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Use of Prohexadione-Calcium, Vitamin E, and Glycerine for the Reduction of Frost Injury in Apple (*Malus domestica)* Flowers and Leaves

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Summary

The protective effects of the growth regulator prohexadione-calcium and a mixture of the antioxidant α-tocopherol with the cryoprotector glycerol against freezing injury were examined in leaves of apple (*Malus domestica* Borkh.) seedlings and in flowering branches of various apple varieties. Apple seedlings were treated with prohexadione-Ca two and nine days before freezing incidences. The results were compared with the data obtained, from plants pretreated with α-tocopherol/glycerine and exposure to artificial and natural frost. Chlorophyll fluorescence was measured for the determination of intensity of cold damage in apple seedlings cv. 'Golden Delicious'. Frost injury was significantly reduced due to the application of prohexadione-Ca. The photochemical efficiency of the electron transport system was slightly elevated in leaves of pre-treated apple seedlings. Artificial cold treatments of flowers resulted in reduced damage in plants treated with prohexadione-Ca compared to the control. The protective effect was also observed in flowers of orchard trees, especially after natural freezing events, which turned out to be more destructive than artificial freezing. In addition, the formulations containing α-tocopherol/glycerol gave significant protection against freezing injury, in leaves of apple seedlings as well as in flowers of orchard trees.

Zusammenfassung

Einsatz von Prohexadion-Calcium, Vitamin E und Glycerin zur Minderung von Frostschäden bei Apfelblüten und -blättern (*Malus domestica*). Ein möglicher Effekt des Wachstumsregulators Prohexadion-Calcium wurde im Vergleich zu einer α-Tocopherol/Glycerin-Formulierung hinsichtlich der Wirksamkeit gegenüber Frost-induzierten Pflanzenschäden bei Apfelsämlingsblättern sowie bei Blüten verschiedener Apfelsorten geprüft. Die Apfelsämlinge wurden mit Prohexadion-Calcium zwei bzw. neun Tage vor dem Auftreten des Frostes behandelt. Die Tocopherol/Glycerin-Applikationen erfolgten 24 h bzw. 48 h vor dem Eintreten des Frostereignisses. Das Pflanzenmaterial wurde sowohl einem künstlichen, aber auch einem natürlichen Frost ausgesetzt. Chlorophyllfluoreszenzmessungen an Apfelsämlingen haben gezeigt, dass die Frostschäden durch Applikation von Prohexadion-Ca reduziert werden konnten. Die relative Chlorophyllfluoreszenz war in den mit Prohexadion-Ca behandelten Pflanzen geringfügig erhöht im Vergleich zu den unbehandelten Pflanzen nach Frostexposition. Eine Prohexadion-Ca-Vorbehandlung resultierte auch bei Apfelblüten in einem verminderten Auftreten von Frostschäden nach Einwirkung von künstlichem, aber auch von natürlichem Frost im Freiland, wobei dem natürlichen Frost im Vergleich zum künstlichen Frostereignis ein höheres Schadpotential zugeschrieben wurde. Auch die Vorbehandlung mit α-Tocopherol/Glycerin-Lösungen hatte bei Apfelsämlingen wie auch bei Apfelblüten eine mindernde Wirkung gegenüber künstlichem und natürlichen Frost zur Folge.

Key words. Prohexadione-Ca – freezing injury – α-tocopherol – glycerine – *Malus domestica*

Introduction

In Central Europe, late frost is meteorologically defined as freezing events that occur in the period between the onset of the vegetative period and the end of May.

On average, severe damage of flowers and young apple fruits caused by late frost events occurs in this region at least once every three years (MITTELSTÄDT 1994). Climatic conditions during bloom often determine the final yield level and fruit losses may reach 100 % (WÖLFEL and NOGA 1998). Many plant species have developed effective mechanisms to protect themselves against freezing injury, such as morphological barriers (WISNIESWSKI et al. 1997; WORKMASTER et al. 1999), antifreeze proteins (ANTIKAINEN and GRIFFITH 1997; GRIFFITH et al. 1997) or antioxidative compounds (SCHMITZ-EIBERGER et al. 2001a, b, and 2002) for the protection against free radicals induced by freezing temperatures (WÖLFEL and NOGA 1998). Effi-

ciency of these protective mechanisms is limited, and damage occurs if temperatures exceed critical levels.

