Use of *Aureobasidium pullulans* for Resistance Management in Chemical Control of *Botrytis cinerea* in Berries

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Abstract

Infections with *Botrytis cinerea* cause serious damage in strawberry production. The infection starts already at blossom. For *Botrytis* control several treatments are necessary. After repeated treatments, chemical fungicides are highly at risk to loose efficacy due to the development of resistant strains of *B. cinerea*. A simple and effective measure for resistance management is the replacement of chemical fungicides with single side mechanisms by biocontrol agents containing antagonistic microorganisms. *Aureobasidium pullulans* used in Boni Protect forte has a strong potential to fulfill this mission. Its efficacy in the control of *B. cinerea* could be shown in two years trials in strawberries at two different locations in Germany and in a one year trial in Poland on raspberries. Spray strategies in which Boni Protect forte was used in alternation with chemical fungicides revealed comparable control of *B. cinerea* as the standard treatments with chemical fungicides.

INTRODUCTION

Fruit decay is one of the most serious diseases in plants and causes enormous economic losses, especially in commercial fruit production. The control of the disease is very complex and successes are often just moderate because the appearance of infections is subjected to various factors.

Botrytis cinerea, causing grey mold, is the main pathogen in strawberries and infects already at the state of opening blossoms. The occurrence of infections depends very much on weather conditions during harvest. The pathogen can only be combated protectively. Several fungicide applications have to be done to protect all open blossoms.

Distinctive for the active ingredients nowadays used in *Botrytis* control is the specific mode of action (so called single side inhibitors). The repeated use of these substances led to a selection of resistant strains in the pathogen population.

The occurrence of resistant strains against all common used chemical fungicides in strawberry production in Germany has been described (Weber, 2011).

The complete renounce of products with substances at risk for resistance would have enormous implication for integrated fruit production. Furthermore, also products with new active ingredients are usually acting as single side inhibitors (Prochnow et al., 2010) and have to be shielded as well from resistance development in the pathogen population to keep their effectiveness.

One opportunity to prevent the risk for resistance will be the tightly focused use of highly specific fungicides and the application of products with a different mode of action like antagonistic bacteria or yeasts.

Boni Protect forte based on the yeast like fungus *Aureobasidium pullulans*, has antagonistic potential against several pathogens in fruit-growing. In numerous trials on different fruits *Aureobasidium pullulans* showed its high efficacy. (Kunz, 2004; Weiss et al., 2006; Achleitner, 2010; Weiß et al., 2012). Therefore the use of Boni Protect forte in spray strategies will be a helpful manner to prevent resistances against chemical fungicides in *B. cinerea*.

MATERIAL AND METHODS

Field trials were done in strawberry fields (cultivar 'Clery') in 2011 and 2012 at two locations in Baden Württemberg, Germany, and in a raspberry field (cultivar 'Polana') 2011 located in the Lublin area in Poland.

Experiments were performed from April to May 2011 and 2012 in strawberries and from July to September 2011 in raspberries. Treatments were done in strawberries between beginning of bloom (BBCH 62 (Meier et al., 1994)) until end of bloom (BBCH 69). The dates, products and doses used in the trials for *B. cinerea* control are shown in Table 1.

At ripeness of strawberry fruits (BBCH 85) disease incidence was evaluated by counting infection sites caused by *B. cinerea* on remaining flowers and fruits of 28 plants in every replicate of each treatment.

In raspberries applications were done from the beginning of flowering (BBCH 61 (Schmid et al., 2001)) to the end of flowering were the majority of the flowers faded and first fruits were visible (BBCH69). Detailed trial data are shown in Table 2.

For evaluation, fruits of every treatment were harvested eight times during the ripening period and the masses of healthy fruit and of fruit infected by *B. cinerea* were determined. For each picking the disease incidence of every replicate in each variant was calculated. Incidences of the eight pickings were averaged in each replicate and an overall average per variant was calculated.

Data Analyses

All trials were conducted in a randomized block design with four repetitions. Efficiencies of treatments against *B. cinerea* were calculated according to Abbott (Abbott, 1925). Statistical analyses of the data were done using one-way analysis of variance, and mean separation was accomplished using Tukey's Multiple Comparison Test ($p<0.05$).

RESULTS AND DISCUSSION

The application of *Aureobasidium pullulans* to strawberry blossoms or green fruits reduced *Botrytis* incidence on fruits in a study in Australia (Adikaram et al., 2002). Mayr and Späth (2008) demonstrated the positive effect of Boni Protect forte treatments (containing *A. pullulans*) on yield and on shelf life of fruits in three years trials in organic strawberry orchards. The effect of *A. pullulans* against *B. cinerea* was also demonstrated in strawberry tunnels, in which *A. pullulans* was dispersed by bumble bees (Weiß et al., 2012).

