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Control of Pathogen Incidence in Pome Fruits and Other Horticultural Crop Plants with Prohexadione-Ca

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Summary

Products containing prohexadione-Ca represent a considerable alternative to antibiotics for the control of secondary fire blight *(Erwinia amylovora)* in pears as well as in apples. Furthermore, less pathogen incidence is also induced by this compound in apples against scab *(Venturia inaequalis)*, in grapevines against grey mould *(Botrytis cinerea)*, and in several other host-pathogen systems. Clear evidence is available that these effects are primarily due to induced physiological resistance. However, it should not be ruled out that morphological and histological changes caused by prohexadione-Ca might support this effect.

Zusammenfassung

Kontrolle von Pathogenen im Kernobst und anderen gartenbaulichen Kulturpflanzen mit Prohexadion-Ca. Für die Kontrolle von sekundärem Feuerbrand *(Erwinia amylovora)* im Birnen- und Apfelanbau stellen Prohexadion-Ca-haltige Produkte eine relevante Alternative zu Antibiotika dar. Behandlungen mit dem Wirkstoff führen außerdem zu einem geringeren Befall bei Apfel mit Schorf *(Venturia inaequalis)*, bei Reben mit Graufäule *(Botrytis cinerea)* und in weiteren Pflanzenarten mit anderen Pathogenen. Diese Effekte lassen sich primär auf induzierte physiologische Resistenz zurückführen. Es sollte jedoch nicht ausgeschlossen werden, dass den durch Prohexadion-Ca bewirkten morphologischen und histologischen Effekten eine unterstützende Bedeutung zukommt.

Key words. *Botrytis* – *Erwinia* – *Malus* – *Pyrus* – resistance – *Venturia* – *Vitis*

Introduction

Significant losses in agricultural and horticultural crops are caused by bacterial and fungal pathogens and continuous efforts are being made to minimise incidence and severity of diseases caused by such microorganisms. Besides breeding resistant genotypes or using fungicides and bactericides, an innovative approach is the induction of defence mechanisms by treating plants with suitable triggering agents. For instance, systemic acquired resistance may be induced by acibenzolar-S-methyl (OOSTENDORP et al. 2001). A new chemical to induce pathogen defence in distinct plant species is prohexadione-Ca (ProCa). This compound possesses very favourable toxicological and eco-toxicological features (EVANS et al. 1999) and is primarily used to control excessive vegetative growth (RADEMACHER and BUCCI 2002). In fruit production, the products REGALIS® (10 % ProCa) and APOGEE® (27.5 % ProCa) have recently been introduced in a number of countries. The underlying mode of action for shoot growth reduction consists in blocking the formation of growth-active gibberellins (RADEMACHER 2000).

During the development of suitable application protocols for ProCa in apple orchards, it was surprisingly detected that treated trees were significantly less infected by fire blight *(Erwinia amylovora)* (WINKLER 1997; FERNANDO and JONES 1999; MOMOL et al. 1999; YODER et al. 1999; ALDWINCKLE et al. 2002; MAXSON and JONES 2002). Soon afterwards, reduced incidence of fire blight was also found in pears (COSTA et al. 2001a, 2002; BUBÁN et al. 2002; DECKERS and SCHOOFS 2002). Orchard trials carried out from 1995 to 1999 showed that the best results were obtained when ProCa was applied approximately 2 weeks prior to infection. Due to difficulties with treating trees very early in the season, shoot infections could be much better controlled (71 % efficiency – 45 trials) than flower infections (27 % efficiency – 19 trials) (STAMMLER 2000). Ongoing research revealed that treatments with ProCa led also to reduced infection with scab *(Venturia inaequalis)* (COSTA et al. 2001b), powdery mildew *(Podosphaera leucotricha)* (E. Ammermann and W. Rademacher, unpublished), sooty blotch (K. Yoder, Virginia Tech, Winchester, VA, USA, personal communication) in apples, with powdery mildew *(Sphaerotheca pannosa)* in peaches (A. Erez, The Volcani Center, Bet Dagan, Israel, personal communication), with downy mildew (*Plasmopara viticola*) (BAZZI et al., in this issue), and grey mould (*Botrytis cinerea)* (J. B. Speakman, E. Ammermann and W. Rademacher, unpublished) in grapevines, and with bacterial spot (*Xanthomonas vesicatoria)* and bacterial speck *(Pseudomonas syringae* pv. *tomato)* in tomatoes

