Field trials with non-bactericide products to control fire blight in apple orchards

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Summary: Recently, novel strategies and chemical agents for prophylactic protection against the bacterial (*Erwinia amylovora*) disease fire blight are being sought. Resistance-inducing compounds, such as prohexadione-Ca represent promising alternatives. Prohexadione-Ca is the active ingredient of the bioregulator Regalis, currently being introduced in several European countries and overseas. Another product used in this study was Biomit Plussz, a leaf fertiliser providing harmonic supply of nutrient elements, the complete supply is assumed to improve the tolerance against diseases.

Treatments' effects of both of these products were compared to the effectiveness of treatments with antibiotics repeated twice, three or four times a season.

In the years of 2001 and 2002, the effectiveness of both Regalis and Biomit Plussz in reduction of incidence of shoot blight was similar, or proved to be superior to the check treatments consisting of repeated sprayings of antibiotics. Last year (2003) treatments of streptomycin resulted – although within the same magnitude – in a somewhat better control of shoot blight than sprayings with the other compounds.

As regards severity of blossom blight, inconsistent results were recorded concerning both Regalis and Biomit Plussz.

In general, prohexadione-Ca is less efficient for controlling flower infection by *E. amylovora* as compared to shoot infections, since successful prophylactic treatments are difficult to carry out early in the season. The highest effectiveness in fire blight management can, therefore, be achieved by using prohexadione-Ca (as preventive protection) in combination with streptomycin or other suitable antibiotics (as curative protection).

Key words: fire blight, *Erwinia amylovora*, Regalis, prohexadione-Ca, Biomit Plussz, apple, resistance inducers, preventive plant protection

Introduction

Erwinia amylovora is the causative agent of fire blight, a bacterial disease existing in most countries where pome fruits like apple and pear, or ornamental plants of *Rosaceae* are grown.

First description of 'fire blight' in fruit trees date back to Denning (1794; cited by *van der Zwet*, 2002). Fire blight as a danger for fruit production, however, was recognized by Cox in 1817 (cited by *van der Zwet and Keil*, 1979). Nevertheless, the prevention of this disease has long remained an unsolved problem. Losses caused by fire blight in the North-West USA in 1998 alone were estimated to be in excess of $68 million (*Vanneste*, 2000). In Italy, 500,000 fruit trees were destroyed as a consequence of the disease in 1997 (*Calzolari* et al., 1999). Fire blight was identified in Hungary in early 1996 (*Hevesi*, 1996) and by the end of that year 47,000 apple, 8,700 quince (*Cydonia oblonga*), 8,200 pear, 1,000 medlar (*Mespilus germanica*) trees and 600 shrubs of various ornamental plants were eradicated at a cost of $1.1 million (*Fishl*, 1997; Bonn and *van der Zwet*, 2000). Due to the fast spread of the disease, after just another two years only 2 of the 19 counties of Hungary could be considered as free of fire blight (*Németh*, 1999). Presently fire blight has occurred in over 40 countries of the world (*van der Zwet*, 2002).

The most important chemicals for controlling fire blight in pome fruit trees are copper compounds and antibiotics (*Psallidas* and *Tsiantos*, 2000; *McManus et al.*, 2002). Today, it is a common practice, for instance in California, to apply streptomycin sulphate every 4–5 days during bloom. As a consequence, resistance to streptomycin has developed in *E. amylovora* population in almost all of Californian apple and pear orchards (*Holtz et al.*, 2002).

Because of the lack of publicly acceptable, effective and non-phytotoxic plant protection chemicals to combat fire blight, novel control strategies have gained much interest in recent times, which trigger defence mechanisms in the host plants (*Maxson and Jones*, 2002). Such effects can, for instance, achieved by phosetyl-Al (Aliette), harpin protein (Messenger), acibenzolar-S-methyl; ASM (Bion or Actigard), and prohexadione-Ca; ProCa as Regalis or Apogee (*Momol et al.*, 1999; *Steiner*, 2000; *Aldwinckle et al.*, 2002, *McManus* et al., 2002; *Bazzi et al.*, 2003; *Norelli et al.*, 2003).

