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Biocontrol of fire blight with the antagonist *Rahnella aquatilis* and a natural product of *Thymbra spicata*, BioZell-2000B

Abstract

As alternative products to the antibiotic streptomycin against fire blight, bacterial antagonists and a natural product were tested for their efficacy in greenhouse and field experiments. The antagonistic strain of *Rahnella aquatilis* Ra39 in combination with aromatic compounds could increase the efficacy up to 68%. The etheric oil compound, BioZell-2000B was able to reduce blossom infection by up to 52% and is proposed as a bioagent for ecological farming.

Key words: Fire blight, biocontrol, bacterial antagonists, natural products

Introduction

Research on alternatives to the antibiotic streptomycin for the control of fire blight in pome fruits has become of main interest in German and European fruit growing, since the permission for the use of antibiotics in EEC-countries will be withdrawn in the future. Thus the development of biologicals on the basis of natural products and antagonistic bacteria against the pathogen has been undertaken in Germany, especially by the Federal Research Centre for Agriculture and Forestry (BBA), in cooperation with the plant protection service. Latest results with antagonistic bacteria and a natural product based on an etheric oil of the thyme species *Thymbra spicata*, BioZell-2000B, which was developed together with Turkish colleagues (Yegen et al. 2002), will be presented.

Material and Methods

Studies with bacterial antagonists: In this study the virulent strain of *Erwinia amylovora* Ea 7/74 and the antagonistic strain *Rahnella aquatilis* Ra39 were used. *In vitro* studies, for estimating the effect of aromatic compounds and the antagonistic strain on the growth of *E. amylovora* were carried out in buffered nutrient sucrose (NS) medium. The used benzoate concentration was between 10 and 100 mM; the degradation of Na-benzoate by *R. aquatilis* Ra39 was measured spectrophotometrically (UV) over 9 days after addition of the bacteria to the medium. Field studies were done during full blossom of apple `Golden Delicious` applying to a spraying schedule with the antagonistic strain Ra39 in different combinations with Na-benzoate and the growth regulator of BASF REGALIS (APOGEE) under natural infection conditions. Infected blossom clusters were counted after blossom time and the efficacy was calculated in %.

Studies with natural products

Plant material and application of treatments: As host plants, M26 rootstock apple plants were grown in pots under greenhouse conditions until a shoot length of 20-30 cm. BioZell-2000B was used at a concentration of 0.05 % (diluted with tap water) as inducing agent by spraying on leaves 48 h before inoculation. Control plants were treated with tap water.

Inoculation: After cutting off the tip of the youngest two leaves of the shoots, the leaves were inoculated by dipping into a suspension of 10^8 cfu/ml of the virulent strain of *Erwinia amylovora* Ea 7/74 (Zeller and Meyer 1975).

Determination of symptom development: Symptom development was evaluated according to a rating system from 0 (no symptom) to 10 (whole shoot infected). From the data the disease index (%) was calculated according to Baysal and Zeller (2000).

Results and Discussion

Studies on bacterial antagonists: Screening of several potential antagonists among more than 120 isolates showed inhibitory effects mainly of the species *Pseudomonas fluorescens*, *Pantoea agglomerans* and *Bacillus subtilis* (Zeller and Wolf 1996), later also of the epiphytic bacterium *Rahnella aquatilis* (Laux et al. 2002). Moreover, in field experiments efficacies up to 61% could be observed (Table 1) but without a control effect similar to streptomycin. In order to increase the efficacy of strain Ra39, the antagonist was combined with aromatic compounds, since it was less sensitive to benzoate than the fire blight pathogen *in vitro* and since it was able to use this bactericidal substance as nutritive source (Figure 1). In field experiments the combination of Ra39 and Na-benzoate was nearly comparable in the efficacy to streptomycin with 68 to 77%. Also another combination with the growth regulator REGALIS (APOGEE) showed a high control effect of 68% (Figure 2).