(Bazzi et al., in this issue). However, in other cases, no effects on infection severity could be detected after application of ProCa: against powdery mildew (*Blumeria graminis*) in wheat, grey mould *(Botrytis cinerea)* in pepper, and late blight (*Phytophthora infestans)* in tomatoes (E. Ammermann and W. Rademacher, unpublished). It should also be noted that fruit trees treated with ProCa were less infested with insect pests, such as aphids (Krawczyk and Greene 2002) or leafhoppers (Leahy et al. 2002). From the experience available so far, it appears that ProCa predominantly induces reduction of disease and insect pest incidence in woody plant species. – This contribution gives a survey of the effects of ProCa against fire blight in pears and against scab in apples. Additionally, first results with controlling *Botrytis cinerea* in grapevines under vineyard conditions are reported.

Nature of resistance induced by ProCa

ProCa has no direct activity against bacteria, fungi and insects. Therefore, the effects found against diseases and insect pests have, initially, been difficult to explain. Clear evidence is meanwhile available that ProCa induces pathogen resistance primarily by affecting the spectrum of phenylpropanoids in treated plants: ProCa blocks flavanone 3-hydroxylase, a key enzyme in flavonoid metabolism. As a result, significant changes in the spectrum of flavonoids occur and 3-deoxyflavonoids, in particular luteoforol, with phytoalexin-like properties are formed. Details on these aspects are given in other contributions in this issue. The reasons for lowered insect attack have not yet been specifically investigated. One may suppose that luteoliflavan is involved, since it reaches relatively high concentrations in apple shoots after ProCa treatment (Roemmelt et al. 1999) and has a structure related to viscutin-3, which is known to possess insect growth inhibitory effects (Kubo and Kim 1987).

The lowered susceptibility of leaves and shoots to pathogens and insect pests may also result from morphological and histological changes induced by the growth retardant ProCa. However, there is no hint in the pertinent literature that growth retardants like daminozide, chlormequat chloride (CCC) or paclobutrazol (PBZ), which exhibit equivalent morphological and histological effects and which have been widely used in pome fruit production, cause any remarkable effect on fire blight or scab. In order to obtain better knowledge of this aspect, growth retardants with different effects on gibberellin biosynthesis were compared to ProCa (Costa et al. 2001b): CCC (an inhibitor of *ent*-kaurene formation), PBZ (an inhibitor of *ent*-kaurene oxidation into *ent*-kaurenoic acid), and trinexapac-ethyl (TrixE – like ProCa an inhibitor of dioxygenases involved in late stages of gibberellin metabolism – for details see Rademacher 2000). Susceptibility to scab was tested in apple seedlings under greenhouse conditions at different times after application of dosages giving roughly equivalent degrees of shoot growth control: only ProCa and TrixE lowered their susceptibility. Therefore, reduced scab infection is not necessarily a consequence of a more compact shoot growth. ProCa and TrixE, both being inhibitors of 2-oxoglutarate-dependent dioxygenases, would rather act by inducing physiological changes. In the experiments, the plants needed approximately three weeks after treatment to display lowered scab susceptibility: this relatively long time span was observed in plants under rather cool conditions. When apple seedlings were kept at slightly higher temperatures, it took approximately ten days to observe a significant degree of lowered scab incidence (Fig. 1). These results are in line with other observations on the effect of ProCa against scab, fire blight, and further diseases. A time span of approximately 7 to 20 days is required to build up resistance and, once established, resistance lasts for several weeks. Most likely, differences are primarily due to different temperatures during the trials.