It is obvious that frost protection is of great economic relevance, especially when fruit trees are grown in high-risk or marginal areas. Several methods for protection against frost are available (PERRY 1998). However, all of them require relatively high expenditures and none of them is giving absolutely satisfying results. The most commonly used method in many parts of Europe and elsewhere is frost irrigation. However, frost irrigation may even aggravate frost damages if the temperature in the floral tissue drops below –2 °C, because water-soaked tissue is more susceptible to frost (WISNIEWSKI et al. 1997). Moreover, frost irrigation often has a damaging effect on the soil structure (SCHUHMACHER 1989). It requires the installation of expensive over-the-top irrigation equipment. The main disadvantage, however, is the immense water consumption that, in many areas, exceeds local availability.

Decreasing temperatures cause oxidative stress in plant tissues (LEVINE 1999). Hence, agents with antioxidative properties are discussed to reduce frost damage. Prohexadione-calcium (ProCa), recently introduced as a growth regulator for pome fruits, is an inhibitor of distinct dioxygenases, which require 2-oxoglutaric or ascorbic acid as co-substrates. It inhibits the formation of growth-promoting gibberellins and of ethylene. In apple and pear shoots, it also blocks flavanone 3-hydroxylase, thereby inducing a completely new spectrum of flavonoids and other phenolic plant constituents, which, obviously, leads to reduced pathogen incidence (RADEMACHER 2000; HALBWIRTH et al. 2002; ROEMMELT et al. 2002). Flavonoids and other phenols are known to act as scavengers of free oxygen radicals (ELSTNER 1990). Vitamin E is a lipophilic antioxidant (NOGA and SCHMITZ 1998); among its components α-tocopherol has the highest biological activity in its function as scavenger and quencher (FRYER 1992). Furthermore, α-tocopherol is incorporated in membranes, contributing to their biophysical stability and functioning (FRYER 1992). Its practical use against frost damage has been restricted for a long time due to the limited uptake after exogenous foliar application. However, combined with an appropriate formulation, cuticle penetration can be facilitated (NOGA and SCHMITZ 1998). Cryoprotectants like glycerol are widely used for industrial and biotechnological purposes, e.g. for the conservation of germplasm (LEIBO et al. 1970; CHOPRA 1993). Attempts are also being made to expand these positive effects on frost injury into the production of fruits and vegetables (KETCHIE et al. 1973). The aim of this study was to evaluate the frost-protecting effects of the above-mentioned substances in flowers and young leaves of apple (*Malus domestica)* under controlled as well as under field conditions.

Materials and Methods

Expt. 1: Experiment with apple-seedlings

Plant material. Seedlings raised from cold-stratified seeds of 'Golden Delicious' apples were cultivated in a growth chamber at 20 °C and 65–70 % relative humidity under an illumination of 200 µmol photons/m^{-2} s^{-1} for 12 h/d. Plants were irrigated according to demand. Nutrient solution was added to the irrigation water once a week.

Treatments. Seedlings were sprayed until run-off with 50 ppm of an aqueous solution of prohexadione-calcium (ProCa; prepared from Regalis®) 21, 8 and 2 days before cold treatment. The first application was carried out four weeks after seedling emergence. A mixture of α-tocopherol/glycerol (0.25 % a.i./5 % w/v) (α-toc/glyc.) was sprayed as an aqueous solution made from emulsifiable concentrate. Spraying was performed 24 h before cold treatment as previous experiments had demonstrated that maximum tocopherol content in the tissue is accomplished within this period of time (NOGA and SCHMITZ 1998). Seedlings were placed 50 cm above the ground in a freezing chamber (8 m^3) with constant and uniform air movement, and subjected to cold treatment. The temperature was gradually lowered to a min-