Tab. 1 Efficacy of bacterial antagonists against blossom blight of *Erwinia amylovora*, 1998-2001

Antagonist	Origin	Efficacy %
Bacillus subtilis BsBD 170 (BIOPRO)		30-60
Rahnella aquatilis Ra39	Apple blossom (Steinbrenner, 1991)	39-53
Pseudomonas spp	Shoot tissue	45-58
Pantoea agglomerans Pa21889	Apple Blossom (Steinbrenner, 1991)	50-61
Control		
Streptomycin	*Streptomyces griseus*	68-80

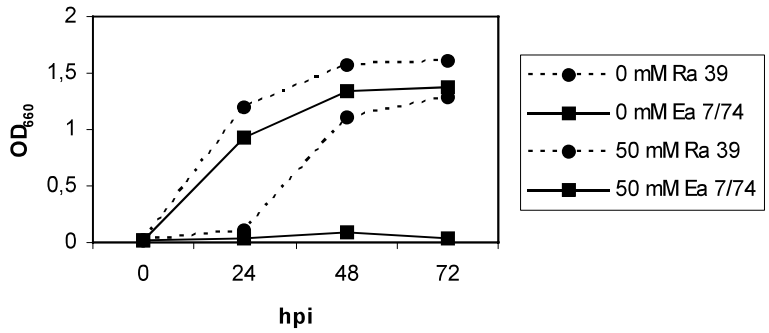
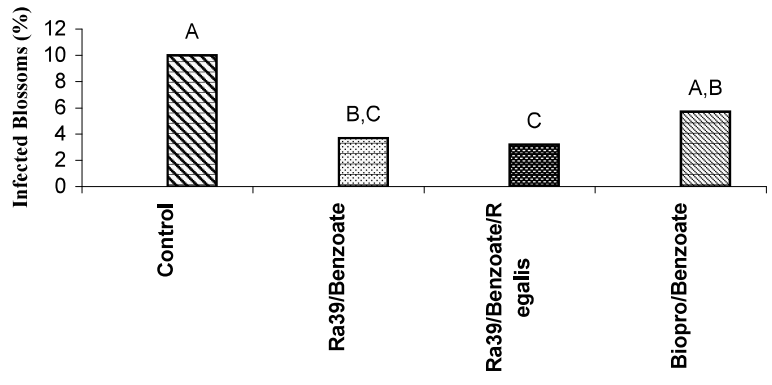


Fig. 1 Effect of Na-benzoate on the growth of the antagonistic strain



Ra39 and *E. amylovora* strain Ea 7/74 in vitro Control 63 68 43

Fig. 2 Fire blight control with the antagonist Ra39 in different combination on the apple variety `Golden Delicious` after natural infection, 2003

Studies on natural products: As another biological alternative, the natural product BioZell-2000B, a compound prepared of 70% thyme oil from *Thymbra spicata*. It has been registered in Turkey as a plant protection substance (Yegen et al. 2002) and in Germany as a plant strengthener. Here we tested it for its efficacy against fire blight under greenhouse and field conditions.

Under *in vitro* conditions no direct effect could be observed on the pathogen with BioZell-2000B at a concentration of 0.05% (Figure 3), indicating that the plant strengthener had no direct effect. In the greenhouse, symptom development on leaves of the highly susceptible apple rootstock M26 was markedly reduced after 4 days induction time with BioZell-2000B (Figure 4). Under field conditions a significant reduction of blossom infection of 52% was found on the apple variety Boskoop (Table 2). In a further control experiment with a highly susceptible *Cotoneaster* variety of *C. salicifolius* the control effect could be confirmed with 85% (Zeller and Laux 2002).