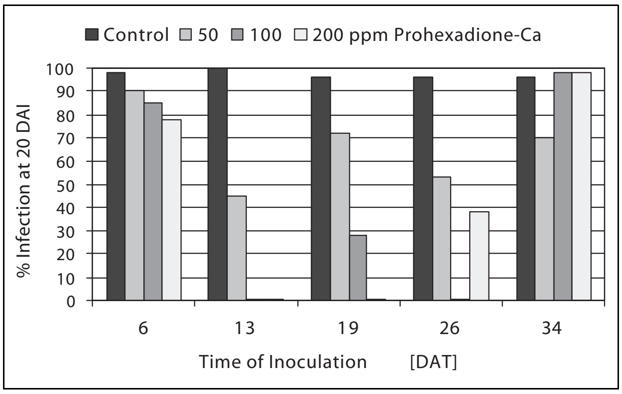

Fig. 1 Effect of prohexadione-Ca (applied as REGALIS®) on the incidence of scab (*Venturia inaequalis*) in apple seedlings: Effects of dosages and time difference between treatment and inoculation (greenhouse trial). (DAT = days after treatment; DAI = days after inoculation)

In addition to the physiological type of resistance induced by ProCa, morphoregulatory effects may also be of relevance in disease control, since pathogens and insect often prefer young leaves. Therefore, earlier bud set, as caused by ProCa, will reduce susceptibility accordingly. The canopies of treated trees are less dense, which allows a more efficient use of fungicides and insecticides. Spray penetration turned out to be improved by 8 % as a mean of 11 independent trials in different apple orchards (J. R. Evans and R. R. Evans; BASF Corp., personal communication). In a mature apple orchard (9-year-old 'Elstar' on M.9, planted at 1.25 x 3.25 m), a sequential treatment with 100 plus 150 g ha^{-1} ProCa reduced tree row volume from 14,530 to 11,720 m^3. Using a blast sprayer, the nozzles of which were controlled by optical sensors, the spray volume for plant protection could be reduced by 8 % [J. Hilbers, Horticultural Research Institute (OVA), Jork, Germany, personal communication].

Fire blight in pears

Fire blight is of particular concern in pear cultivation because, in general, pear trees are more susceptible than apple trees (Lespinasse and Aldwinckle 2000). Many trials and observations have demonstrated that pear trees pre-treated with ProCa are significantly less affected by fire blight (Costa et al. 2001a, 2002; Bubán et al. 2002; Deckers and Schoofs 2002). Hence, it is now possible to set up control strategies without the controversial use of antibiotics (McManus et al. 2002). We report in this contribution on some specific aspects on fire blight control in pears by using ProCa in order to enable a better understanding of the underlying processes.

Trials conducted under greenhouse conditions

Table 1 shows the results of experiments carried out under greenhouse conditions on 1-year-old pear trees cv. 'Abbé Fétel' grafted on quince BA29. Two weeks after application of ProCa (250 ppm), the apical portions of the two youngest leaves on each actively growing shoot were cut with scissors dipped in an aqueous suspension of a local virulent strain of *Erwinia amylovora* (about 10^8 cfu ml^{-1}). The application of ProCa lowered disease incidence (determined as the % of shoots showing typical blight symptoms) and severity (quantified on the length of necrotic leaves expressed as the % of shoot length) and significantly reduced the length of necrotic lesions in the inoculated shoots (Costa et al. 2001a). In further experiments, conducted under similar conditions, scions of different pear cultivars were treated with 250 ppm of ProCa 15 days prior to inoculation with *E. amylovora.* A certain degree of disease control was achieved, which, however, varied among the cultivars used. In particular, in cv. 'Conference', blight incidence on treated shoots was significantly lower than on control shoots (Table 2). A trend for reduced fire blight severity was observed in the four pear cultivars tested. Actively growing shoots of micropropageted pear trees cv. 'Williams' were treated with ProCa (125 ppm and 250 ppm), and experimentally inoculated in the greenhouse after 15–20 days with a local virulent strain of *E. amylovora* by cutting the apical portion of the three youngest leaves with contaminated scissors: ProCa clearly reduced disease incidence and severity. In particular, none of the plants treated with 250 ppm developed fire blight symptoms 9 days after inoculation while disease incidence in the control was 83.3 % (Sabatini et al. 2002). Other experiments indicated an optimisation of practical application: ProCa was applied (125+125+100 ppm) at different times (14-day intervals) to young trees of the pear cv. 'Abbé Fétel' grown in pots, and 10 days after the last application, the three youngest apical leaves of actively growing shoots were wounded (3 wounds per leaf) with a special hand device (Galasso et al. 2002) and sprayed with a suspension of *E. amylovora* (7 x 10^8 cfu ml^{-1}); water and streptomycin sulphate (100 ppm) were sprayed 24 h before inoculation and used as positive and negative controls, respectively. ProCa application gave a significant resistance response against fire blight, not different from that obtained with the antibiotic: Mean values of disease incidence and severity were reduced from 56 and 77 % to 4 and 40 %, respectively (Fig. 2). The dynamics of endophytic *E. amylovora* populations, as well as their re-isolation frequency, were determined in symptomatic and asymptomatic pear shoot tissues (Roemmelt et al. 2002). Analyses were carried out at 2, 6, 9 and 13 days after inoculation on extracts from segments of treated control shoots, taken at different distances from the apex or the necrotic front, by quantitative isolation on CCT semi-selective medium (Ishima-

