PROHEXADIONE-CA AND CROP PROTECTION IN POME FRUIT TREES

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ABSTRACT

Prohexadione-Ca is a new plant bioregulator for use in pome fruits. Trees pre-treated with prohexadione-Ca are less affected by diseases such as fire blight and scab and by different types of insect pests although the compound is inactive as a bactericide, fungicide or insecticide. Prohexadione-Ca leads to growth reduction by blocking gibberellin biosynthesis. However, it can largely be ruled out that its effect on morphological or histological structures is of major direct relevance for disease and insect pest incidence. In treated shoots, prohexadione-Ca causes also considerable changes in the spectrum of flavonoids. Convincing evidence is now available that prohexadione-Ca triggers pathogen resistance by inducing the formation of 3-deoxyflavonoids, in particular luteoforol, with phytoalexin-like properties. Luteoliflavan, another flavonoid, is also found only after treatment with prohexadione-Ca. This compound is known to be an inhibitor of insect growth, which may explain the observed effects on insect pests. Another advantage with regard to plant protection results from the fact that prohexadione-Ca-treated trees have a more compact but open canopy, thereby enabling better spray penetration and coverage for fungicide or insecticide treatments.

PATHOGEN AND INSECT PEST INCIDENCE IN TREATED TREES

Prohexadione-calcium (calcium 3-oxido-4-propionyl-5-oxo-cyclohexene carboxylate - abbreviation: ProCa) is contained in the products APOGEE® (27.5% ProCa) and REGALIS® (10% ProCa), new plant bioregulators for fruit trees, which are currently introduced to the market. Control of excessive shoot growth had initially been the only target for the products. However, in the course of developing ProCa for use in pome fruits, it turned out that pre-treated trees were less infected by fire blight (*Erwinia amylovora*), scab (*Venturia inaequalis*), and powdery mildew (*Podosphaera leucotricha*). Additional trials revealed that similar effects could also be induced in other, but not all, host-pathogen-combinations. Table 1 gives a survey of these findings. It was also found that trees treated with ProCa were less infested with insect pests, such as aphids and potato leafhoppers (Table 2). In general, the degree of efficiency in controlling diseases or attacks by insects is in the range of 30 to 80%.

Most insect pests and fungal pathogens can be effectively controlled by modern insecticides and fungicides. In contrast, prevention of fire blight, caused by the bacterium *E. amylovora*, is still not solved satisfactorily. Products containing copper and sulfur often lead to fruit russeting and are, therefore, only of restricted value. Likewise, bacterial antagonists and other biological control products do not perform sufficiently efficiently and reliably. In many countries, the use of the antibiotic streptomycin has become the standard, particularly for controlling blossom infections by *E. amylovora* (Psallidas and Tsiantos, 2000; McManus et al., 2002). However, resistance to streptomycin has developed in *E. amylovora* populations, for instance in the western part of the USA and Canada and in Michigan (Norelli et al., 2003). In order to overcome resistance, growers use oxytetracycline, either alone or in combination with streptomycin. In the USA, approximately 20% of the apple and 40% of the pear growing area is treated with streptomycin and/or oxytetracycline, mainly to combat fire blight (McManus et al., 2002). Other

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antibiotics used in some countries against fire blight comprise gentamycin, oxolinic acid, and kasugamycin.

Table 1. Effect of pre-treatment with prohexadione-Ca on disease incidence in different host-pathogen combinations (for sources see Bazzi et al., 2003a,b).

Host plant	**Pathogen**	**Incidence reduced?**
Apple *Malus domestica*	Fire blight *Erwinia amylovora*	Yes
Pear *Pyrus communis*	Fire blight *Erwinia amylovora*	Yes
Apple *Malus domestica*	Scab *Venturia inaequalis*	Yes
Apple *Malus domestica*	Apple powdery mildew *Podosphaera leucotricha*	Yes
Peach *Prunus persica*	Peach powdery mildew *Sphaerotheca pannosa*	Yes
Rose *Rosa ssp.*	Black spot *Diplocarpon rosae*	Yes
Grape vine *Vitis vinifera*	Grey mould *Botrytis cinerea*	Yes
Grape vine *Vitis vinifera*	Downy mildew *Plasmopara viticola*	Yes
Pepper *Capsicum annuum*	Grey mould *Botrytis cinerea*	No
Wheat *Triticum aestivum*	Cereal powdery mildew *Blumeria graminis*	No
Tomato *Lycopersicon esculentum*	Late blight of tomato *Phytophthora infestans*	No

