-Ritter, M., Schmitt, A. and Haug P. 2006. Feuerbrandbekämpfung im ökologischen Obstbau - Ergebnisse der Bekämpfungsversuche 2006. Ökoobstbau: (4) 3-7.

Kunz, S., Mendgen, K., Haug, P. and Schmitt, A. 2009. Entwicklung von Strategien zur Feuerbrandbekämpfung im ökologischen Obstbau, Organic E-Prints.

Matteson-Heidenreich, M.C., Corral-Garcia M.R., Momol, E.A. and Burr T.J. 1997. Russet of apple fruit caused by *Aureobasidium pullulans* and *Rhodotorula glutinis*. Plant Disease 81:337-342.

Pusey, P.L. and Smith T.J. 2008. Relation of Apple Flower Age to Infection of Hypanthium by *Erwinia amylovora*. Plant Disease 92:137-142.

Scheer, C. and Bantleon G. .2009. Feuerbrandsituation und Feuerbrandfreilandversuche am Bodensee. Obstbau 34:626-629.

Spotts R.A. and Cervantes L.A. 2002. Involvement of *Aureobasidium pullulans* and *Rhodotorula glutinis* in russet of d'Anjou pear fruit. Plant Disease 86:625-628.

Sundin G.W., Werner N.A., Yoder K.S. and Aldwinckle H.S. 2009. Field Evaluation of Biological Control of Fire Blight in the Eastern United States. Plant Disease 93:386-394.

Voegele, R. T., Kunz S., Olbrecht L., Hinze M., Weißhaupt S., Schmid A., Ernst M., Joos M., Matschinsky M. and Mendgen K. 2010. Monitoring *E. amylovora* using real time PCR, p. 110-117 in: FÖKO e.V. (Ed.), 14th International Conference on cultivation technique and phytopathological problems in organic fruit-growing, FÖKO e.V., Weinsberg.

Tables

Table 1: Type of treatments and applied concentrations, number of treatments (No.), fire blight incidence and efficiency in an apple field trial in Darmstadt 2010. 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	-	23.0	(a)	-
BlossomProtect™ (12 g/L)	4	4.2	(b)	82
Tank mix: BlossomProtect (12 g/L) + Netzschwefel Stulln (2.5 g/L)	4	6.1	(b)	74
LX4630 (15 g/L)	4	4.1	(b)	82
Tank mix: LX4630 (15 g/L) + Myco-Sin® (10 g/L)	4	2.3	(b)	90

Table 2: Efficiency (%) of BlossomProtect™ and spray strategies in field trials in Karsee (KA) and Darmstadt (DA) in the years 2004-2010. Only 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. + = tank mix; altern. = alternating treatments.

Treatments	**KA 04**	**KA 06**	**DA 06**	**KA 07**	**KA 08**	**DA 09**	**DA 10**
BlossomProtect (12 g/L)	85 (4)	86 (4)	85 (4)	83 (3)	80 (4)	81 (4)	82 (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 (2.5 g/L)		88 (4) (3)	85 (4) (1)	84 (3) (3)			
BlossomProtect (12g/L) altern. wettable sulphur (2.5g/L) + Myco-Sin ® (10g/L)				87 (3) (3)	70 (3) (2)	74 (2) (2)	
tank mixture: BlossomProtect (12 g/l)+ wettable sulphur (2.5 g/l)					77 (4)		74 (4)