Table 1. Effect of pre-treatment with prohexadione-Ca (250 ppm – applied as REGALIS®) on incidence and severity of fire blight infections on 'Abbé Fétel' pear shoots inoculated with *E. amylovora* (greenhouse experiment) (Costa et al. 2001a).

Treatment	% Incidence	% Severity	Lesion extension (cm)
Control	70.0 a	21.8 a	12.1 a
Prohexadione-Ca	57.9 a	15.1 a	6.5 b

Values with different letters are significantly different at P≤0.05 (Duncan's Test).

Table 2. Severity and incidence of fire blight on shoots of different pear cultivars inoculated with *Erwinia amylovora* 15 days after application of prohexadione-Ca (250 ppm – applied as REGALIS®) (greenhouse experiment).

	% Severity resulting from inoculation after			% Incidence
	6 days	9 days	13 days	
'Abbé Fétel'				
Control	15.1	44.9	57.7	81.2
Treated	17.6	47.2	47.9	64.3
'Conference'				
Control	11.2	20.4	36.5	68.6 a
Treated	10.5	18.0	23.1	45.2 b
'Williams'				
Control	16.6	30.5	40.2	88.3
Treated	12.3	22.4	34.9	76.4
'Tosca'				
Control	n.d.	n.d.	n.d.	69.7
Treated	n.d.	n.d.	n.d.	69.2

Values with different letters are significantly different at P≤0.05 (Duncan's Test); n.d. = not determined

RU and KLOS 1984). A number of extracts were also tested by chem-PCR-ELISA (MERIGHI et al. 2000); representative bacterial colonies from direct isolation were identified by agarose gel electrophoresis (PCR-AGE) using the primers of BERESWILL et al. (1992). The effect of ProCa application was particularly evident in symptomatic shoots, where *E. amylovora* was detected at a shorter distance from the necrotic front than in the controls (23 cm and 29 cm, respectively, 9 days after inoculation) (Fig. 3). Application of ProCa had also an effect in limiting *E. amylovora* re-isolation frequency in four pear cultivars tested (ROEMMELT et al. 2002).

Orchard experiments

In orchard trials (COSTA et al. 2002), ProCa was applied to pear trees cv. 'Abbé Fétel' at 4 x 50 ppm and 3 x 125 ppm with multiple spray applications: Four sprays, performed according to the Cougarblight forecasting model (SMITH 1996), were ineffective against fire blight, while the three sprays performed at pre-bloom stage, 3

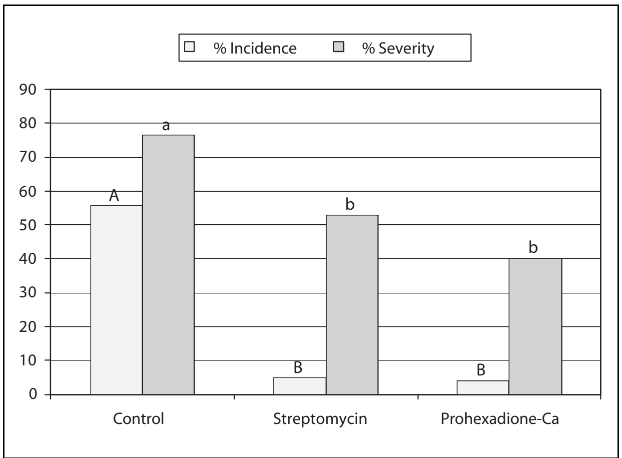

Fig. 2 Effect of sequential applications of ProCa (125+125+100 ppm – applied as REGALIS®) followed by inoculation with *E. amylovora* on fire blight incidence and severity in young pear trees cv. 'Abbé Fétel': Water and streptomycin (100 ppm) were used for comparison (greenhouse experiment). [Columns with different letters are significantly different at P≤0.05 (Duncan's Test)].