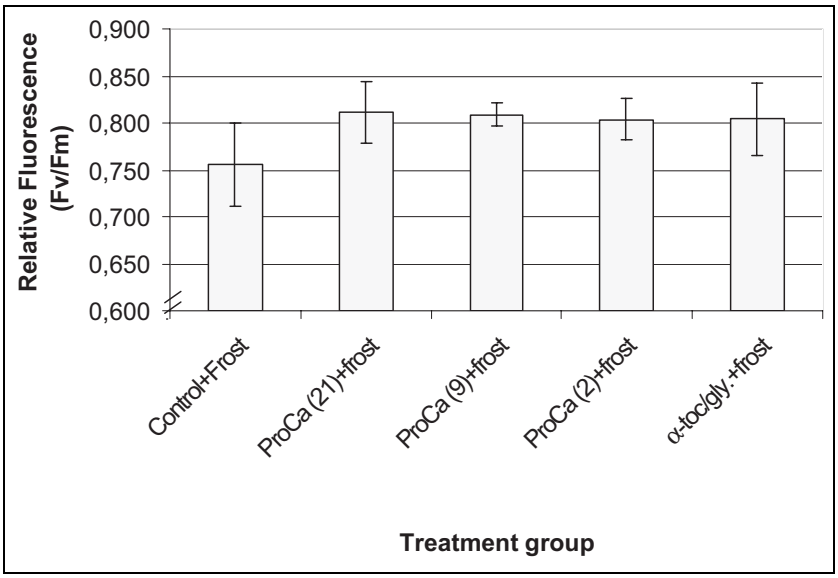


Fig. 1. Relative fluorescence (Fv/Fm) of 'Golden Delicious' seedling leaves after treatment with different spray solutions and 4h after frost exposure; mean ± SE.

imum of –3 °C, and maintained for 3 h 15 min. In our studies under controlled conditions we did not employ temperatures much below –3 °C because tissue damage at this temperature at controlled conditions in the freezing chamber compares to damage induced in the field at a much lower temperature (–6 ° to –7 °C). Additionally, we avoided freezing of the soil, which could lead to shoot desiccation. Therefore, the pots were embedded in soil and further insulated with plastic bubble wrap.

Chlorophyll-fluorescence measurements. As described by SCHMITZ-EIBERGER and NOGA (2001a) measurements were conducted with a 'pulse-amplitude-modulation-fluorometer' (PAM, model 2000, Walz, Effeltrich, Germany). After the seedlings had been dark-adapted for 20 min, the second fully developed leaf from the top was exposed to a weak modulated measuring beam (<0.1 $\mu mol/m^{-2}\ s^{-1}$) to ascertain the basic fluorescence F_0 followed by an 800 ms saturation pulse of white light for assessment of the maximum fluorescence F_m. The ratio $(F_m–F_0)/F_m$ represents the photochemical efficacy of the electron flow of the photosynthetic yield. Chlorophyll fluorescence was measured immediately (30 min) and 24 h after cold treatment of plants.

Expt. 2: Orchard experiments

Plant material. The orchard experiments were carried out in 13-year-old *Malus domestica* trees cv. 'Red Cox'/M9 and 18-year-old trees cv. 'Jonagold'/M9 at the Fruit Research Station in Klein-Altendorf, Germany, and in 8-year-old cv. 'Gala'/M9 and 18-year-old cv. 'Boskoop'/M9 at the Department of Horticulture, University of Bonn, Germany, in spring 2002.

Treatments. At both locations, whole trees were sprayed on March 25th and April 2nd 2002 until run-off with 250 ppm of ProCa (prepared from Regalis®). The combination α-toc/glyc. (0.25 % a.i./5 % w/v) was applied on April 2nd 2002. In Klein-Altendorf each treatment group consisted of 18 trees being sprayed with a knapsack sprayer, in Bonn six trees were used per treatment group.

Artificial freezing. In Klein-Altendorf, one branch per tree was sampled from the upper to middle part of the canopy on April 3rd. In Bonn, two branches from each tree were harvested on April 4th. All branches were at full bloom with approximately 50 % of the flowers open and the first petals falling (growth stage 65 BBCH). Applications with ProCa had taken place at both locations 9 days (1st ProCa) and 2 days (2nd ProCa) before artificial freezing. Application of α-toc./glyc. was performed 24h before artificial freezing.

