Development of a strategy for fire blight control in organic fruit growing

Entwicklung einer Strategie zur Feuerbrandbekämpfung im ökologischen Obstbau

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Abstract

Effective control strategies are needed in organic fruit growing to prevent blossom infections by the fire blight pathogen *Erwinia amylovora*. There are many potential control agents under discussion. 18 preparations have been tested for their efficiency against *Erwinia amylovora.* Twelve of them were highly effective against *E. amylovora in vitro*. Nevertheless only five of them led to a symptom reduction of more than 50% on detached apple blossoms. These results and data on field performance from the literature indicate that Blossom-Protect is the most effective preparation to prevent blossom infections by fire blight. Therefore further experiments have been performed to develop a strategy for the use of Blossom-Protect in organic fruit growing. The side effect of Blossom-Protect on fruit russeting was tested in two field trials in 2005. No increase of fruit russet was detected in the varieties \`Jonagored\` and \`Golden Delicious\` after 2 or 3 applications of Blossom-Protect during bloom.

Keywords: Fire blight, *Erwinia amylovora*, Blossom-Protect, Mycosin, Copper, Russeting

Introduction

Fire blight caused by *Erwinia amylovora* is the most serious bacterial disease in apple trees. During the last three decades it has spread throughout Europe. Since pruning of diseased material and other sanitation methods are not sufficient to stop the spread of the disease, efficient control agents are needed. Primary infection occurs in the blossom, where the pathogen enters through natural openings after multiplying on the stigmas. To prevent blossom infections in organic fruit growing several potential control agents are under discussion. In this study 18 preparations have been tested for their ability to suppress the multiplication of *E. amylovora* in shaken cultures and to reduce the symptom development on detached blossoms. The mode of action of effective preparations has been evaluated. The most promising preparation, Blossom-Protect, was further investigated for its integration into the spray schedules of organic farmers.

Material and Methods

Reduction of *E. amylovora* growth in shaken cultures

E. amylovora strain Ea385 was sub-cultured on NBS-A (8 g/l Nutrient Broth, 50 g/l Sucrose, 20 g/l agar) at 27℃. Inoculum suspensions of Ea385 were standardised by measurement of their optical density at 660 nm. 1×10^7 cells/ml of Ea385 have been added to 25 ml NBS (8 g/l Nutrient Broth, 50 g/l Sucrose) in a 100 ml Erlenmeyer flask containing the test preparation. After 24 h of incubation on a rotary shaker at 27℃, the Ea385 concentration in the culture was calculated by dilutions plated on petri-dishes with NB-A (8 g/l Nutrient Broth, 20 g/l agar). If the test preparations contained *Bacillus subtilis* or *Aureobasidium pullulans* the culture was plated on MacC-A (40g/l Mac Conkey broth, 20g/l Agar) or NBAc-A (8 g/l Nutrient Broth, 0,05g/l Actidione , 20 g/l agar) respectively.

Reduction of fire blight symptoms on detached blossoms

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An *in vivo* test-system with detached apple blossoms was established according to Pusey [1]. Apple trees (`Gala`) were stored at 2℃ in the dark from January to August. Every week a group of trees was transferred to the greenhouse to force them to bloom. The blossoms were cut and maintained with cut peduncle submerged in 10 % sucrose in plastic racks (23℃, 100% rH). Blossoms were sprayed with a suspension of Ea385 (10^6 cfu/ml) in water until runoff. Treatments were sprayed 1h after inoculation. The number of blossoms with bacterial ooze at the peduncle was counted 6 days after the inoculation.

Efficiency of the preparations in field experiments

The results from the laboratory studies were compared to efficiencies found in field experiments conducted from 1997 to 2004 in Germany [2-10] in accordance with EPPO guideline PP1/166(3). In the test orchards one tree per parcell was inoculated with the pathogen, from this tree *E. amylovora* was spread over the entire orchard by natural vectors [5]. Only the results from trees which had not been inoculated were taken into account.