This study was devoted to estimate the effectiveness of both Regalis; a new, multifunctional bioregulator and Biomit Plussz; a leaf fertiliser with presumable plant activator activity.

Material and methods

Orchard

Experiments were carried out in a high-density orchard of 8 hectares, planted with trees on M.9 and M.26 rootstocks at

3.5 by 1.4 and 3.5 by 2.3 m, respectively. While planning the cultivar composition of the orchard, we have decided to plant trees of cultivars possessing different sensitivity to *E. amylovora*. Cultivars actually involved in the field trials are listed in Tables 1 to 5.

Products used

Regalis (a.i.: prohexadione-Ca 10%) is a bioregulator developed by the BASF Corporation (Germany). It has been registered as a plant growth regulator in Belgium, Chile, France, Hungary, Italy, The Netherlands, Poland and Spain, as well as with the trade name Apogee (a.i.: prohexadione-Ca 27.5%) in the USA. Furthermore, Regalis is permitted to use against secondary fire blight in Austria and Germany (with a time limit of 120 days per year).

Prohexadione-Ca inhibits certain steps in the biosynthesis of gibberellin and it may transiently intervene in the phenylpropanoid metabolism. The induction of pathogen resistance by prohexadione-Ca is attributable, at least in part, to qualitative and quantitative changes in the spectrum of flavonoids and their phenolic precursors in the treated plants (*Rademacher,* 2000; *Roemmelt* et al., 2003). The physiological mode of action of the prohexadione-Ca and its effectiveness in reducing infection with other bacterial and fungal pathogen in many, but not all, crop plant species has been reviewed elsewhere (*Bubán et al.,* 2003).

As for environmental saving points of view, prohexadione-Ca represents reduced risk to human health and environment, i.e. prohexadione-Ca is a candidate for "reduced risk" registration by the U.S. Environmental Protection Agency; EPA (*Winkler,* 1997). Its acute oral toxicity is LD50>5000 mg/kg, and no carcinogenic, mutagenic or teratogenic effects were observed. Animal and plant metabolism studies show that the active ingredient is bioconverted to naturally occurring products.

Biomit Plussz (Ponton Ltd, Hungary) is a leaf fertiliser containing macro-, meso- and microelements, as well as more than 60 plant extracts. Its involving in our experiments was based on the supposition that a complete and/or harmonic supply of nutrient elements should increase the resistance against diseases. Furthermore, the results of laboratory tests on fruit slices of the pear cv. Beurré Bosc (*Németh,* 1997) and apple seedlings inoculated with *E. amylovora* (*Németh–Kovács,* 1999) were promising enough for its effectiveness to be checked under orchard conditions.

Vektafid R (Rogator Ltd, Hungary) is a copper-oil pesticide containing paraffin oil (79%) and copper oleate (6%). It is patented in Hungary (*Ilovai et al.,* 1995). The aim of its spraying together with Biomit Plussz was to decrease the dose of the latter.

Treatments

2001

Bloom stages: pink bud April 17, beginning of petal fall May 8,

Spray volume: 600 litres/hectare
Regalis 100 g/100 litres: April 17, May 8 and 30,
50 g/100 litres: June 15, July 20
Additive: Citowett 30 ml/100 litres.

Biomit Plussz 0.5 litre/100 litres: April 5 and 17,
2.0 litres/100 litres: April 25, May 2, 8, and 30, June 15, July 5,
1.0 litre/100 litres: July 20.

2002

Bloom stages: pink bud April 19, beginning of petal fall May 6,
Spray volume: 600 litres/hectare
Regalis 150 g/100 litres: April 19,
100 g/100 litres: May 28.
Additive: Dash HC 50 ml/100 litres.
Biomit Plussz 1.0 litre/100 litres, from green bud stage to beginning of petal fall: 4 treatments,
after that (till end of July) another 5 treatments,
Additive: Vektafid R 0.5 litre/100 litres.