Immediately after cutting, branches were placed into 10 l plastic buckets with a diameter of 40 cm. Fifteen branches of the different treatment groups were each uniformly arranged over the area of each bucket. The buckets were uniformly distributed on the floor of the freezing chamber. Branches were exposed to a 7–8 hours freezing program, which was simulating a naturally occurring freezing event. The temperature was gradually lowered over 3–4 h and then maintained at a minimum temperature of –3 °C. Fifty percent of the branches were held at this temperature for 3 h, the remaining 50 % for 4 h. After cold treatment, branches were transferred to a glasshouse with a temperature of 20 °C±2 °C. Rating of flower injury was performed after 2–3 days in order to let potential freezing injuries become visible in the tissue. Flowers were cut lengthwise using scalpels. All flowers containing ovaries with brownish tissue were considered dead (Fig. 2). The total number of intact and irreversibly freeze-injured flowers was recorded for each branch and percentage of damaged flowers according to total flowers were calculated and given in the table.



Fig. 2. Evaluation of freezing damage (upper flower: irreversible tissue damage; lower flower: healthy).

Natural freezing events. In spring 2002 the apple trees used in the experiments underwent a series of natural freezing events. These events were more intense in Klein-Altendorf as compared to Bonn where the climate is generally milder. On April 9th, after the incidence of a freezing event, branches were detached from the upper to middle part of the canopies. In Klein-Altendorf, 1 branch was taken from a total of 12 trees each of cvs. 'Red Cox' and 'Jonagold'. In Bonn, 2 branches each were cut from 6 trees of cv. 'Boskoop'. Rating of injury was performed immediately after sampling as described above.

Statistical analysis. All data were tested for homogeneity of variance. Significance was evaluated subjecting the data to Duncan`s test at $P \leq 0.05$ using the SPSS

program for windows (SPSS GmbH Mat Soft., Munich, Germany).

Results

Expt. 1: Experiment with apple seedlings

Effect of treatment on fluorescence characteristics. Directly after cold treatment, a yield of Fv/Fm=0.750 was measured in non-treated seedlings (Fig. 1). In all treated seedlings, Fv/Fm was slightly higher (Fv/Fm=0.803 to 0.814). However, only the Fm-value obtained from seedlings, which were sprayed with ProCa 21 days before cold treatment was significantly different (Fv/Fm=0.814). This effect was less pronounced as the interval between ProCa application and cold treatment decreased (application 9 and 2 days before cold treatment) and was similar to the fluorescence yield in leaves after treatment with α-toc./glyc.

Twenty-four hours after cold treatment, Fv/Fm had further declined in all seedlings. The untreated seedlings and those being treated with vitamin E/glycerol showed the strongest decline (–5.5 % and –4.9 %, respectively), whereas in all other treatments the reduction was only ≤–0.4 % (data not shown). The results were corresponding to those obtained directly after cold-treatment. The differences between the treatments had increased. As the mean variation within each treatment had also increased, there was no subdivision into different statistical groups.

Expt. 2: Field experiments

Flower injury after artificial freezing. Three hours of freezing killed 29.6 % of the non-treated flowers of 'Red Cox' apple trees (Table 1). All treated flowers showed reduced damage compared to the untreated flowers. The strongest decrease (–52.7 %) was observed after ProCa application 9 days before cold treatment.

After 4 hours of freezing the percentage of injuries among the non-treated flowers was 34.6 %. Differences between the treatments were more intense than after 3 hours of freezing. As before, the first application of ProCa had the greatest effect on reducing cold damage. Injuries were significantly reduced by –82.7 %. Also treatment with α-toc/glyc. resulted in a significant decrease in injured flowers as compared to the control. A treatment 2 days before the cold treatment (2. ProCa) did not have any effect.