Field experiments on the influence of treatments on fruit russet

Two experiments were conducted in organic apple orchards in a randomised block design with four replications per treatment. Treatments were applied with a backpack sprayer when fire blight infection periods were forseen (tab. 2). Full bloom was reached in both orchards by the 2nd of may. All of the fruit from 4 trees per lot were classified into 4 classes according to the russeted area. For each lot the russet index was calculated [11].

Table1: Location, variety and treatments in the field trials 2005

Location	Stetten	Lindau
Variety	Jonagored	Golden Delicious
No. of treatments	2	3
Application dates	29.4. ; 2.5.	28.4. ;1.5. ; 4.5.
Evaluation date	31.08.	31.08.

Results

18 preparations were tested in shaken cultures and on detached apple blossoms on their efficiency against *E. amylovora*. The use of Cutisan (15 g/l), Kaolin Tec (15 g/l), Phyto-Vital (20ml/l), Quassia extract (2g/l), Biplantol Erwinia (2ml/l) or Fungend (0.25 ml/l) neither reduced the growth of *E. amylovora* in shaken cultures nor prevented symptom development on detached blossoms. With Serenade WPO (10 g/l), Protex-Cu (1ml/l), 0.03% Funguran (0.3 g/l), Copper-Protein (10 g/l), BioZell 2000B (0.5 ml/l), slaked lime (20 g/l), DoMoF/Lysozym (20 ml/l) or lime sulphur (15 ml/l) the growth of the pathogen in shaken culures was inhibited completely, but the symptom reduction on detached blossoms was below 50 %. Elot-Vis (100 ml/l), Funguran (3 g/l), 1.0% Mycosin (10 g/l), BPASC (12 g/l) and Blossom-Protect (12 g/l) had a high efficiency in both test systems.

Results from field experiments in Germany during the last 8 years confirm the laboratory results (tab. 2). Preparations, which did not inhibit growth in vitro, did not lead to a significant disease reduction in field experiments. Elot-Vis and Mycosin, which showed good results on detached blossoms, gave no or only slight disease reduction in the field.

Table 2: Efficiency of test preparation in shaken cultures and on detached blossoms in comparison to results from field experiments. Data from field trials perfomed in Kirschgartshausen (KGH), Neustadt (NST), Schlachters (SCH), Amtzell (AMT), Groß-Umstadt (G-U) and Karsee (KAR) in the years 1997-2004 were taken from the indicated literature. Only the results from trees not inoculated with the pathogen are considered.

Eficiency (%)	**Plantomycin**	**Blossom-Protect**	**BPASC**	**Mycosin**	**Serenade WPO**	**Elot-Viss**	**Slaked lime**	**Lime sulfur**	**Protex-Cu**	**Fungend**	**Biplantol Erwinia**	**Kaolin Tec1**
Shaken culture	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**66**	**0**	**0**
Detached blossoms	**84**	**70**	**78**	**78**	**46**	**54**	**14**	**0**	**34**	**0**	**2**	**17**
Field experiments average	**84**	**72**	**83**	**38**	**45**	**19**	**48**	**28**	**49**	**11**	**-16**	**7**
KGH 97 [5]	88			53								
KGH 98 [2]	84			20								
KGH 00 [3]	89			12	55							40
KGH 01 [4]	93			28	51							
NST 01 [7]	76				-8							
SCH 01 [8]	83			46								
AMT 03 [6]	86	74										
KGH 03 [6]	72				56							
AMT 04 [9]	82	61			65							
G-U 04 [10]		66		54		19	48			7	-18	-25
KAR 04 [10]		85	83	56	51			28	49	15	-15	