Figures

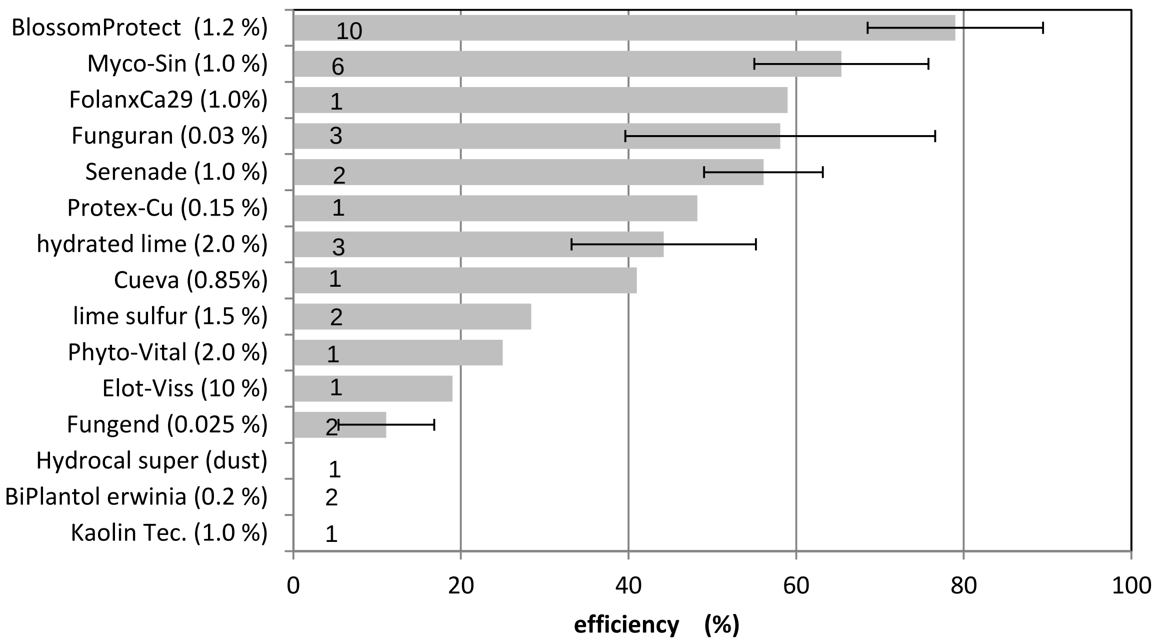

Figure 1: Efficiency against fire blight blossom infections of preparations commercially available in Germany in apple field trials. Trials were carried out from 2004 to 2010, efficiencies are based on evaluation of trees with secondary infections. The preparations were applied 4 to 5 times according to the phenological stage of the blossoms. The numbers in the column show the number of trials.

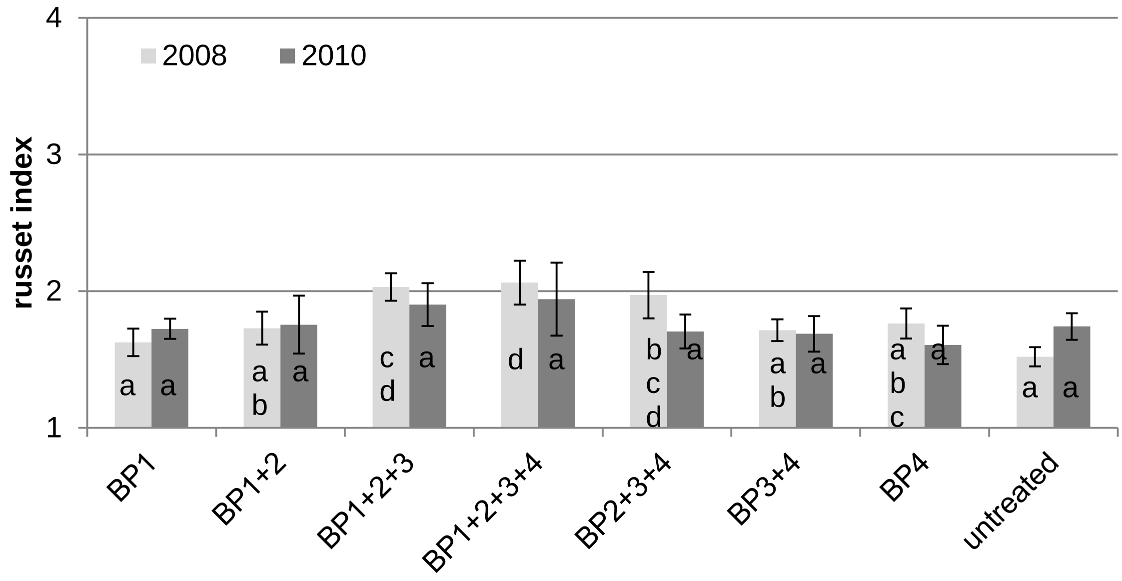

Figure 2: Russet index on apple fruit of the variety ´Santana´ in Mainau 2008 and 2010 after varying numbers of treatments with BlossomProtect™ (BP) in comparison to an untreated control**.** The numbers 1-4 represent the dates of applications. Different letters indicate significant differences according to Tukey´s Multiple Comparison test ($P \leq 0.05$) per year.