2003

Bloom stages: pink bud April 28, beginning of petal fall May 6,
Spray volume: 800 litres/hectare
Regalis 179 g/100 litres: April 28 and May 19,
Additive: Dash HC 100 ml/100 litres.
Biomit Plussz 2.0 litres/100 litres: April 24, 28 and May 5,
1.0 litre/100 litres: May 19, June 2, 19 and 30.

Treatments in check plots with antibiotics:

2001 Erwin 25WP (a.i.:streptomycin) 0.5 kg/hectare: May 1 and May 4,
Kasumin 2L (a.i.: kasugamycin) 4.0 litres/hectare: May 31 and June 25,
2002 Erwin 25WP, 0.5 kg/hectare: April 29 and May 3,
Kasumin 2L, 4.0 litres/hectare: May 16,
2003 Erwin 25WP, 0.5 kg/hectare: May 2 and May 6.
Important note: antibiotics were never used in plots of Regalis or Biomit treatments.

Evaluating incidence of fire blight

Fire blight is a sporadic disease in both space and time, which makes epidemiological studies and spray trials difficult to plan (*Billing,* 2000). A patchy distribution of fire blight symptoms is frequently observed, even in relatively small plots (i.e. replicates) of a trial established in the customary way. Large plots were therefore treated: at least 0.3 hectares involving a number of trees of several cultivars (listed in *Tables 1 to 5*).

Within the experimental plots all the trees were one-by-one and thoroughly supervised for incidence of fire blight. The 'quotient of infection' refers the number of symptoms to

Table 1 Incidence of fire blight* in trees of apple cultivars Újfehértó, 2001

Number of cultivars	Treat-ments	Date of investigations				Average
		June 5	June 18	July 6	August 21	
6	Regalis	2.7	0.0	0.0	0.0	0.7
6	Biomit Plussz	1.7	1.0	2.5	2.5	1.9
6	Check	4.3	0.4	1.2	3.5	2.3

*On June 5: (pieces of shoots and flower clusters infected/pieces of trees investigated) × 100
after June 5: (pieces of shoots infected/pieces of trees investigated) × 100
Cultivars: Sampion, Freedom, Jonagold, Jonagold deCosta, King Jonagold, Elstar
Number of trees checked on fire blight incidence in plots treated with: Regalis = 258, Biomit Plussz = 344, Check = 910

Table 2 Incidence of shoot blight* in trees of apple cultivars Újfehértó, 2002

Number of cultivars	Treat-ments	Date of investigations				Average
		May 23	June 6	June 20	July 30	
4	Regalis	7.8	8.5	4.1	1.1	5.4
6	Biomit Plussz	0.1	5.7	8.3	0.6	3.7
6	Check	4.0	8.7	7.6	1.2	5.4

*(pieces of shoots infected/pieces of trees investigated) × 100
Cultivars: Sampion, Freedom, Jonica, Jonagold deCosta, King Jonagold, Elstar (no trees of cultivars Freedom and Elstar were in plots treated with Regalis)
Number of trees checked on fire blight incidence in plots treated with: Regalis = 1410, Biomit Plussz = 910, Check = 829

Table 3 Incidence of blossom blight* in trees of apple cultivars Újfehértó, 2002

Number of cultivars	Treatments	Date of investigations			Average
		May 23	June 6	June 20	
4	Regalis	42.0	18.5	0.2	20.2
6	Biomit Plussz	0.7	4.0	0.5	1.7
6	Check	11.3	9.1	0.5	7.0

*On May 23: (pieces of flower clusters infected/pieces of trees investigated) × 100
after May 23: (pieces of young fruitlets infected/pieces of trees investigated) × 100
Cultivars and pieces of trees checked on fire blight: see *Table 2*