'Jonagold' flowers were further developed at freezing than 'Red Cox' flowers; 1/3 was assigned to the stage "green bud" (BBCH 56), 1/4 to "red bud" (BBCH 57), and another 1/4 to "balloon" (BBCH 59). Consequently, damage caused by artificial freezing was more severe (Table 1); 71.9 % of the untreated flowers was destroyed after 3 hours of freezing. A significant reduction to 54.4 % and 56.1 %, respectively, was obtained by an early treatment with ProCa and with α-toc/glyc. Applications of ProCa 2-days before the cold treatment did not show any effect. After 4 hours of freezing 77.1 % of the flowers damaged were found on untreated branches. Differences between the treatments were more apparent. All treatments resulted in a significant reduction of flower damage. Maximum protection was conferred by an application of ProCa 9 days before cold treatment (43.8 % injured). Convincing effects were also observed after treatment with α-toc/glyc. (49.3 % injured). Applications with ProCa 2 days before cold treatment led also to a reduction of damaged flowers to 66.0 %.

Among all varieties included in this experiment, flowering was most advanced in cv. 'Boskoop', in which the mean developmental stage of the inflorescences was

Table 1. Flower injury of 'Red Cox', 'Jonagold' and 'Boskoop' apple trees after pre-treatment with ProCa and α-toc/glyc. and exposure to an artificial frost event for 3 and 4 h, respectively; mean±SE, n=80.

Treatment	3 h Artificial frost	Treatment	4 h Artificial frost
	Frost injury (%)		Frost injury (%)
'Red Cox'			
Control (untreated)	29.6±2.6	Control (untreated)	34.6±2.6
ProCa, 9 days	15.6±2.5	ProCa, 9 days	6.0±1.2
ProCa, 2 days	23.1±2.4	ProCa, 2 days	33.3±2.6
α-toc./glyc.	15.6±2.1	α-toc./glyc.	24.3±2.1
'Jonagold'			
Control (untreated)	71.9±2.7	Control (untreated)	77.1±2.2
ProCa, 9 days	54.4±3.2	ProCa, 9 days	43.8±4.2
ProCa, 2 days	69.0±2.9	ProCa, 2 days	66.0±2.7
α-toc./glyc.	56.1±2.7	α-toc./glyc.	49.3±2.7
'Boskoop'			
Control (untreated)	69.4±4.4	Control (untreated)	69.4±4.7
ProCa, 9 days	33.6±4.1	ProCa, 9 days	76.6±3.6
ProCa, 2 days	36.8±4.2	ProCa, 2 days	61.1±5.2
α-toc./glyc.	61.8±4.2	α-toc./glyc.	42.6±6.0

in the beginning of bloom (BBCH 62; 1/3 of the flowers opened). Three hours of freezing killed 69.4 % of the non-treated flowers (Table 1). In contrast, only 33.6 % of the flowers treated with ProCa 9 days before cold treatment and 36.8 % of those treated with ProCa 2 days before cold treatment were injured. No significant effect was observed after application of α-toc/glyc. Results obtained after 4 hours of freezing were not in line with the other findings: again, 69.4 % of the non-treated flowers were injured. The first ProCa treatment led to an significant increase (76.6 %) of flower damage, the second brought about a non-significant reduction to 61.1 %. Positive effects were only observed after application of α-toc/glyc., which resulted in a significant reduction to 42.6 % frost injured flowers.

Flower injury after natural freezing events. In spring 2002 the apple tree orchards in Klein-Altendorf and Bonn were affected by two series of frost events. When branches were examined on April 10, 1/3 of the flowers cv. 'Red Cox', were at growth stage BBCH 56 ('green bud'), 1/3 at BBCH 57 ('red bud') and another 1/3 at BBCH 59 ('balloon'). Full damage (100 %) was found in the untreated flowers (Table 2). In all other treatment groups damage was significantly lower. Positive effects (23.4 % survival) were obtained due to application of ProCa 9 days before the frost event, the other treatments resulted in a survival of 11.7 % and 10.8 %, respectively.

The average stage of development of the flowers cv. 'Jonagold' was BBCH 57 ('red bud') to BBCH 59 ('balloon'). Again, freezing injury was significantly higher in non-treated flowers (74.5 %) compared to treated ones, among which a general reduction by about 7–9 % was found, the mixture α-toc/glyc. being slightly better than ProCa (Table 2).

Table 2. Flower injury of 'Red Cox', 'Jonagold' and 'Boskoop' apple trees after pre-treatment with ProCa and α-toc/glyc. and exposure to a natural frost event for about 4h; mean±SE, n=80.

