

Summary of an eight year research project on fire blight control

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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 “BundesprogrammÖkologischerLandbau” from 2004 to 2011, 64 different preparations have been tested in laboratory trials for efficiency against the fire blight pathogen. 26 of them were further tested in field trials according to EPPO guideline PP1/166(3). From the products, which are commercialised in Germany, BlossomProtect had the highest efficiency, followed by Myco-Sin, FolanxCa 29, Funguran and Serenade.*

*Blossom Protect contains blastospores of the yeast-like fungus *Aureobasidium pullulans*. Therefore strategies were developed to integrate Blossom Protect applications in commercial spray schedules for apple scab control. In addition the influence of the selected products on fruit russet was evaluated. The increase in fruit russetting depended on the number of applications of Blossom Protect and on the variety treated. Combined control of fire blight and apple scab control could be achieved using tank mixtures of Blossom Protect and wettable sulphur. With a strategy of alternating Blossom Protect applications with sprays of a mixture of wettable sulphur and Myco-Sin, fire blight and apple scab were both controlled significantly. In addition, this strategy reduced the risk of fruit russetting.*

*In field trials, carried out since 2006, the abundance of *E. amylovora* was measured in blossoms with qPCR and was correlated with the disease incidence in untreated plots. These data indicate that control measures can be limited to orchards infested with the fire blight pathogen.*

Keywords: Fire blight control strategy, *Erwinia amylovora*, Blossom Protect, Myco-Sin

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 (Voegelé *et al.*, 2010). 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. In addition information on the risk for infections is needed to successfully schedule the applications.

A three-step evaluation procedure was established consisting of 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) and was used to select control agents to be tested under field conditions.

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Products used for fire blight control have to be integrated into spray schedules of organic pome fruit producers. Moreover, strategies are needed for the control of both, fire blight and apple scab, and strategy components should not have any negative side effects. Therefore, control agents showing high efficacy against fire blight were also tested for phytotoxicity (fruit russetting) and compatibility with fungicides.

Besides the weather conditions, which are considered in fire blight prediction models, presence of the pathogen in the orchard is a prerequisite for infections. Real Time PCR is a fast and sensitive method that allows quantification of *E. amylovora* in blossoms (Voegelé *et al.*, 2010). Since 2007 the abundance of *E. amylovora* in blossoms of untreated trees was measured and correlated with the disease incidence. Implementation of measurement of the abundance of *E. amylovora* in the orchard in decision making for control measures is discussed.

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 in Kunz *et al.* 2009.

Field trials to test the efficiency of products and strategies 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 symptoms from trees, which had not been inoculated, were taken into account. Results from field trials conducted in the year 2004 in Groß-Umstadt, 2006-2008 in Karsee and Darmstadt (Kunz *et al.*, 2009 and literature cited therein), 2009-2010 in Darmstadt (Kunz *et al.*, 2010; Kunz *et al.*, 2011) and from 2012 in Mühlhingen and Darmstadt (Kunz *et al.*, 2012) have already been published.

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. All of the fruit from 4-6 trees per plot were classified into 4 classes (cl) according to the russeted area per apple (cl1= 0% of the surface russeted; cl2=0-10%; cl3=10-30%; cl4= >30%). For each plot the russet index (RI) was calculated ($RI = (N_{cl1} \times 1 + N_{cl2} \times 2 + N_{cl3} \times 3 + N_{cl4} \times 4) / (N_{cl1} + N_{cl2} + N_{cl3} + N_{cl4})$). (Kunz *et al.*, 2009).

Real Time PCR analyses were performed as described by Voegelé *et al.* 2010. Samples consisted of washing fluids from blossoms containing intact bacteria. No DNA extraction was performed prior to PCR analysis. Absolute quantification of bacteria in samples was done by standardization with respect to serial dilutions of pure cultures of *E. amylovora*.

Twenty blossoms were collected in Whirl-Pak bags (Carl Roth GmbH, Karlsruhe), and incubated with 2 ml H₂O per blossom for 15 min. A 1 ml aliquot was removed and centrifuged for 1 min at 15,000 g. The supernatant was discarded and pellets resuspended in an equal volume of H₂O. Samples were either analysed directly or stored at -20°C.

Results

In a systematic evaluation 64 control agents have been tested in different test systems during the last eight years. From these, 38 control agents suppressed *E. amylovora* in vitro, illustrating their bacteriostatic behaviour. However, on detached apple blossoms only 19 preparations were able to reduce symptom development by more than 60%. Six of them were copper products, five products contained *Aureobasidium pullulans*, two were from other fungal origin and three products contained *Bacillus* sp.. The group of efficient products furthermore included Myco-Sin, Chitoplant and LX4630.