Table 4 Incidence of shoot blight* in trees of apple cultivars Újfehértó, 2003

Number of cultivars	Treatments	Date of investigations			Average
		May 28	June 13	June 16	
7	Regalis	4.3	13.7	7.2	8.4
8	Biomit Plussz	3.3	11.9	5.2	6.8
8	Check	1.4	7.5	8.9	6.0

*(pieces of shoots infected/pieces of trees investigated) × 100
Cultivars: Sampion, Freedom, Jonica, Jonagold deCosta, King Jonagold, Elstar, Gala Must, Pinova (no trees of the cultivar Pinova were in plots treated with Regalis)
Number of trees checked on fire blight incidence in plots treated with: Regalis = 1601, Biomit Plussz = 694, Check = 1096

Table 5 Incidence of blossom blight* in trees of apple cultivars Újfehértó, 2003

Number of cultivars	Treatments	Date of investigations			Average
		May 28	June 13	June 16	
7	Regalis	7.7	8.0	0.0	5.4
8	Biomit Plussz	11.9	11.5	0.0	7.8
8	Check	4.5	3.7	4.0	4.1

On May 28: (pieces of flower clusters infected/pieces of trees investigated) × 100
after May 28: (pieces of young fruitlets infected/pieces of trees investigated) × 100
Cultivars and pieces of trees checked on fire blight: see *Table 4*

the number of trees investigated. For example, while investigating 70 trees and as much as 55 blighted shoots were found, this quotient is $(55/70)\times100 = 78.6$. Practically, a quotient less than 10 represents a rather moderate incidence of fire blight.

Results

The primary use of prohexadione-Ca is the control of shoot growth, which was achieved in pome and – sometimes – in stone fruit trees, too. Proper control of shoot growth by Regalis is highly beneficial as regards the reduced need for pruning; increased efficiency in the use of fungicides, insecticides and foliar nutrients and improved fruit quality due to the better light penetration into central parts of the canopy (*Rademacher and Kober,* 2003).

Growth control is not only important because of the aspects mentioned above. Under the influence of Regalis, shoot growth will be completed earlier by setting terminal buds and, thereafter, actively growing succulent shoot tips will no longer be present. It can be assumed that the risk of infection by *E. amylovora* is also diminished to some extent by these morphological changes. Because growth control by prohexadione-Ca is most intense in shoots developing from terminal buds, the degree of acrotony is reduced to some extent, which would enable a higher number of lateral buds to grow and lead to improved feathering. Among the cultivars evaluated for this phenomenon, an increased number of spurs is, indeed, induced in trees of cultivars Jonagold deCosta, and Sampion (*Bubán et al.,* 2003), similar observation were reported by *Basak and Rademacher* (2000).

Effectiveness of the products used in our study are well comparable mith those of antibiotics (except a clearly negative experience concerning incidence of blossom blight).

There was a generally low incidence of fire blight in the year of 2001 *(Table 1),* especially in plots of Regalis treatments.

Next year, incidence of both shoot blight *(Table 2)* and blossom blight *(Table 3)* was reduced most efficiently by Biomit Plussz treatments. The perfect overlap between the bloom period and the first four sprayings with Biomit Plussz (from green bud stage to petal fall), after that another five treatments carried out during the intensive phase of shoot growth could provide a better prevention of the disease than Regalis, or conventional treatments with antibiotics. The heavy blossom blight in trees of Regalis treatments indicates that controlling *E. amylovora* infection with prohexadione-Ca will remained difficult.

Since Regalis works primarily by inducing resistance, prophylactic applications are indispensable. According to its physiological mode of action prohexadione-Ca has to be applied preventive against pathogen infection, i.e. it should be used 1 to 3 weeks prior to a possible infection risk (or inoculation) by *E. amylovora* (e.g. *Bazzi et al.*, 2003). However, carrying out treatments, for instance, at green to pink bud stage runs a high risk of failure since there is a little absorbing surface for uptake of the compound and weather conditions are often unfavourable at that time of the year. It is worth mentioning, too, that in this year (2002) there was a well detectable *E. amylovora* population (10^2–10^3 genome equivalent/flower) in the apparently symptomless flowers. The size of epiphytic population was similar in flowers of cultivars Sampion and Freedom known as sensitive and tolerant ones to *E. amylovora* (in details see: Bubán, Dorgai and Thomson in this issue).

