

## Development of "Boni-Protect" - a yeast preparation for use in the control of postharvest diseases of apples

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### Abstract

Postharvest pathogens cause major losses in apple production. *Gloeosporium album* (Pezicula alba) and *Gloeosporium perennans* (Pezicula malicorticis) are the main pathogens in organic fruit growing. There are several microorganisms with an antagonistic efficiency against these fungi. Out of these organisms two yeast isolates have been chosen to develop the plant strengthener Boni-Protect. Boni-Protect reduced the symptom development of *P. malicorticis*, *B. cinerea* and *P. expansum* after artificial inoculation of apple wounds. In several field trials preharvest applications of Boni-Protect led to reduced disease development during storage.

**Keywords:** Postharvest disease of apples, Boni-Protect, Gloeosporium, Botrytis, Penicillium

### Introduction

Postharvest pathogens cause major losses in apple production. More than 90 fungal species have been described which cause decay of apples during storage. The relative importance of each pathogen depends on climatic and storage conditions as well as on the growing system. *Gloeosporium album* (Pezicula alba) and *Gloeosporium perennans* (Pezicula malicorticis) are the main pathogens in organic fruit growing [1]. Several microorganisms with high efficiency against postharvest pathogens have been selected during a screening procedure of fungal and bacterial isolates at the University of Constance in the 1990's [2, 3]. Mixtures of different antagonists were tested in field trials on Golden Delicious. The efficiency of the antagonistic mixtures were comparable to chemical fungicides [4]. Out of this set of antagonists, two fungal isolates (*Aureobasidium pullulans*) have been selected for further investigations. Production procedures for the two strains and a suitable formulation were developed to create the plant strengthener Boni-Protect. Boni-Protect was evaluated in laboratory and field trials for its ability to prevent apple decay during the past four years.

### Material and Methods

#### a. laboratory experiments

Apples (cultivar Jonagold) were used for inoculation tests. The apples were surface-sterilized by soaking in 70 % ethanol for 3 min, wounded by removing plugs of 5mm diameter and 3mm in depth from the surface. Each apple was wounded four times halfway between the calyx and the stem end. Two wounds were treated with 5 µl of 0.1% Boni-Protect, 0.15% of Euparen MWG, 0.01% Flint or 0.25% Kumulus. The other two wounds were treated with 5 µl distilled water (control). All wounds were inoculated with 10 µl of a conidial suspension ( $10^5$  spores/ml) of a pathogen (*Pezicula malicorticis*, *Botrytis cinerea* or *Penicillium expansum*), afterwards. The apples were placed in sterile boxes and kept at 4°C. Diameters of lesions were determined after 4 weeks. For each apple the reduction in lesion diameter was calculated. Minimum 10 apples were used per treatment.