In 13 field trials since 2004, 26 different preparations have been tested for efficacy against fire blight, from which 16 products were commercially available in Germany as plant protection agents, plant strengtheners or fertilizers. In the following we focus on the results with the commercialised products. 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 (fig. 1). FolanxCa29 (calciumformate) reduced blossom blight by 59% in our trials. The copper fungicide Funguran applied with 135g metallic copper per ha, showed 58% efficiency (fig. 1). Other copper formulations (Protex-Cu and Cueva) used with 100g metallic copper/ha showed lower effects against fire blight in field trials. Serenade (*Bacillus subtilis*) reduced fire blight symptoms by 56%. All the other products, which had efficiencies lower than 50% (fig. 1) cannot be recommended for use in fire blight control.

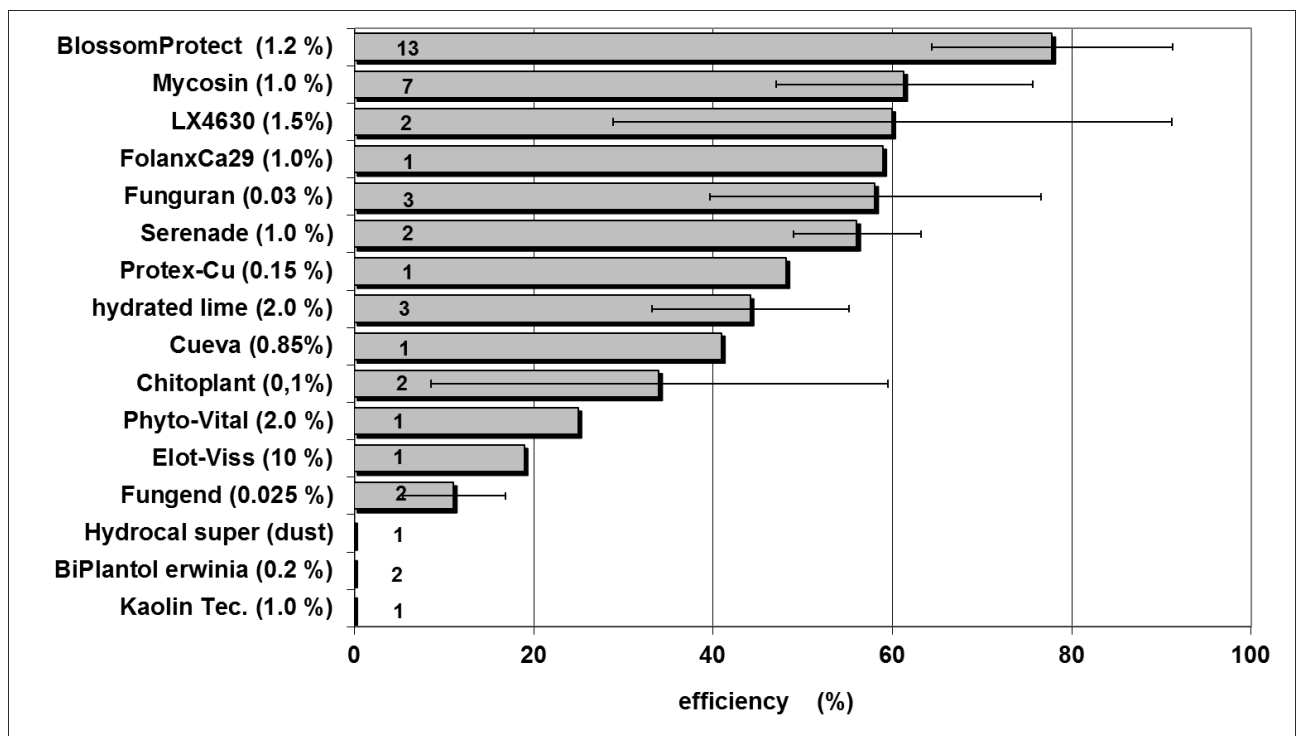


Figure 1: Efficiency against fire blight blossom infections in field trials 2004 to 2011 of products commercially available in Germany. Efficiencies were calculated from evaluations of disease incidence of trees with secondary infections. The products were applied with the given concentration 3 to 5 times according to the phenological stage of the blossoms. The number of trials for each product is shown in the column.