Treatment	Natural frost
	Flower injury (%)
'Red Cox'	
Control (untreated)	100.0±0.1
ProCa, 9 days	76.6±1.7
ProCa, 2 days	88.3±1.3
α-toc./glyc.	89.2±1.2
'Jonagold'	
Control (untreated)	74.5±2.2
ProCa, 9 days	67.5±2.3
ProCa, 2 days	67.7±2.6
α-toc./glyc.	65.1±2.1
'Boskoop'	
Control (untreated)	94.5±2.4
ProCa, 9 days	84.3±3.3
ProCa, 2 days	74.7±3.0
α-toc./glyc.	75.5±4.1

At the Bonn test site, flowers of cv. 'Boskoop' had already reached growth stage BBCH 59 ('balloon') to BBCH 61 ('beginning of bloom'). Freezing had caused damage in 94.5 % of the untreated flowers. A significant reduction was only observed after the second application of ProCa two days before the frost event (74.7 %) and α-toc/glyc. (75.5 %). ProCa applied nine days before frost incidence led to a non-significant reduction (84.3 %) (Table 2).

Discussion

Common methods for cold and frost protection in fruit orchards include the use of water sprays or foam, wind machines and burning of organic matter (PERRY 1998). All these methods are based on providing mechanisms by which the temperature of the plant tissue is prevented from freezing.

Another approach for frost protection is lowering the temperature at which a plant tissue will freeze by spraying agrochemicals, such as Vit.E or glycerine (WÖLFEL and NOGA 1998). This approach is based on the premise that plant structures can supercool without damage to a temperature below 0 °C. The extent of supercooling is determined by the presence of extrinsic or intrinsic ice-nucleating agents of plant or bacterial origin (LINDOW 1995), the presence of cryoprotective and other components or the presence of morphological barriers (WISNIEWSKI et al. 1997). Further factors involved in determining the freezing temperature are the presence of moisture on the tissue surface, the cooling rate, and the plant species involved (ASHWORTH and KIEFT 1995).

Recently, infrared thermography has been used to monitor ice nucleation and propagation in herbaceous and woody plants (WISNIEWSKI et al. 1997). These studies have confirmed the significance of barriers to ice propagation. The data from these studies have shown that the presence of external surface moisture and internal ice-nucleating agents are the major factors that limit the ability to supercool to temperatures lower than 0 °C. An inhibition of ice nucleation would result in higher freezing tolerance. These observations led to our study, in which the ability of the antioxidative component α-tocopherol in combination with the cryoprotector glycerol (WÖLFEL and NOGA 1998) and ProCa was compared regarding their potential to increase supercooling and moderate freezing damage in apple shoots and flowers. Earlier studies conducted by WÖLFEL and NOGA (1998) have shown, that freezing damage on apple branches could be reduced, when plants were pretreated with α-tocopherol/glycerol. These positive results were compared with the results obtained with ProCa pretreatments in our study. Application of both types of agents was followed by a small increase of chlorophyll fluorescence parameter Fv/Fm in leaves of apple seedlings after frost exposure and by the protection to flowering shoots of several apple varieties (Tables 1–2). While the function of α-tocopherol is to scavenge reactive oxygen radicals (FRYER 1992), the application of ProCa induces alteration of the flavonoid spectrum in the plant tissue compared to the control (HALBWIRTH et al. 2002; ROEMMELT et al. 2002). Similar to tocopherols, flavonoids can inactivate