The two yeast preparations BPASC and Blossom-Protect led to a high symptom reduction on detached blossoms and to the highest disease reduction in the field experiments. Both preparations contain blastospores of A*ureobasidium pullulans* [12]. Due to reports on *A. pullulans* causing fruit russet on apple and pear, two field experiments on the influence of Blossom-Protect on fruit russet were performed in the region of Lake Constance. In Stetten Blossom-Protect was tested in comparison to Funguran and an untreated control. Neither Blossom-Protect (12 g/l) nor Funguran(0.3 g/l) led to a significant increase of russeted fruits. The addition of Cutisan (15 g/l) to Blossom-Protect or Funguran tended to reduce the russet index in comparison to the use of the control agent alone (tab. 3). In Lindau the different components of Blossom-Protect and BPASC were sprayed as single treatments in comparison to Blossom-Protect and the untreated control. None of the treatments led to an increase in fruit russet (tab. 3).

Table 3: Russet index (mean ± deviation) after application of test preparations for fire blight control in the field.
Different letters in brackets indicate a significant difference ($p \leq 0,05$) between the treatments in one experiment (LSD-Test).

Treatment	**Russet index**	
	Stetten	**Lindau**
Untreated	1.24 ± 0.24 (ab)	1.18 ± 0.07 (a)
1.2 % Blossom-Protect (*A. pullulans* + buffer P)	1.51 ± 0.12 (ab)	1.16 ± 0.08 (a)
0.75 % BPASC5 (*A. pullulans* + buffer C)		1.14 ± 0.03 (a)
0.75 % BPA105 (*A. pullulans* CF10+ buffer C)		1.19 ± 0.02 (a)
0.6 % buffer C		1.15 ± 0.04 (a)
0.15 % *A. pullulans* CF10		1.14 ± 0.03 (a)
1.2% Blossom-Protect + 1.5% Cutisan	1.34 ± 0.06 (a)	
1.2% Blossom-Protect + 0.4% AlgoVital Plus	1.39 ± 0.12 (ab)	
0.03% Funguran	1.60 ± 0.13 (b)	
0.03% Funguran + 1.5% Cutisan	1.48 ± 0.20 (ab)	

Discussion

Many preparations are under discussion for use in fire blight control. In a systematic evaluation 18 control agents have been tested in different test systems. Twelve control agents suppressed *E. amylovora in vitro*, illustrating their potential for bacteriostatic behaviour. The *in vitro* activity was necessary but not sufficient to predict a high effectiveness on detached blossoms, or in the field. Even preparations as ElotVis and Mycosin, which showed high efficiency on detached blossoms, showed no or only slight disease reduction in the field. ElotVis acts by alcohol. The alcohol is active in the shaken culture, but due to evaporation it is not active following application in the field. The copper fungicide Funguran gave good results on detached blossoms at high concentrations (0,3%). The application of copper at high concentrations during bloom causes fruit russet. The use of a reduced copper concentration (0.03% Funguran) did not cause fruit russet in our experiment (tab. 3). The reduction of the copper concentration also resulted in a decrease of the efficiency on detached blossoms. This indicates that the field performance of the reduced copper concentration may not be satisfactory.

The two yeast preparations BPASC and Blossom-Protect showed the best results on detached blossoms and in the field experiments. Therefore the yeast preparations are promising tools for a fire blight control strategy in organic apple growing. Both preparations contain blastospores of *A. pullulans.* Despite reports on *A. pullulans* being able to cause fruit russet [13, 14], no increase in russeted fruits after the application of Blossom-Protect could be determined from our experiments. In field trials with Blossom-Protect, carried out over nearly 20 ha by organic farmers in 2004 and 2005 no increase of russeting was found [11].

A. pullulans is as a yeast sensitive to fungicides including sulphur and lime sulphur which are used for apple scab control. Blossom-Protect can not be mixed with these fungicides without reducing its efficiency against fire blight. Therefore fungicides should not be used on the day and two days after the application of Blossom-Protect. However, sulphur or lime sulphur can be used before and after this period, making it possible for Blossom-Protect to be integrated into the spray schedule of organic apple growers.

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