weeks later, and at the beginning of the secondary blossoming, showed an interesting reduction of disease incidence as compared to the controls (0.2 vs. 0.7 infections per tree, respectively). Other trials conducted under orchard conditions on 10-year-old pear trees cv. 'Williams', grafted on 'Kirchensaller', gave further indications of the efficacy of prohexadione-Ca (125 ppm, 3–4 applications, 10-day intervals): 2 and 4–5 infections per tree were monitored in the treated and in the control trees, respectively (SABATINI et al. 2002). Under natural fire blight infection conditions, ProCa (used as APOGEE®) was applied to 12-year-old pear trees cv. 'Abbé Fétel' as a spray at 125, 2 x 125, 4 x 125, and 250 ppm at 15-day intervals; on cv. 'Conference', the chemical was applied at 2 x 125 ppm (COSTA et al. 2001a). These investigations confirmed the effectiveness of ProCa in significantly reducing relative disease incidence and severity: in particular, the highest application rate (4 x 100 ppm) was clearly the most effective and reduced disease incidence and severity by approximately 75 %. At each inspection in these orchards, asymptomatic pear leaf samples from each treatment were collected and analysed in the laboratory to detect the presence of "epiphytic" *E. amylovora* populations. Moreover, samples of dormant cuttings were washed under vacuum (BAZZI et al. 1987) and xylem sap extracts were analysed to assess endophytic *E. amylovora* populations by direct isolation on CCT medium and chem-PCR-ELISA. These results gave further evidence that i) the pathogen is unable to live as a resident epiphyte on leaf surfaces, ii) it can live in apparently healthy 1–2-year-old pear twigs, iii) on cv. 'Abbé Fétel', relative frequencies of *E. amylovora*-positive xylem extracts, obtained from vacuum washings from asymptomatic cuttings treated with ProCa, were lower than those from the controls (ROEMMELT et al. 2002). ProCa was confirmed to be a valuable tool for controlling secondary fire blight in pears as reported by DECKERS and SCHOOFS (2002) and by BUBÁN et al. (2002). As in apples, the best results are generally obtained under natural infection conditions. Most likely, the resistance induced by ProCa is not sufficient to cope with high inoculum concentrations. This might explain why post-symptom sprays with ProCa are only of limited value (SCHUPP et al. 2002). The time span needed to develop resistance seems to be of special relevance as well.