In practice, spray strategies have to control both, fire blight and apple scab. In general, sulphur did not hamper the efficiency of Blossom Protect against fire blight, when applied in alternation with Blossom Potect. However, the use of lime sulphur tended to be more critical than the use of NetzschwefelStulln and the total number of applications during bloom was too high (tab. 1). The use of tank mixtures of Blossom Protect with NetzschwefelStulln gave good control of both, fire blight and apple scab, in three field trials and reduced the number of applications (tab. 1). As Myco-Sin is known to enhance the efficacy of sulphur against apple scab and since it has a proven effect against fire blight, a strategy to alternate Blossom Protect applications with sprays of a mixture from NetzschwefelStulln and MycoSin was tested. In four field trials this strategy was almost as effective against fire blight as Blossom Protect as stand alone treatment. 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 Blossom Protect treatments per year from 4 to 2, which reduces costs and the risk of fruit russet.

Table 1: Efficiency (%) of BlossomProtect and spray strategies in field trials 2004-2011 in Karssee (KA), Darmstadt (DA) and Mühlingen (MÜ). Only results on disease incidence from trees not artificially inoculated were considered. The numbers in brackets indicate the number of applications of the agents used in the described strategies.

	KA 04	KA 06	DA 06	KA 07	KA 08	DA 09	DA 10	MÜ 11
BlossomProtect (12 g/l)	85 (4)	86 (4)	85 (4)	89 (4) 83 (3)	80 (4)	81 (4)	82 (4)	91 (4) 84 (3)
BlossomProtect (12 g/l) altern. lime sulphur (15 ml/l)	68 (4) (4)		87 (3) (1)	77 (3) (3)				
BlossomProtect (12 g/l) altern. wetable sulphur (3 g/l)		88 (4) (3)	85 (4) (1)	84 (3) (3)				
BlossomProtect (12g/l) altern. wetable sulphur (3g/l) + MycoSin (10g/l)				87 (3) (3)	70 (3) (2)	74 (2) (2)		76 (2) (2)
tank mixture: BlossomProtect (12 g/l)+ wettable sulphur (3 g/l)					77 (4) (4)		74 (4) (4)	89 (4) (4)
Vacciplant (0.375 ml/l) before Blossom Protect (12 g/l)								90 (2) (3)