## b. field experiments

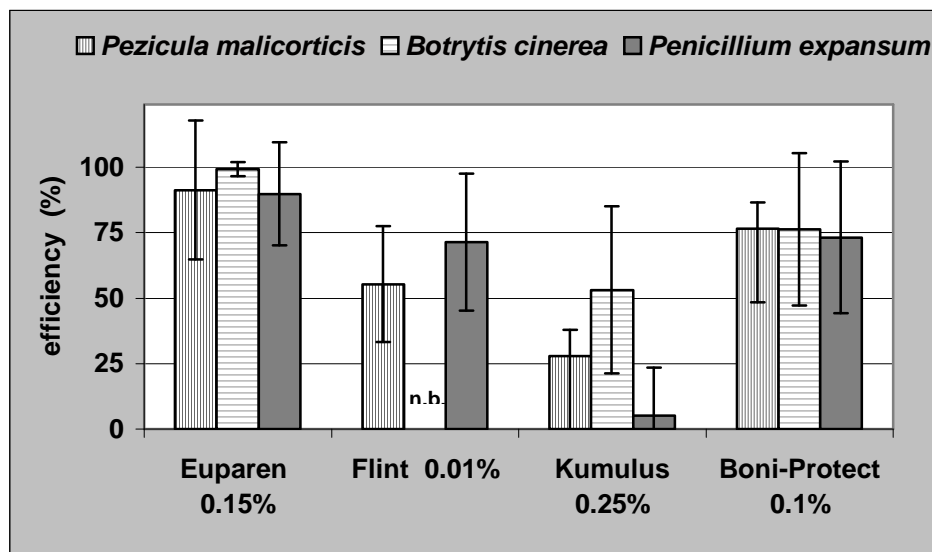
The experiments with Boni-Protect were placed in an apple orchard (cultivar Cox-Orange) near the lake of Constance during the years 2002 – 2005. The orchard was managed following the guidelines of Integrated production. The untreated control, the treatment with Boni-Protect (500 g/(ha x m Kh [crown height])) and Boni-Protect (500 g/(ha x m x Kh)) + Düngal Calcium (6l/(ha x m x Kh)) was arranged in a randomized block design with four replicates. The experimental block was not treated with chemical control agents after two weeks before the first application of Boni-Protect. The apple grower sprayed the bordering trees with standard chemical treatments (tab 1). Each year 1500-2300 apples were picked per treatment and stored at 2°C for 3 to 4 month (tab 1).

**Table 1:** Dates of application of treatments (Du Pont Benomyl 150 g/ha x m KH; Flint 50 g/ha x m KH; Malvin 600 g/ha x m KH;.Euparen MWG 600 g/ha x m KH; Merpan 80 MWG 600 g/ha x m KH; Düngal-Calcium 2,5 /ha x m KH) and harvest and rating of the apples.

<b>Treatment</b>	<b>2002</b>	<b>2003</b>	<b>2004</b>	<b>2005</b>
Boni-Protect	07.08. 19.08.	30.07. 11.08.	10.08. 23.08.	17.08. 01.09.
Boni-Protect+ Düngal-Ca	05.09.	25.09.	07.09.	09.09.
Chemical Standard	05.08. Du Pont Be- nomyl	26.07. Flint, Düngal- Calcium 15.08. Flint, Düngal Calcium	05.08. Malvin 17.08. Flint, Düngal-Calcium 28.08. Euparen, Düngal- Calcium	08.08. Merpan 80 WG 28.08. Euparen, Düngal- Calcium
Harvest	11.09.	09.09.	14.09.	19.09.
Rating	27.11.	20.11.	22.11.	01.12.