Therefore in this study spray applications of Boni Protect forte were tested in strawberries and raspberries as stand-alone treatments and in spray strategies with chemical fungicides for their ability to control infections with *B. cinerea*. In 2011 the *Botrytis* incidence was low at the Buchholz trial at BBCH 85 evaluation. All treatments showed significant disease reduction with efficiencies of more than 59% (Fig. 1). At a higher infection pressure resulting in an incidence of 180 infection sites per 28 plants in 2012, again all three treatments reduced the disease incidence significantly. The stand-alone treatment with Boni Protect forte as well as the spray strategy using chemical fungicides in alternation with Boni Protect forte was comparable to the chemical standard in both years (Fig. 1).

At the Oberkirch trial the *Botrytis* incidences in the control were comparable in both years. In both years in all treatments (control, chemical standard, Boni Protect forte) an initial chemical treatment with Switch took place at the beginning of flowering at BBCH state 62. In 2011 the chemical standard as well as the Boni Protect forte variant showed an efficiency of 73%. In 2012 efficiency of 57% in the chemical standard treatment and of 63% in the Boni Protect forte variant were observed. All treatments showed significant effects in both years (Fig. 2).

The mean *Botrytis* incidence in the raspberry trial was 29% in untreated control. All three treatments showed significant reduction of *B. cinerea* incidence (Fig. 3). The spray strategy (eff. 86%) and the chemical standard treatment (eff. 89%) were significantly better than the stand alone treatment with Boni Protect forte (eff. 69%).

In all five trials Boni Protect forte reduced the *B. cinerea* incidence significantly compared to untreated control. Spray strategies of Boni Protect forte and chemical fungicides showed also significant effects and tended to be better than the stand-alone treatment with Boni Protect forte. The use of Boni Protect forte instead of one to three chemical fungicide treatments denotes no losses in plant protection. Furthermore the use of Boni Protect forte instead of one to three chemical treatments will reduce chemical residues on the harvested fruits and reduce the risk for selection of *B. cinerea* strains resistant to chemical fungicides. Therefore Boni Protect forte represents a promising tool for integrated pest management. The results with Boni Protect forte showed also reproducible good effects as stand-alone treatment. So it could also be used for control of *B. cinerea* in organic berry growing.

CONCLUSION

The yeast like fungus *Aureobasidium pullulans* used in Boni Protect forte has a strong potential in the reduction of *B. cinerea* incidence. Therefore *A. pullulans* is qualified to be used in organic berry production as well as in spray strategies in integrated pest management of *B. cinerea* in berries. The use of Boni Protect forte in spray strategies can reduce chemical residues on the harvested fruit and can prevent the rapid increase of resistant strains of *B. cinerea* to chemical fungicides.

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Tables

Table 1. Application dates, BBCH states, products and doses used for *B. cinerea* control in the strawberry trials.

Trial	Treatment	Application: product/dose (kg/ha)			
Buchholz 2011[a]		02.04.2011 (BBCH 60)	11.04.2011 (BBCH 63)	18.04.2011 (BBCH 65)	26.04.2011 (BBCH 69)
	Control	-	-	-	-
	Chemical standard	Signum / 1.8	Switch / 1.0	Switch / 1.0	Switch / 1.0
	Boni Protect forte	BPf / 1.0	BPf / 1.0	BPf / 1.0	BPf / 1.0
	Spray strategy	Switch /1.0	Teldor / 2.0	BPf / 1.0	BPf / 1.0
Buchholz 2012[a]		07.04.2012 (BBCH62)	13.04.2012 (BBCH64)	19.04.2012 (BBCH65)	26.04.2012 (BBCH69)
	Control	-	-	-	-
	Chemical standard	Signum / 1.8	Switch / 1.0	Switch / 1.0	Switch / 1.0
	Boni Protect forte	BPf / 1.0	BPf / 1.0	BPf / 1.0	BPf / 1.0
	Spray strategy	Signum / 1.8	Switch / 1.0	BPf / 1.0	BPf / 1.0
Oberkirch 2011		08.04.2011 (BBCH62)	15.04.2011 (BBCH64)	23.04.2011 (BBCH67)	
	Control	Switch / 1.0	-	-	
	Chemical standard	Switch / 1.0	Flint / 0.3 + Teldor / 2.0	Switch / 1.0 + Topas / 0.5	
	Boni Protect forte	Switch / 1.0	BPf / 1.0	BPf / 1.0	
Oberkirch 2012		10.04.2012 (BBCH62)	13.04.2012 (BBCH64)	26.04.2012 (BBCH67)	04.05.2012 (BBCH69)
	Control	Switch / 1.0	–	–	–
	Chemical standard	Switch / 1.0 +Malvin / 2.0	Switch / 1.0 + Malvin / 2.0	Flint / 0.3 + Teldor / 2.0	Teldor /2.0
	Boni Protect forte	Switch / 1.0	BPf / 1.0	BPf / 1.0	BPf / 1.0

BPf = Boni Protect forte (*Aureobasidium pullulans*).