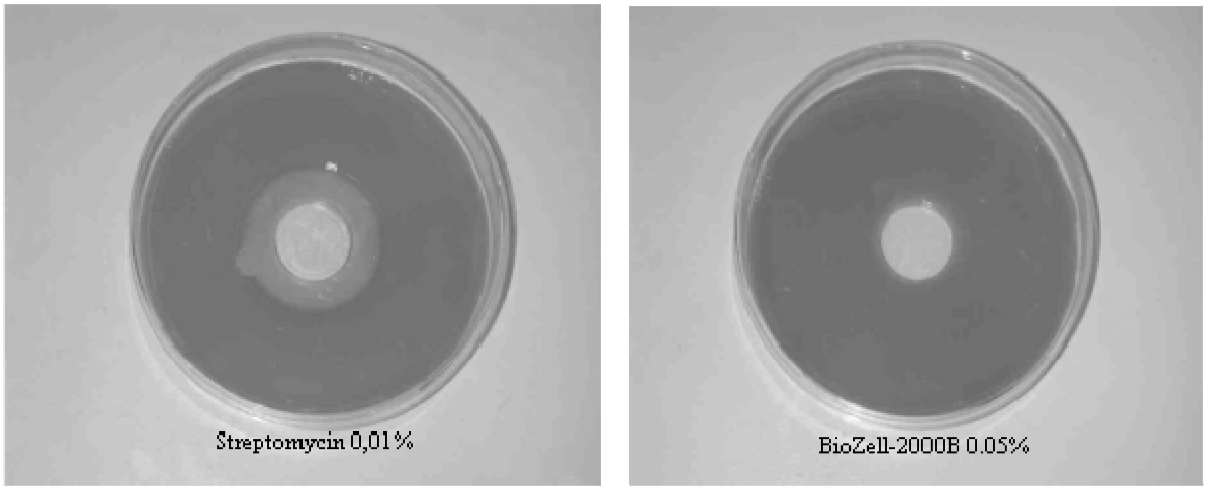


Fig. 3 Test of BioZell-2000-B (0.05%) in comparison to streptomycin (0.01%) in the agar-diffusion-test against *E. amylovora*

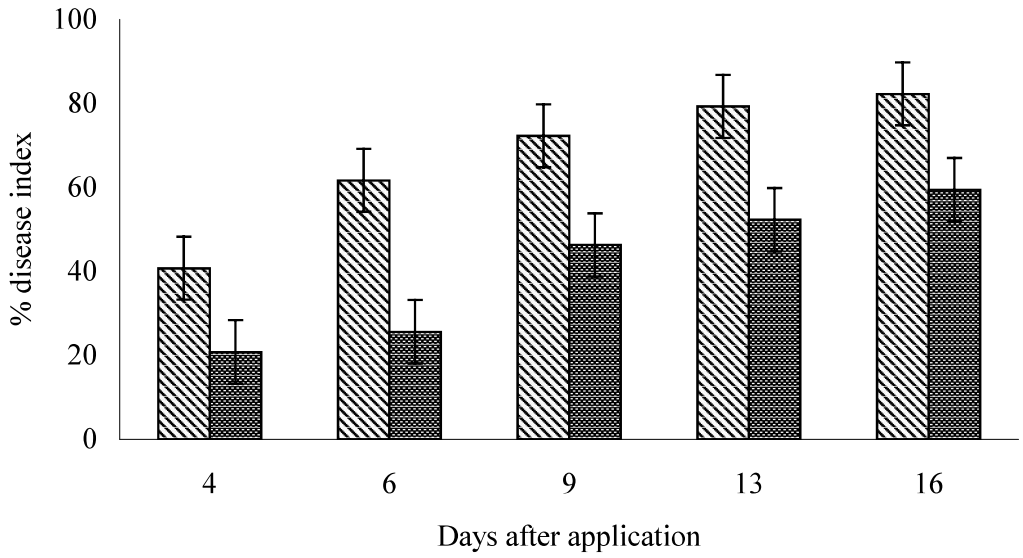


Fig. 4 Disease Index of fire blight on shoots of M26 apple rootstock after treatment with BioZell-2000-B (hatched) and of controls without treatment (dark)

Tab. 2 Fire blight control with BioZell-2000B on apple variety `Boskoop` after natural blossom infection, 2001

Treatment	No. of blossom clusters		Infection %	Efficacy %
	Total	Infected		
Inf. control	720	70	9,7 a	-
BioZell-2000B	680	30	4,7 b	52

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