

## Development of "Blossom-Protect" - a yeast preparation for the reduction of blossom infections by fire blight

Entwicklung von "Blossom-Protect"- ein Hefepräparat zur Reduktion von Blüteninfektionen durch Feuerbrand

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### Abstract

In organic apple-growing control agents are necessary to prevent the blossoms being infected by the fire blight pathogen *Erwinia amylovora*. Detached apple blossoms were used as an experimental model to develop preparations based on yeast isolates for use in the control of fire blight. Several yeast isolates reduced disease incidence in apple blossoms. The efficiency of yeast isolates was increased by developing a suitable formulation. This yeast preparation exhibited high efficiency in the control of fire blight in field-trials and will be marketed under the tradename "Blossom-Protect".

### Keywords:

Fire blight, Blossom-Protect, *Biopro Erwinia amylovora*, *Aureobasidium pullulans*,

### Introduction:

Fire blight caused by *Erwinia amylovora* is the most serious bacterial disease in apples. During the last three decades it has spread through nearly all European countries. 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 infections occur in the blossom where the pathogen enters through natural openings after multiplying on the stigmas. To prevent the epiphytic multiplication by the pathogen several micro organisms were examined for their ability to colonise apple blossoms (3, 4, 6).. Yeast isolates showed a promising ability to colonise apple stigmas and nectar (7)..

In this study yeast strains, isolated from apple fruits, were tested for their ability to suppress symptom development on detached apple blossoms after inoculation with *E. amylovora*.. For these strains, production procedures were developed, providing storable powder formulations with high numbers of viable blastospores. Additives were found, which increased the efficiency of the yeast isolates on detached blossoms and in field trials.

### Material and Methods:

#### Micro organisms used and composition of test preparations

Names and origin of the isolates used are given in table 2. From these isolates three test preparations were produced (tab. 1).

**Table 1:** Composition of Test- preparations

Name	Microbial strains (spores/g)	Formulation	Conc. for application (%)
BIOPRO®	<i>BsBD170</i> ( $2 \times 10^{10}$ )	WP	0,1
<i>BPAxxx</i>	<i>ApCF10</i> ( $1 \times 10^{10}$ ) + <i>ApCF40</i> ( $1 \times 10^{10}$ )	WP	0,2
<i>BPASxxx</i>	<i>ApCF10</i> ( $1,5 \times 10^9$ ) + <i>ApCF40</i> ( $1,5 \times 10^9$ )	WP+buffer	1,3

### Reduction of fire blight symptoms on detached blossoms

An in vivo test-system with detached apple blossoms was established according to Pusey (6, 7). From January to August apple trees (`Gala`) were stored at 2°C in the dark. Weekly 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 solution in plastic racks (23°C, 100% rH). Blossoms were sprayed with a suspension of *E. amylovora* ( $10^6$  cfu/ml) in water until runoff. Depending on the experiment, treatments were sprayed 24h before, 1h after or 22h after inoculation. The number of blossoms with bacterial ooze at the peduncle was counted 6 days after the inoculation.

### Reduction of fire blight symptoms on blossoms taken from a field trial

The field trial was performed on the variety `Jonagold` and was arranged in a completely randomised block design with 4 replicates per treatment. Applications were timed according to the development of the blossoms and the infection risk predicted with the Maryblyt model. An infection risk day was predicted for the 29<sup>th</sup> April. Therefore, the first application was made on 28<sup>th</sup> April when approx. 50% of the blossoms were open. From the 4<sup>th</sup> May onwards the temperature necessary for infection was reached. On the 6<sup>th</sup> May the second application was made, when approx. 95% of the blossoms were open, as rain was predicted for the following days. During blossoming (26.04.03-08.05.03) no other pesticides were applied in the test orchard. The orchard was controlled for fire blight symptoms regularly from the end of July.

To see, if the blossoms were protected by treatment at a certain time, 4x24 blossoms per treatment were cut at the 28<sup>th</sup>, 29<sup>th</sup>, 30<sup>th</sup> of April and the 5<sup>th</sup> and 6<sup>th</sup> of May and inoculated with *E. amylovora* in the laboratory as described above.