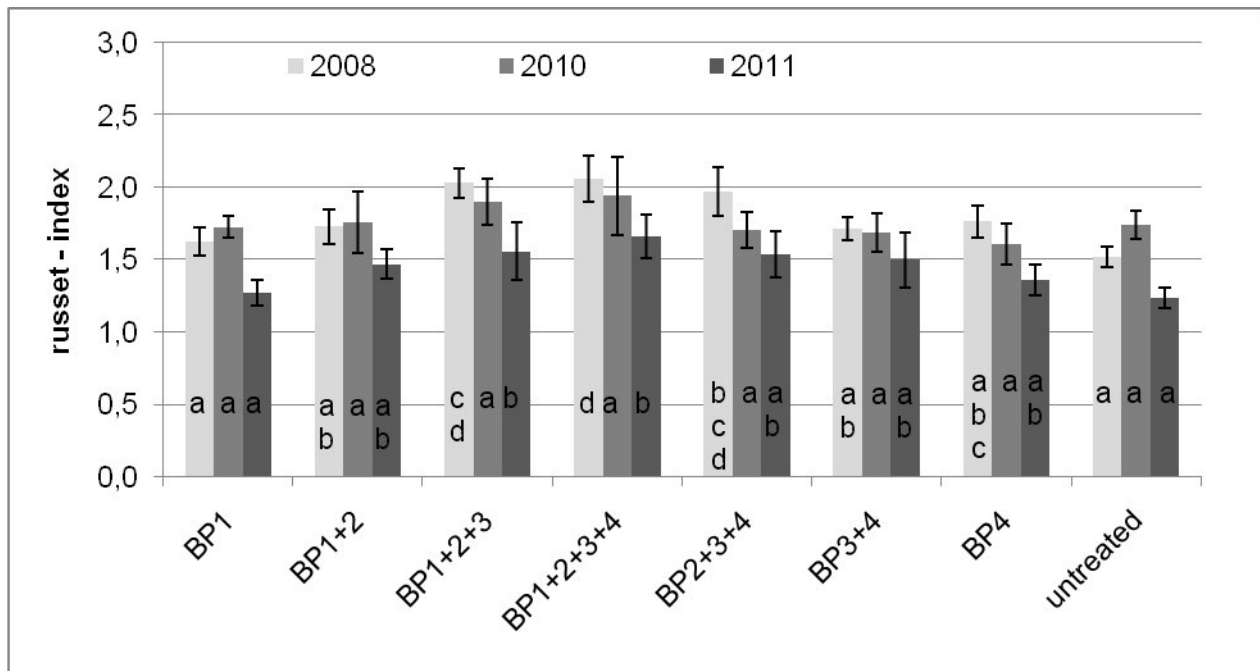


Figure 2: Russet index on fruits of the variety 'Santana' in Mainau 2008, 2010 and 2011 after varying numbers of treatments with 12 g/l Blossom Protect (BP) in comparison to an untreated control. The numbers 1-4 represent the dates of the treatment. Different letters indicate a significant difference in Tukey's Multiple Comparison test ($p \leq 0.05$) in one year

The influence of products and strategies on fruit russet was tested in organic apple orchards. Copper or Blossom Protect increased fruit russet significantly on some varieties ('Jonagored', 'Jonagold', 'Golden Delicious', 'Idared', 'Sansa', 'Santana') whereas in 'Gala', 'Goldrush', 'Summerred', 'Pinova', 'Braeburn' and 'Topaz' no effect was visible (Kunz *et al.*, 2010; Kunz *et al.*, 2009). In a three years trial in Mainau Blossom Protect (12g/l) was applied 1 to 4 times during bloom at different application dates on the variety 'Santana'. 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 in 2008 and 2011 (fig. 2).

The addition of wettable sulphur to Blossom Protect or strategies alternating Blossom Protect with a mixture of Myco-Sin and wettable sulphur did not further increase the risk for russetting on 'Jonagold'. Tank mixtures of copper and Blossom Protect or a mixture of Vacciplant with Blossom Protect increased fruit russet compared to Blossom Protect applied as a stand alone treatment on the variety 'Jonagold', but not on 'Topaz' (data not shown).

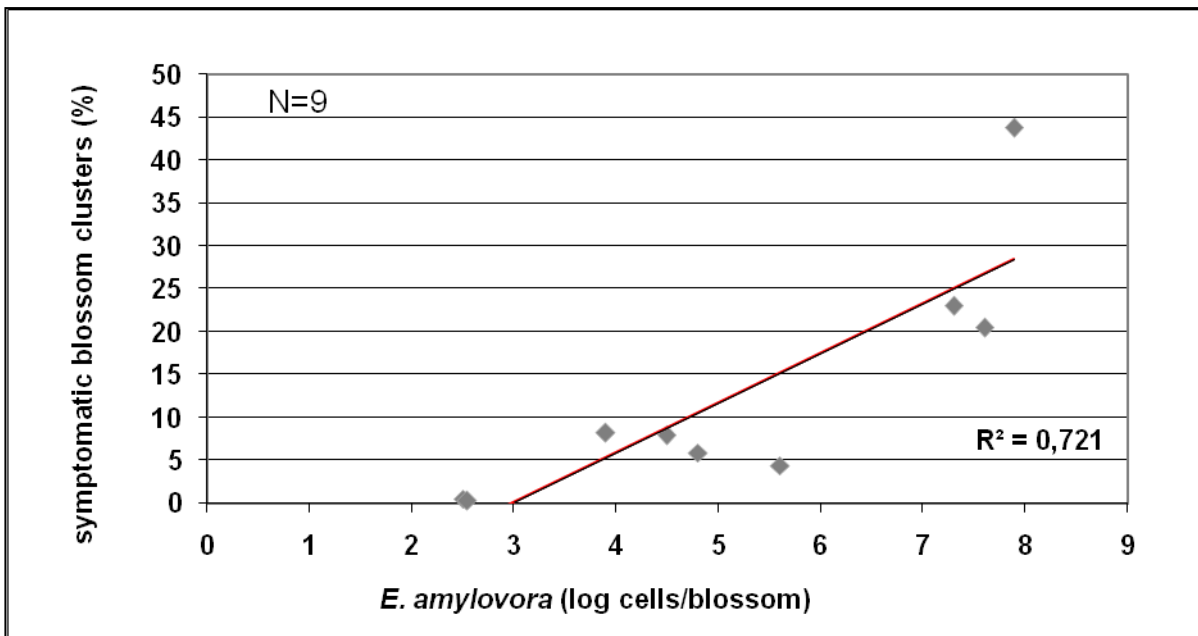


Figure 3: Correlation of fire blight incidence (% symptomatic blossom clusters) and maximum abundance of *E. amylovora* (log cells/blossom) in blossoms during the flowering period in 9 field trials from 2006 to 2011.