Table 2. Incidence of insect pests in pome fruit trees pre-treated with prohexadione-Ca

Host plant	**Insect pest**	**Source(s)**
Apple *Malus domestica*	Green apple aphid *Aphis pomi* + Green citrus aphid *Aphis spiraecola*	Krawczyk and Greene (2002)
Apple *Malus domestica*	Woolly apple aphid *Eriosoma lanigerum*	D. Wilson (BASF Australia) (personal communication)
Apple *Malus domestica*	Potato leaf hopper *Empoasca fabae*	Leahy et al. (2002)
Apple *Malus domestica*	Apple psylla *Psylla mali*	Paulson and Hull (1999)
Pear *Pyrus communis*	Pear psylla *Psylla pyri*	Paulson and Hull (1999); D. Bylemans (Royal Research Station, St. Truiden, Belgium) and P. Francois (BASF France) (personal communications)

However, using antibiotics in plant production is highly controversial due to the potential risk of promoting the development of antibiotic resistance in human pathogens (McManus et al., 2002). For instance, a restricted use of streptomycin against fire blight is presently allowed in the European Union only in Germany, Greece, and The Netherlands. From the foregoing, it is obvious that there is an urgent need for alternative and advanced procedures to control fire blight. Consequently, a considerable amount of work has been invested to exploit the effects caused by ProCa for fire blight control under practical conditions.

All available data of trials with ProCa against fire blight conducted between 1995 and 1999 have been collected and summarized by Stammler (2000) (Table 3). It turned out that the best results were obtained when ProCa was applied approximately two weeks prior to infection. Most likely due to difficulties with treating trees very early in the season, effects on blossom infections were less pronounced than the control of shoot (secondary) infections. Outbreaks of secondary fire blight were much more effectively controlled as compared with streptomycin. With advanced knowledge for optimized application (Rademacher and Kober, 2003), even higher degrees of efficiency can, meanwhile, be obtained. In contrast, control of blossom blight is, in general, insufficient for practical use. Here, streptomycin shows a better technical performance. - At present, ProCa is registered for use against secondary fire blight in the USA, Germany, and Belgium.

Table 3. Summary of all available data from trials conducted from 1995 to 1999 with prohexadione-Ca and streptomycin to control fire blight (after Stammler, 2000).

Type of Fire Blight	Number of Trials	Involved Species	Growing Conditions	Type of Infection	% Control (Average)
Prohexadione-Ca					
Blossom	20	Apple: 9x Pear: 11x	Glasshouse: 9x Orchard: 11x	Artificial: 17x Natural: 3x	27
Shoot	46	Apple: 38x Pear: 6x Quince: 1x Cotoneaster: 1x	Glasshouse: 6x Orchard: 40x	Artificial: 38x Natural: 8x	71
Streptomycin					
Blossom	15	Apple: 6x Pear: 9x	Glasshouse: 9x Orchard: 6x	Artificial: 14x Natural: 1x	60
Shoot	15	Apple: 10x Pear: 3x Quince: 1x Cotoneaster: 1x	Glasshouse: 5x Orchard: 10x	Artificial: 13x Natural: 2x	41

MODE OF ACTION: MORPHOREGULATION OR INDUCED RESISTANCE ?

Since ProCa is absolutely inactive as a bactericide, fungicide or insecticide, its effects against diseases and insect pests have, initially, been difficult to explain. Morphological and histological changes as the underlying mechanisms for reduced disease incidence could be ruled out to a large extent, since no comparable results with compounds causing equivalent effects could be found in the pertinent literature. Compounds such as daminozide, chlormequat chloride or paclobutrazol, which had been used for many years in apple and pear production had not shown up, for instance, with side activities against fire blight. Trials with scab on apple seedlings (Costa et al., 2004) confirmed, that reduction of shoot growth was not the relevant cause for reduced disease incidence. In these trials, lowering of infection susceptibility could only be achieved with ProCa and the closely related trinexapac-ethyl. Chlormequat chloride and paclobutrazol also reduced shoot length and caused smaller but thicker leaves with a higher pigment concentration, but no effects against scab infection could be recorded. In spite of these finding, one should not rule out that part of prohexadione's activity against pathogens or insect pests is due, for instance, to earlier bud set, which will often reduce susceptibility to pathogens or pests.