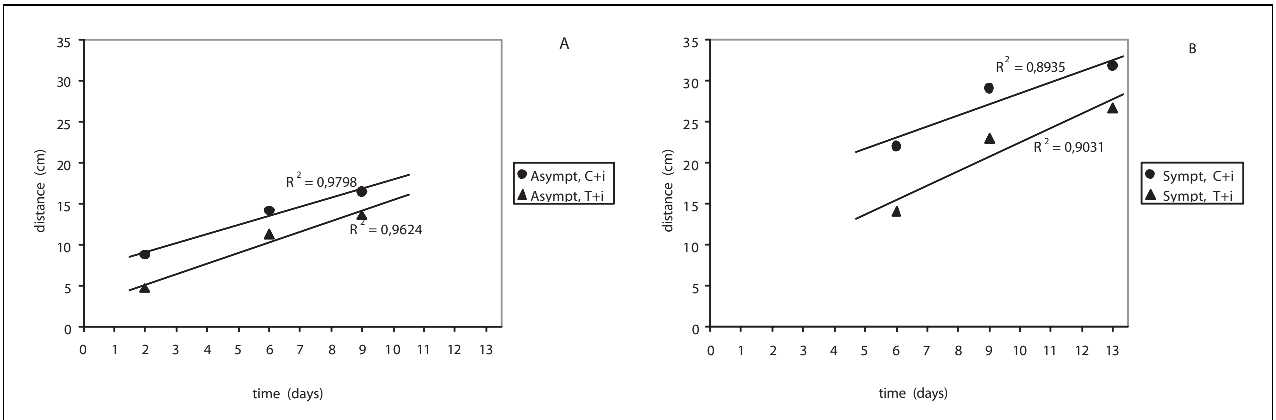

Fig. 3 Spatial and temporal movement of *E. amylovora* in inoculated pear shoots and linear fitting of experimental data: A) asymptomatic, control (C+i) and treated (T+i); B) symptomatic, control (C+i) and treated (T+i).

Table 3. Activity of prohexadione-Ca (applied as REGALIS®) against scab *(Venturia inaequalis)* in apple cv. 'Summered': two applications at 175 ppm associated with a reduced fungicide schedule (based on applications at 10-day intervals of tebuconazole + mancozeb) in comparison with a standard schedule based on kresoxim-methyl (orchard trial).

Treatment	Infected fruits (%)	Scab spots per fruit (No.)	Infected leaves (%)	Scab spots per leaf (No.)
Reduced schedule (tebuconazole 125 ppm + mancozeb 1200 ppm)	27.25 b	1.09 b	54.68 b	3.46 b
Reduced schedule +ProCa	4.34 a	0.18 a	27.99 a	0.96 a
Standard schedule (kresoxim-methyl 70 ppm)	8.42 a	0.13 a	31.47 a	0.74 a

Values with different letters are significantly different at P≤0.05 (Duncan's Test)

Effect of ProCa on apple scab incidence

The first experiments to control scab in apple were conducted with seedlings under greenhouse conditions and gave promising results (Fig. 1; Costa et al. 2001b). A very high degree of efficiency is required to control apple scab, since only "perfect" fruits without any fungal spot can be successfully marketed. Such quality requirements cannot be achieved by using ProCa alone. In order to investigate its usefulness against fungal diseases, experiments were, therefore, carried out by integrating ProCa into reduced fungicide schedules. Intensive testing over several years in different cultivars and at different locations gave very promising results. However, in some cases effects against scab were not robust enough. This could be due to an insufficient time span between treatment and infection, not allowing a proper induction of resistance. Very encouraging results came from orchard trials conducted in the districts of Bologna and Ferrara (Bazzi et al. 2000): ProCa was sprayed twice at 175 ppm (15-day intervals) on apple trees cvs. 'Golden Delicious', 'Gala', 'Granny Smith', 'Mutsu', and 'Summerred' in combination with a reduced fungicide schedule consisting of four applications at 10-day intervals of tebuconazole plus mancozeb. This was compared with the same reduced schedule and with a standard schedule based on the fungicide kresoxim-methyl (70 ppm). In all cases, ProCa significantly reduced incidence and severity of natural scab (*Venturia inaequalis*) infections on leaves and fruits, improving the effectiveness of the reduced control schedule. Table 3 shows the results obtained with cv. 'Summerred'. In other trials (conducted in 2001 on cv. 'Golden Delicious'/M.9, six years old, location: Limburgerhof, Rhineland-Palatinate, Germany), the related compounds ProCa (REGALIS®) and TrixE (Moddus®) were compared. Additionally, different volumes of spray liquid (800, 400, and 200 L ha^{-1}) were tested to study uptake and biological performance. Significant scab infections were provoked by employing a reduced fungicide schedule. Irrespective of the volume of liquid, trees treated with ProCa showed infections on leaves and on fruits ranging from 22 to 36 % and 4 to 8 %, respectively, whereas no effect on disease incidence could be observed on trees treated with TrixE. In the control trees, 46 % of the leaves and 11 % of the fruits were infected (Fig. 4).