Taking into considerations the average values for the season of 2003 *(Table 4)* there were no considerable differences among the treatments' effects. It is true concerning the occurrence of blossom blight in trees of plots treated with Regalis and antibiotics, respectively *(Table 5)*. Nevertheless, the preventive efficiency of the Biomit Plussz was much less than a year before.

Besides cultivars' sensitivity to fire blight, the incidence of blossom- and shoot blight are seriously determined by the actual weather related infection risk and the size of epiphytic *E. amylovora* population in the open flowers. The role of these decisive circumstances has also been studied in the same orchard and in the same years (see the paper of Bubán, Dorgai and Thomson in this issue).

It cannot be denied that, due to the sporadic appearance of fire blight, it is hard-to-achieve a resounding success in preventing this disease under orchard conditions. Results of experiments by artificial infection in apple trees pre-treated with Regalis (*Bubán et al.*, 2003; Sobiczewski and Bubán, in this issue), or in pear trees preventive treated with other compounds (*Tsiantos and Psallidas*, 2002) proved to be more reproducible and convincing.

Discussion

During the development of prohexadione-Ca as a growth regulator for use in apple and pear orchards, treated trees turned out to be significantly less infected by fire blight; *Erwinia amylovora* (*Winkler*, 1997; *Fernando and Jones*, 1999; *Momol et al.*, 1999; *Yoder et al.*, 1999; *Stammler*, 2000; *Sobiczewski et al.*, 2001; *Costa et al.*, 2000, 2001; *Aldwinckle et al.*, 2002; *Bubán et al.*, 2002b; *Cline and Hunter*, 2002; *Deckers and Schoofs*, 2002; *Holtz et al.*, 2002; *Maxson and Jones*, 2002; *Bazzi et al.*, 2003).

In general, prohexadione-Ca is less efficient to control flower infections by *E. amylovora* as compared to shoot infections, since successful prophylactic treatments are difficult to carry out early in the season (*Stammler*, 2000; *Bazzi et al.*, 2003; *Bubán et al.*, 2003). The highest effectiveness in fire blight management can, therefore, be achieved by using prohexadione-Ca in combination with streptomycin or other suitable antibiotics, i.e.: streptomycin applied at bloom time if *E. amylovora* infection risk is predicted by the Maryblyt or another forecasting model (*Billing*, 2000; *Bubán et al.*, 2002a) and prohexadione-Ca sprayed at the beginning of vegetative growth to induce resistance against shoot infections. The magnitude of improvement in fire blight control as a result of simultaneously applying streptomycin and prohexadione-Ca suggests a complementary effect (*Winkler*, 1997). Synergistic effects of combinations of prohexadione-Ca with streptomycin, or of prohexadione-Ca followed by streptomycin at late bloom have been reported by *Yoder et al.* (1999) and hold particular interest for reducing disease pressure.

Advanced management strategies integrating uses of prohexadione-Ca, or acibenzolar-S-methyl (ASM; as Actigard or Bion 50WG) with streptomycin for fire blight control have recently been suggested by *Norelli et al.* (2003). However, for the time being the importance of non-antibiotic products is increasing because the use of streptomycin and other antibiotics against fire blight becomes more and more questionable (*McManus et al.*, 2002). Against this background, it can be forecast that, particularly in current and future member states of the European Union, prohexadione-Ca will become an important component in new procedures for fire blight control.

Prohexadione-Ca possesses very favourable toxicological and eco-toxigological features (*Winkler*, 1997; *Evans et al.*, 1999; *Rademacher and Kober*, 2003) and, overall, fits well into Integrated Fruit Production programs (*Krawczyk and Greene*, 2002; *Bazzi et al.*, 2003).

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