free radicals in the plant tissue. Most representatives of the large group of flavonoids (more than 4500 compounds are known) are hydrophilic and are primarily located in the cytosol and vacuole. The present study shows that freezing injury is reduced in plant material exposed to artificial freezing under controlled conditions as well as to natural frost under orchard conditions, when treatments are made with ProCa or α-toc/glyc. ROEMMELT et al. (2002) measured a qualitative and quantitative shift in flavonoids and other phenolics in apple leaf tissues after application of ProCa. The authors have also shown, that a period of several days is required to obtain the most pronounced effects and that the resistance of plant material increases with the changes in flavonoids and phenolic compounds. This indicates that physiological changes need to occur as a prerequisite for tissue resistance. The effect of ProCa in this study can possibly be explained by an alteration of flavonoids and other phenolic compounds as affected by the ProCa treatment, which may reduce oxidative stress, decrease the freezing point or scavenge frost-induced radicals. So far, there are no indications for significant histological changes induced by ProCa. In contrast, α-toc/glyc gives a more rapid effect: The cryoprotector glycerol most likely decreases the freezing point in treated plant tissue, whereas α-tocopherol has an immediate antioxidative effect after passing the cuticular barrier (SCHMITZ-EIBERGER et al. 2002). A higher antioxidative activity in plant tissue treated with ProCa was also reflected by experiments with apple seedlings treated with paraquat. Paraquat is an herbicide, which induces the synthesis of free radicals in plant tissue. This is a part of reactions, which are activated by the exposure of plants to freezing temperature. Under natural conditions, the flow of energy along the electron transport chains is central for any living cell (LEVINE 1999). In an ideally tuned system, energized electrons are passed from a protein donor to a specific acceptor along a gradually decreasing gradient of redox potential. Such precise transfer of excited electrons requires perfect positioning of the donor and acceptor molecules. Perturbations of membrane fluidity, e.g. affected by cold stress or other temperature changes, will interfere with the precise alignment of membrane proteins and result in leakage of electrons. These electrons can react with available molecular oxygen and create reactive oxygen species and lipid hydroperoxides, the same reaction products, which are induced by the application of paraquat to the plant tissue. Minor damage in plant tissue pretreated with ProCa indicates on a positive effect of the treatment solution against oxidative stress. Here, pre-treated plants were much less affected by oxidative stress induced by the herbicide than the controls (RADEMACHER, unpublished results).

It is well known, that the sensitivity of apple flowers to frost injury depends on several factors, including the variety and rootstock (PERRY 1998). This and the different phenological stages at which treatments were carried out is a possible explanation for the different intensities of freezing injury in our field experiments (see Tables 1 vs. Table 2). Results of the experiments with the same plant material grown under controlled conditions and under field conditions were consistent over the different treatment groups. The need to apply ProCa several days ahead of a frost incidence may represent a disadvantage, because a corresponding reliable weather forecast might be difficult to obtain. It rather appears that prophylactic treatments are mandatory. On the other hand, it is likely that treating pome fruit trees at green to pink bud stage will also decrease susceptibility to flower infections by fire blight. Analogous to the situation with shoot infections (ROEMMELT et al. 2002), this might be a viable alternative to using antibiotics. Therefore, future work will have to consider both aspects and also evaluate other potential effects of early ProCa applications, e.g. impact on fruit set. Furthermore, it might be of advantage to use sequential sprays of ProCa (approximately 5 to 15 days ahead of a potential freezing event) and α-toc/glyc. (24 h before freezing). Due to the different modes of action involved, additive or even synergistic effects may occur.

In summary, our studies demonstrated that the use of ProCa or α-toc/glyc. are valid approaches for protecting fruit trees against freezing injury. The results indicate, that spraying the plants with the different products brought about reduced incidence of freezing damage in leaves as well as in flowers of different apple varieties exposed to natural and artificial freezing events.

References

ANTIKAINEN, M. and M. GRIFFITH 1997: Antifreeze protein accumulation in freeze-tolerant cereals. Physiologia Plantarum **99**, 423–432.

ASHWORTH, E.N. and T.L. KIEFT 1995: Ice nucleation activity associated with plants and fungi. In: LEE, R.E., Jr., WARREN, G.J. and L.V. GUSTA (eds.). Biological ice nucleation and its applications. APS Press, St. Paul, Minn, 137–162.

CHOPRA, V.L. 1993: Biotechnological tools for conserving biodiversity. Indian Farming **43**, 7–16.

ELSTNER, E.F. 1990: Der Sauerstoff. B.I.-Wissenschaftsverlag, Mannheim/Wien/ Zürich.

FRYER, M.J. 1992: The antioxidative effects of thylakoid vitamin E (α-tocopherol). Plant Cell and Environment **15**, 381–392.

GRIFFITH, M., ATIKAINEN, M., HON, W.-C., PIHAKASKI-MAUNSBACH, K., YU, X.-M., CHUN, J.U. and D.S.C. YANG 1997: Antifreeze proteins in winter rye. Physiologia Plantarum **100**, 327–332.