## Results:

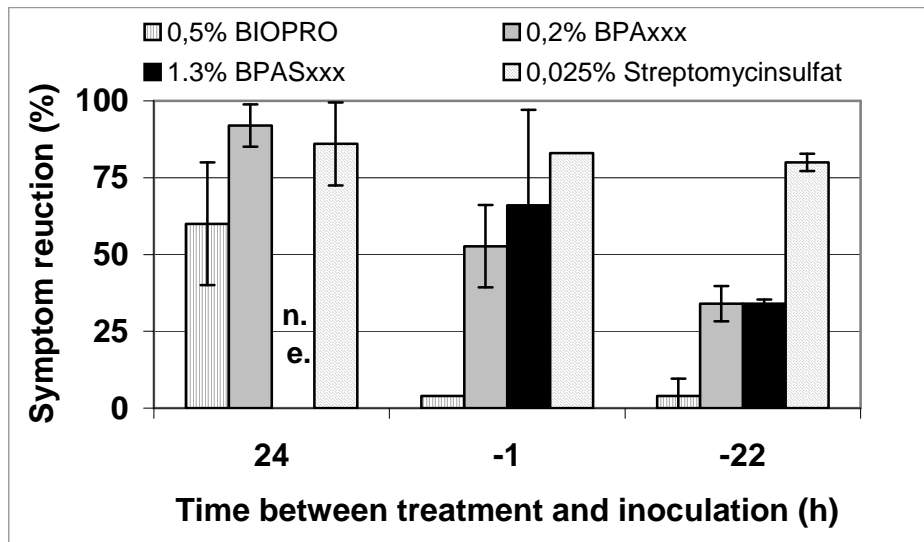
### Reduction of fire blight symptoms on detached blossoms

The ability of bacterial or yeast isolates to reduce fire blight symptoms was determined on detached apple blossoms. The microbial strains were cultivated in shake cultures and applied to the blossoms 24h before inoculation. The yeast isolates were more effective in reducing fire blight symptoms than the bacterial isolates (tab. 3). It was possible to develop production procedures for *ApCF10* and *ApCF40* resulting in storable powders with a high number of viable blastospores. *SrCF35* was not appropriate for mass production. Therefore *ApCF10* and *ApCF40* were used for further testing and to mix test preparations.

**Table 2:** Reduction of fire blight symptoms on detached apple blossoms by different microbial strains or streptomycin. Bacteria ( $10^8$  cfu/ml) or yeast ( $10^7$  cfu/ml) was suspended in water and sprayed on the blossoms 24h before inoculation with *E. amylovora* ( $10^6$  cfu/ml).

	Name	Species	Origin	No. *	Reduction of symptoms (%)
Bacteria	Bs BD170	<i>Bacillus subtilis</i>	BBA, Darmstadt	8	60
	Bs Hg77	<i>Bacillus amyloliquefaciens</i>	University of Konstanz	2	67
	Ra 39	<i>Rahnella aquatilis</i>	BBA, Darmstadt	2	64
Yeast	Ap CF10	<i>Aureobasidium pullulans</i>	University of Konstanz	2	88
	Ap CF40	<i>Aureobasidium pullulans</i>	University of Konstanz	6	93
	Sr CF35	<i>Sporobolomyces roseus</i>	University of Konstanz	3	79
Antibiotic		Streptomycin-sulfat (0,02%)		6	89

\* No. of experiments



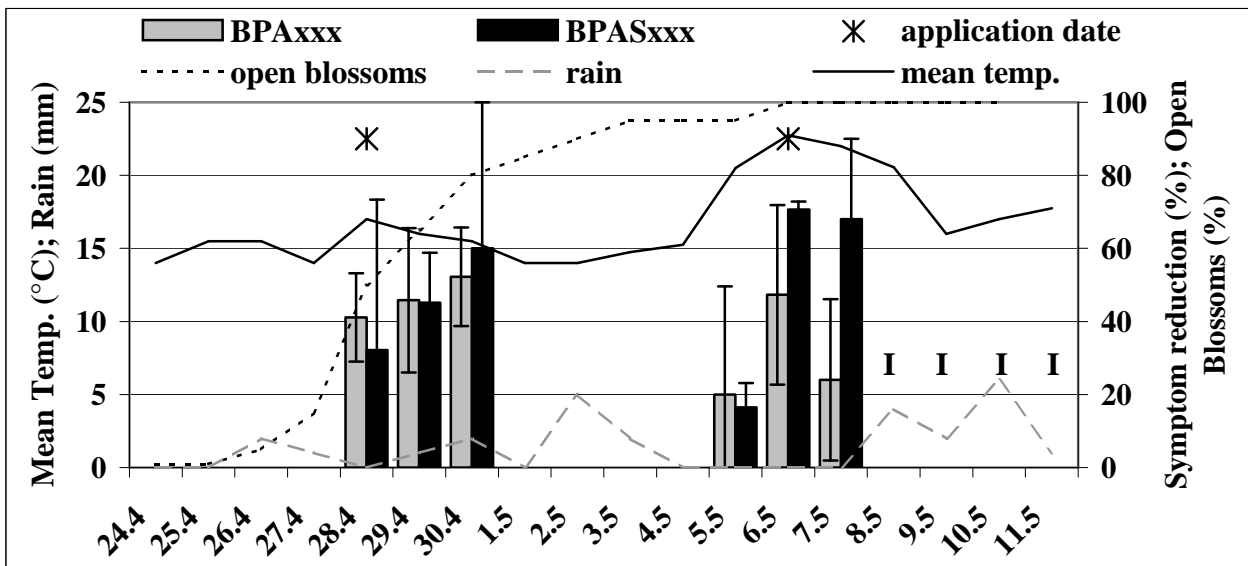
**Figure 1:** Reduction of fire blight symptoms by different test preparations on detached apple blossoms. The blossoms were treated 24h before, 1h after or 22h after the inoculation with *E. amylovora* ( $1 \times 10^6$  Zellen/ml). BPASxxx was not examined at 24h before inoculation.