[a] Before blossoms opened (BBCH 57-59), score (0.4 kg/ha), Calypso (0.25 L/ha) and Milbeknock (1.25 L/ha) were applied against powdery mildew, insects and mites in all treatments except the control.

Table 2. Application dates, BBCH states, products and doses used for *B. cinerea* control in the raspberry trial.

Treatment	Application: product/dose (kg/ha)				
	11.07.2011	23.07.2011	01.08.2011	09.08.2011	12.08.2011
Control	_	_	_	_	_
Chemical standard	Switch / 0.8	Switch / 0.8	Rovral /1.5	_	Teldor /1.5
Boni Protect forte	BPf / 0.8	BPf / 0.8	BPf / 0.8	BPf / 0.8	_
Spray strategy	Switch / 0.8	Switch / 0.8	BPf /0.8	BPf / 0.8	_

BPf = Boni Protect forte (*Aureobasidium pullulans*).

Figures

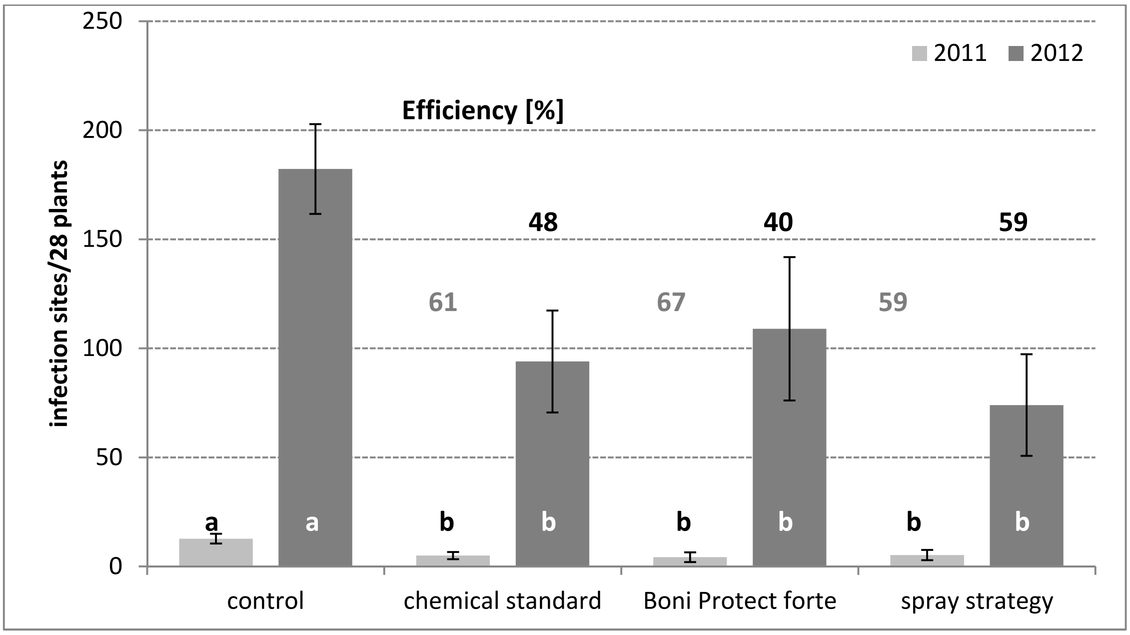

Fig. 1. Number of *Botrytis* infection sites in the strawberry trials in Buchholz in 2011 and 2012 in different treatments. Different letters in columns indicate a significant difference in Tukey's Multiple Comparison Test (p<0.05) in that year; n=4.