When trying to elucidate the mode of action of ProCa against pathogens, it has been suggested from the very beginning that effects on flavonoid metabolism were involved: High dosages of ProCa and other acylcyclohexanediones inhibit the formation of anthocyanins in flowers and other parts of intact higher plants. 2-Oxoglutarate-dependent dioxygenases, in particular flavanone 3-hydroxylase (FHT), involved in the biosynthesis of anthocyanidins and other flavonoids would represent targets for these compounds (Rademacher et al., 1992). Distinct flavonoids function as phytoalexins, and it has be hypothesized that ProCa would induce such active principles by modifying flavonoid metabolism.

Prohexadione is structurally similar to 2-oxoglutaric acid. This enables the compound to inhibit 2-oxoglutarate-dependent dioxygenases, which are involved in the formation of growth-active gibberellins and in flavonoid metabolism (Rademacher, 2000). Work by Roemmelt *et al.* (2003a,b) and Halbwirth *et al.* (2004) with ProCa has demonstrated that FHT is its main target enzyme in the flavonoid biosynthesis of apple and pear shoot tissues. The inhibition of FHT leads to an alternative pathway in phenylpropanoid metabolism and causes considerable changes in the spectrum of flavonoids and related compounds. Most notably, luteoliflavan (3-deoxycatechin) is formed, which does not normally occur in apple and pear tissues. At approximately one week after treatment, luteoliflavan is reaching levels of several milligrams per gram dry weight in young apple leaves, which coincides with fire blight resistance (Roemmelt et al., 2003a). Apigeninidin, luteolinidin and derivatives thereof, which are also 3-deoxyflavonoids, act as phytoalexins in *Sorghum bicolor* (Nicholson et al., 1988; Nicholson & Hammerschmidt, 1992). However, neither luteoliflavan nor several other constitutive as well as induced phenolic compounds showed sufficiently inhibitory effects on *E. amylovora in vitro* to provide a clear explanation for the enhanced fire blight resistance after treatment with ProCa (Roemmelt *et al.*, 2003a). Special attention has then been paid to luteoforol, the unstable and difficult-to-work-with precursor of luteoliflavan. This compound was found to be highly active against all microorganisms tested (Spinelli et al., 2005): It inhibits the growth of all strains of *E. amylovora* tested *in vitro* at concentrations of approximately 300 ppm. Likewise, the growth of further bacterial and fungal species, including *Venturia inaequalis*, is blocked by luteoforol *in vitro* at concentrations between approximately 3 and 300 ppm. It is obvious that ProCa triggers qualitative and quantitative changes in flavonoids or other phenylpropanoids, thereby inducing physiological resistance to fire blight and other diseases in pome fruits. Positive effects found in further host-pathogen combinations are, most likely, also a result of such changes (Bazzi et al., 2003a,b).

The work invested so far to find out the reasons for reduced incidence of insect pests in pome fruit trees treated with ProCa does not yet allow final conclusions. Again, it should not be ruled out that morphological and histological changes are at least partly involved: Trees with a more open canopy having leaves with a thicker epidermis are, most likely, less attractive to a number of pests. However, a major component in pest defense is likely to come from the 3-deoxy-flavonoids induced by ProCa. Luteoliflavan, which accumulates to almost per cent levels in growing shoots (Roemmelt et al., 2003b), could be of major interest: Conjugates of this compound have been described as inhibitors of insect growth (Kubo and Kim, 1987). It can be assumed that luteoliflavan in its free form is even more active.

MORE EFFICIENT CROP PROTECTION IN TREATED TREES

Trees treated with ProCa display a more compact and open canopy. Resulting from this, spray-applying crop protectants gives a more complete and more uniform covering. Systematic trials conducted at 11 different locations in the USA have revealed an average improvement in spray coverage by 18% (J.R. Evans and R.R. Evans, BASF Corp., Research Triangle Park, NC, personal communication).

CONCLUSIONS

Crop protection in pome fruit trees will benefit in different ways from treatment with ProCa: (a) Morphoregulated trees can be treated easier and more effectively with fungicides and insecticides. (b) The performance of fungicides and, most likely, also of insecticides is assisted by induced 3-deoxyflavonoids causing physiological resistance. Resistance induced against secondary fire blight is of special relevance. (c) Morphological and histological changes may additionally lower the spread of diseases and insect pests.

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