Since 2006 in 9 field trials the abundance of *E. amylovora* was measured in blossoms of untreated plots of not inoculated trees. *E. amylovora* showed a typical growth curve starting below the detection level at beginning of bloom and reaching numbers of nearly 10^8 cells/blossom at the end of the blossoming period (Kunz *et al.*, 2009), in most of the trials. In two trials cell counts below 1'000 per blossom were measured and no increase in abundance of *E. amylovora* over the blossoming period was detected. In both trials the fire blight incidence was below 1% infected blossom clusters (fig. 3). In all of the 9 field trials the maximum number of pathogens measured in blossoms correlated with the disease incidence in untreated plots. More than 5'000 *E. amylovora* bacteria per blossom were necessary to result in a measurable disease incidence.

Discussion

Investigation of products for efficacy against fire blight in field trials is expensive and time consuming. Trials under natural infection conditions are seldom successful and trials with artificial inoculation are restricted to certain trial sites as *E. amylovora* is a quarantine organism. Only a small number of products or strategies can be tested in the field each year. Reliable methods to screen products for efficacy are needed to avoid a waste of resources in field trials. Therefore, in this project a three step evaluation procedure was established composed of tests in shaken cultures, tests on detached blossoms and field trials (Kunz *et al.*, 2009). The efficacy of a product on detached blossoms corresponded well with its efficacy in field trials. We suggest to first test all control agents in the detached blossom system and then to evaluate only products with proven efficacy under field conditions.

The aim of the project was to investigate products for fire blight control, which are available for organic growers. Nevertheless test preparations not registered have been included in the study. Although having good efficiencies in some cases (Kunz *et al.*, 2009), these test preparations were not developed further by the companies. Therefore, during the last year, we focused on products commercially available in Germany.

Based on the results of this project, BlossomProtect is recommended for fire blight control in organic orchards in Germany and other European countries. Due to high efficiencies in field trials in the USA, which were comparable to that of antibiotics, the use of BlossomProtect will also be recommended in pome fruit production in the USA after finalization of registration (Kunz *et al.*, 2012). BlossomProtect contains blastospores of the fungus *Aureobasidium pullulans*. Reports on *A. pullulans* causing fruit russeting in apple and pear (Spotts and Cervantes, 2002) have been addressed in several field trials during our project. The results indicate that the enhancement of fruit russeting 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. This can be achieved by using a strategy applying Blossom Protect twice in periods with high risk for fire blight infections and additional applications of a tank mixture of Myco-Sin + wettable sulphur, when additional risk days occur or applications for apple scab control are necessary.

Applications against fire blight were done according to the phenological stage of the blossoms in our field trials in order to achieve the coverage of all blossoms. However, blossoms can only be infected when certain weather conditions are fulfilled during their life span. Under conditions unfavourable for fire blight, applications of control agents are unnecessary. In two field trials we proved that the timing of Blossom Protect applications according to the forecast model Maryblyt (Lightner and Steiner, 1992; Moltmann, 1996) reduced the number of applications in comparison to the timing according to the phenological stage without reducing efficacy. Therefore the timing of applications according to forecast models is recommended. Blossom Protect or Myco-Sin should be applied the day before predicted infection conditions.

All the forecast systems only take physical factors like temperature or moisture/wetness into account, but not the actual presence of the pathogen. With qPCR a fast, specific and quantitative detection of the pathogen in blossoms is possible (Voegelé *et al.*, 2010). In our field trials, the dissemination of *E. amylovora* from artificially inoculated trees to not inoculated trees as well as the multiplication of the pathogen in not inoculated trees was measured. The amount of *E. amylovora* bacteria detected in the blossoms correlated with the disease incidence in nine field trials. In trials without measurable dissemination of bacteria the abundance in blossoms was below 5'000 cells/blossom resulting in no or only sporadic symptom development. In our trials 20 blossoms were taken per sample and four samples per day. In a monitoring established in commercial orchards during the last years

100 blossoms per sample and two samples per day were taken. The correlation between abundance of *E. amylovora* in blossoms and disease incidence was comparable in both studies (Voegelé and Kunz, unpublished). Monitoring of *E. amylovora* in the orchards during bloom can give additional input to the decision process on when and how often control agents should be applied to control fire blight. As long as the pathogen is not detectable by qPCR, the application of control agents can be postponed.

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