Biopro reduced fire blight symptoms on detached apple blossoms by 60%, when applied 24h prior to inoculation with *E. amylovora* whereas BBAxxx reduced it by 92% and streptomycinsulfat by 86% when applied at

the same time (fig.1). The treatment with BPAxxx 1h after the inoculation led to a symptom reduction of 53%. Additives in BPASxxx, which made the application suspension acidic, increased the symptom reduction to 66%.

Following the Maryblight model *E. amylovora* needs a growth phase on the stigma characterised by a sum of degree hours (> 18,3°C) of 110. The incubation of the blossoms at 23°C for 22h leads to a sum of degree hours of 103. Applications at this time with BPAxxx or BPASxxx reduced disease symptoms by approx. 30% whereas a treatment with streptomycin was more efficient (fig. 1). This shows, that the yeast preparations were able to prevent the growth of *E. amylovora* on the blossom surface, but were not able to prevent infections after the pathogen was able to multiply on the stigmas.

### Reduction of fire blight symptoms on blossoms taken from a field trial



**Figure 2:** Reduction of fire blight symptoms on blossoms taken from the field trial by 0,2% BPAxxx or 1,3% BPASxxx. The Application was done at the marked dates (X). Per sampling date and treatment 4x24 blossoms were cut, put in 10% sucrose solution and inoculated with *E. amylovora* in the laboratory. After 6d incubation (23°C ; 100% rH) blossoms with bacterial ooze at the peduncle were counted. The columns indicate the symptom reduction in comparison to the untreated control. Infection risk days (Maryblight) are marked with I.

In the test orchard no fire-blight symptoms were found in 2003. One reason for this, is that on 6<sup>th</sup> May, 100% of the blossoms were already open and infection risk days did not occur until 8<sup>th</sup> May (fig. 2).

The artificial inoculation of the detached blossoms taken from the field trial shows, that the yeast preparations BPAxxx und BPASxxx reduced the fire blight symptoms noticeably (fig. 2). After the first application the symptom reduction reached 60% over a period of three days. The 4<sup>th</sup> sampling date was 7 days after application. In the meantime, the treated blossoms faded and new blossoms had opened - the symptom reduction was correspondingly low. In the samples from the day of the second application and on the following day a symptom reduction of 70% was reached with BPASxxx (fig. 2).

### Discussion:

The experiments on detached apple blossoms showed, that spray treatments with yeast isolates reduced fire blight symptoms significantly. The yeast isolates needed less time to build up a defence barrier on the blossoms than the tested bacteria and the efficiency of the yeast isolates was not dependent on the incubation temperature. The use of a suitable formulation increased the efficiency of the yeast strains.

The symptom reduction shown here on detached blossoms was confirmed by field trials, in which some trees were inoculated with *E. amylovora*. In 2002 a preparation corresponding to BPAxxx achieved a symptom reduction of 37% averaged over 4 trials (1, 2, 5).. In 2003 the use of the formulated preparation BPASxxx resulted in a symptom reduction of 74% (8). As a result of the demonstrated effectiveness of this yeast preparation as a biological control agent against fire blight, it will be marketed under the trade name "Blossom-Protect". Further investigations to integrate "Blossom-Protect" into spray schedules of organic - and integrated apple production will be carried out.

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