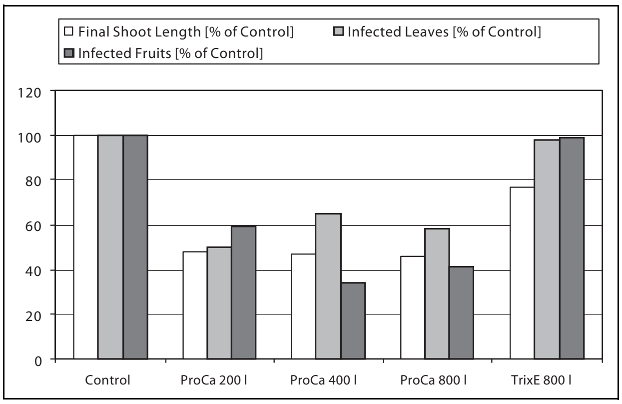

Fig. 4 Effect of 125 g ha^{-1} ProCa (applied as REGALIS® at different spray volumes) or 125 g ha^{-1} TrixE (applied as Moddus®) on shoot growth and incidence of scab *(Venturia inaequalis)* on leaves and fruits of apple, cv. 'Golden Delicious'. (Control values: Final shoot length = 71.6 cm; Infected leaves = 46.3 %; Infected fruits = 10.7 %).

Induction of resistance against grey mould (*Botrytis cinerea*) in grapevines

Previous experiments under greenhouse and laboratory conditions had shown that pre-treatment of grapevines with ProCa led to a reduction of grey mould infection. More detailed trials were performed in 2002 using five cultivars in different vineyards in Rhineland-Palatinate (Germany). ProCa was applied at pre-bloom (growth stage BBCH 57–60 – Stauss 1994) and at early berry formation (growth stage BBCH 71–73). For comparison, a standard botryticide was applied at the beginning of ripening (growth stage BBCH 81), either alone or after a previous treatment with ProCa (growth stage BBCH 71–73). In four of the five trials, treatments with ProCa led to less incidence of grey mould of the berries (Table 4). The best results were obtained with pre-bloom applications. The effects were comparable to, or even better, than the standard fungicide. The trial with cv. 'Huxelrebe' suffered from extreme infection by *B. cinerea*: here, both ProCa and the botryticide showed only a very weak activity.

Table 4. Incidence of grey mould *(Botrytis cinerea)* in different grapevine cultivars after treatment with prohexadione-Ca (applied as REGALIS®) (vineyard trial).

Product	Treatment at growth stage	Dosage [g ha^{-1}a.i.]	'Riesling'	'Pinot Blanc'	'Huxelrebe'	'Dornfelder'	'Portugieser'
			First evaluation (September 7–9)				
Control	–	0	5.0	19.6	52.5	n.d.	3.4
	57–60	180	4.0	5.0	51.8	n.d.	0.3
ProCa	57–60	250	4.1	2.7	48.0	n.d.	n.d.
ProCa	71–73	240	3.5	14.4	45.8	n.d.	n.d.
Fungicide	81	600	3.8	10.9	40.5	n.d.	0.6
ProCa +	71–73	240					
Fungicide	81	600	4.0	8.1	41.8	n.d.	0.7
			Second evaluation (September 20–27)				
Control	–	0	10.9	27.1	61.5	2.7	n.d.
ProCa	57–60	180	6.3	8.9	58.6	2.4	n.d.
ProCa	57–60	250	6.8	8.1	60.5	1.4	n.d.
ProCa	71–73	240	7.3	24.2	56.6	2.7	n.d.
Fungicide	81	600	7.7	18.7	57.8	1.2	n.d.
ProCa +	71–73	240					
Fungicide	81	600	8.7	16.3	53.1	1.8	n.d.

rating: 0 = no fungal infection / 100 = complete infection

Conclusions

The results clearly demonstrate that ProCa is very useful for controlling secondary fire blight in pears. Since antibiotics, such as streptomycin, are increasingly restricted from being used against plant pathogens, fruit growers will have to rely on alternatives, of which ProCa appears to be of special relevance. As in apple, further experience is needed to fully exploit its potential for this use. Additionally, it would be highly relevant to evaluate in more detail a possible use of ProCa against flower infections by *E. amylovora*. ProCa will be primarily used as a plant growth regulator. However, its effects against bacterial and fungal infections and also against insect attacks obviously deserve further attention. The fact that ProCa could allow a more efficient use of fungicides and insecticides would be of great interest for Integrated Crop Production. Furthermore, since ProCa seems to positively "assist" the effects of fungicides and insecticides, the probability of resistance formation against such crop protectants might be reduced.

Acknowledgements

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