HALBWIRTH, H., KAMPAN, W., STICH, K., FISCHER, T. C., MEISEL, B., FORKMANN, G., W. and W. RADEMACHER 2002: Biochemical and molecular biological investigations with respect to induction of fire blight resistance in apple and pear by transiently altering the flavonoid metabolism with specific enzyme inhibitors. Acta Hort. **590**, 485–492.

KETCHIE, D.O., MURREN, C. and T.E. WEEKS 1973: The effect of cryoprotectants on fruit trees. Cryobiology **10**, 533.

LEIBO, S.P., FARRANT, J., MAZUR, P., HANNA, M.G. and L.H. SMITH 1970: Effects of freezing on marrow stem cell suspensions: interaction of cooling and warming rates in the presence of PVP, sucrose or glycerol. Cryobiology **6**, 315–332.

LEVINE, A. 1999: Oxidative stress as a regulator of environmental responses in plants. In: LERNER, H.R.

(ed.): Plant responses to environmental stresses. Marcel Dekker, Inc., New York, Basel.

LINDOW, S.E. 1995: The role of bacterial ice nucleation in frost injury to plants. Annu. Rev. Phytopath. **21**, 363–384.

MÍTTELSTÄDT, H. 1994: Apfelerträge und Spätfröste in Deutschland. Erwerbsobstbau **35**, 204–209.

NOGA, G. and M. SCHMITZ 1998: Uptake characteristics and biological activity of exogenously applied α-tocopherol. In: NOGA, G. and M. SCHMITZ (eds.): Antioxidants in Higher Plants – Biosynthesis, Characteristics, Actions and Specific Functions in Stress Defence. Shaker Verlag, Aachen, 112–122.

PERRY, K.B. 1998: Basics of frost and freeze protection for horticultural crops. HortTechnology **8**, 10–15.

RADEMACHER, W. 2000: Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. Ann. Rev. Plant Physiol. Plant Mol. Biol. **51**, 501–531.

ROEMMELT, S., PETEREK, S., TREUTTER, D., RADEMACHER, W., SPEAKMAN, J.B., ANDREOTTI, C., COSTA, G., SPONZA, G., TORTORETO, L., BAZZI, C., HALBWIRTH, ZIMMERMANN, N., STICH, K. and G. FORKMANN 2002: Alteration of phenylpropanoid biosynthesis of fruit trees as a tool for enhancement of fire blight resistance. Acta Hort. **590**, 477–484.

SCHMITZ-EIBERGER, M. and G. NOGA 2001a: Quantification and reduction of UV-B induced plant damage in *Phaseolus vulgaris* leaves and *Malus domestica* fruits. J. Applied Botany **75**, 53–58.

SCHMITZ-EIBERGER, M. and G. NOGA 2001b: UV-B radiation – Influence on the antioxidative defence system in *Phaseolus vulgaris* leaves. J. Applied Botany **75**, 210–215.

SCHMITZ-EIBERGER, M. HAEFS, R. and G. NOGA 2002: Calcium deficiency – Influence on the antioxidative defence system in tomato plants. Journal of Plant Physiology **159**, 733–742.

SCHUHMACHER, R. 1989: Die Fruchtbarkeit der Obstgehölze. 3. Aufl. Eugen Ulmer Verlag Stuttgart.

WISNIESWSKI, M., LINDOW, S.E. and E.N. ASHWORTH 1997: Observations of ice propagation in plants using infrared video thermography. Plant Physiology **113**, 327–334.

WÖLFEL, D. and G. NOGA 1998: The effect of α-tocopherol and glycerol on preventing blossom freezing injury. In: NOGA, G. and M. SCHMITZ (eds.): Antioxidants in Higher Plants – Biosynthesis, Characteristics, Actions and Specific Functions in Stress Defence. Shaker Verlag, Aachen, 149–155.

WORKMASTER, B.A.A., PALTA, J.P. and WISNIEWSKI, M. 1999: Ice nucleation and propagation in cranberry uprights and fruit using infrared video thermography. J. Amer. Soc. Hort. Sci. **124**, 619–625.

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