## **Results**

### Inoculation tests with wounded apples

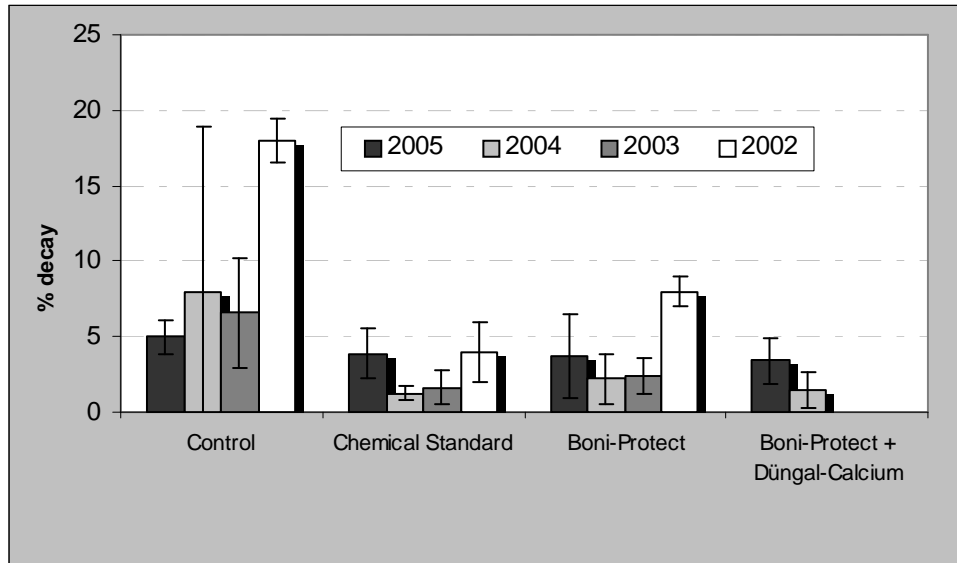


**Figure 1:** Efficiency of Boni-Protect in comparison to fungicides on the lesion development of decay fungi after inoculation of apple wounds.

The treatment of apple wounds with 0.15% Boni-Protect resulted in a high reduction of lesion diameters caused by the decay fungi *Pezicula malicorticis*, *Penicillium expansum* and *Botrytis cinerea*. The efficiency of Boni-Protect is comparable to the efficiency of Euparen MWG and Flint. Kumulus led to only slight reductions in lesion diameters.

#### Field experiments

Boni-Protect was tested in four years on the apple variety Cox Orange in comparison to an untreated control and the chemical standard used by the grower. In the untreated control 5% to 18% of the apples were lost due to decay fungi. The pre-harvest application of Boni-Protect reduced apple decay during storage significantly in the years 2002-2004 and was comparable to the chemical standard. An efficiency of up to 72 % was reached with Boni-Protect. The additive application of Düngal-Calcium has no significant effect on the efficiency of Boni-Protect (fig. 2).



**Figure 2:** Amount of decayed apples after storage of apples which have been treated in the field .

### Discussion

Yeast isolates are described as antagonists to apple decay fungi [4, 5]. Two strains of *Aureobasidium pullulans* were used to develop a biological preparation for use against storage diseases in apple. Production procedures and a suitable formulation for field application were developed. The product named Boni-Protect was evaluated in laboratory and field trials for its ability to prevent apple decay during the past four years. The results of a four-year field experiment showed that Boni-Protect reduced apple decay during storage. The results are comparable to the use of chemical fungicides. In the field experiments conducted so far the main pathogens have been *Botrytis cinerea*, *Penicillium expansum* and *Monilia* sp.

*Gloeosporium perennans* (*Pezicula malicorticis*) and *G.album* (*Pezicula alba*) are the main pathogens in organic fruit growing. They destroy approximately 30% of the stored apples [1]. Therefore Boni-Protect was tested for the efficiency against *Gloeosporium* in apple wounds and led to a reduction of lesion diameter of 75 %. This indicates that Boni-Protect will be a solution for reducing the loss of apples during storage due to *Gloeosporium* infections in organic growing. Field experiments in organic apple orchards started in 2005.

### Literature Cited

- Maxin, P. and K. Klopp, 2004. Die Wirkung des Heißwassertauchverfahrens gegen biotische Lager-schäden im ökologischen Obstbau. *Öko-Obstbau*, (3): p. 3-11.
- Falconi, C.J. and K. Mendgen, 1994. Epiphytic fungi on apple leaves and their value for control of the postharvest pathogens *Botrytis cinerea*, *Monilinia fructigena* and *Penicillium expansum*. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 101(1): p. 38-47.

- Schiewe, A. and K. Mendgen, 1992. Identification of antagonists for biological control of the post-harvest pathogens *Pezicula malicorticis* and *Nectria galligena* on apples. *Journal of Phytopathology*, 134: p. 229-237.
- Leibinger, W., et al., 1997. Control of postharvest pathogens and colonization of the apple surface by antagonistic microorganisms in the field. *Phytopathology*, 97: p. 1103-1110.
- Janisiewicz, W.J., T.J. Tworkoski, and C.P. Kurtzman, 2001. Biocontrol potential of *Metshnikowia pulcherrima* strains against blue mold of apple. *Phytopathology*, 91(11): p. 1098-1108.