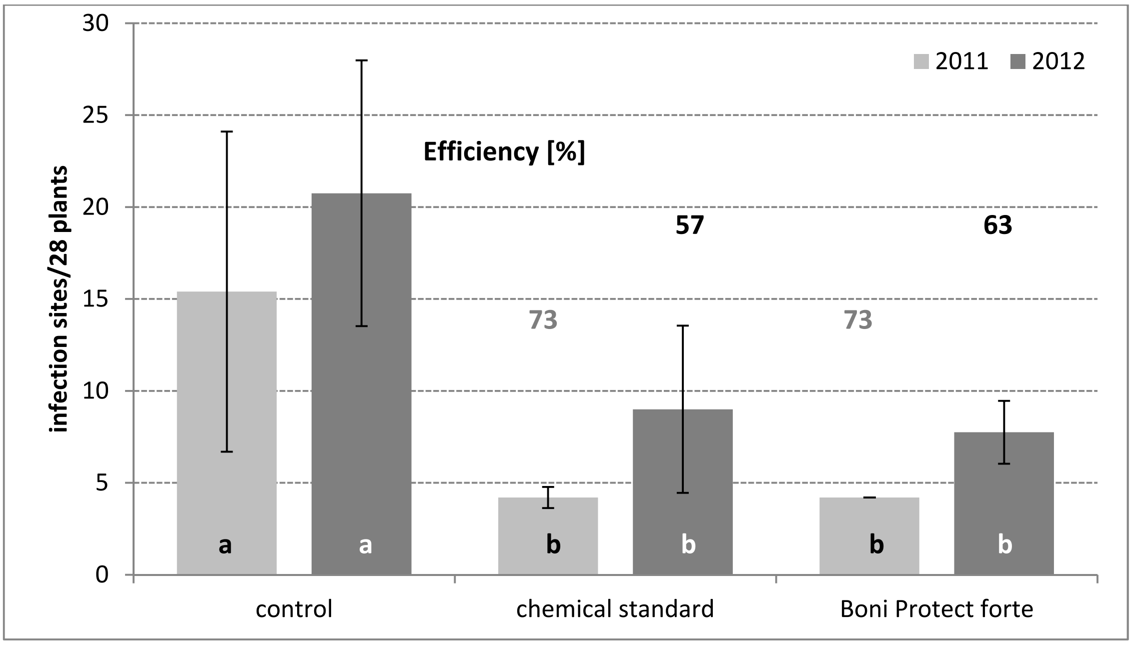

Fig. 2. Number of *Botrytis* infection sites in the strawberry trials in Oberkirch in 2011 and 2012 in different treatments. Different letters in columns indicate a significant difference in Tukey's Multiple Comparison Test ($p<0.05$) in that year; n=4.

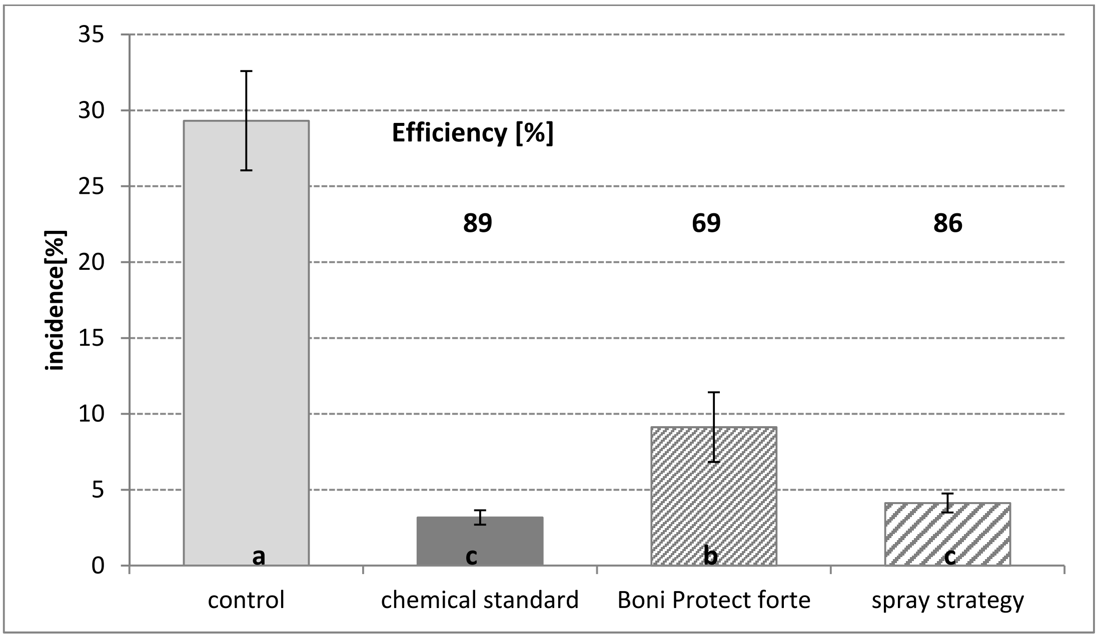

Fig. 3. Incidence of *Botrytis* in raspberries with different treatments in 2011. Different letters in columns indicate a significant difference in Tukey's Multiple Comparison Test ($p<0.05$); n=4.