EIP-AGRI Focus Group
Protecting fruit production from frost damage

MINIPAPER 04: Use of chemicals to help plants tackle frost damages

January 2019

Authors

Sabrina Dreisiebner-Lanz (Coord.), Alois Bilavcik, Roman Chaloupka, Maciej Gąstoł, Susan McCallum, Carlos Miranda

funded by European Commission

Introduction

The aim of this minipaper is to give an overview of the available knowledge about the possible use of chemicals to help plants tackle with frost, identify efficient and promising methods and give – if possible – first practical recommendations. Although some chemicals are already used in growing practise, their effects are not well-understood in many cases. Pros and Cons of interesting methods, side effects, costs, difficulties, etc. are discussed. Furthermore, additional research needs in this field will be highlighted.

In general, two main approaches are discussed:

- Treatments to delay bloom or budburst
- Treatments to help the plant cope with frost

Delay of bloom or budburst is an indirect and preventive method, which must be realized before the risk of frost can be estimated. Depending on the timing of the delay, and the moment of spring frost, the delay can be sufficient to avoid or reduce damage on cultures. Otherwise, active protecting methods are required additionally.

Mechanisms of frost damage

Frost damages occur in susceptible tissues of fruit trees by intercellular formation of ice crystals, which grow and lead to frost dehydration of cells and consequently to injury of flowers, fruits and shoots.

Starting point of ice formation are ice nucleators - particles that act as the origin for the formation of an ice crystal. Ice nucleators are present in plant tissues and on plant surfaces. In the spring period, the exogenous ice nucleators are mainly ice crystals from snow, ice nucleation bacteria, dust and many other inorganic particles. Water in different parts of the plant, including floral tissues of buds of trees, is able to supercool (stay liquid in spite of temperatures below zero), but when ice nucleators are present, ice formation starts. The supercooling ability of floral tissue changes during bud development and the activity of endogenous ice nucleators depends on the type of tissues and plants. When ice nucleation occurs in the flowering fruit tree shoots, the ice crystals propagate in the tissues, depending on the degree of supercooling, the temperature, as well as the possible barriers to the spread of ice crystals. In fully developed flowers, the supercooling state is very unstable.

Substances to delay bloom or budburst

Vegetable Oil[1]

The application of vegetable oil (soya bean, rape and sunflower) produces a film of oil on the surface of the bud, which inhibits the respiration of buds. Consequently, physiological processes in the bud are restricted and bud break will be delayed. Results of field trials in Austria on grapevines (varieties ‘Grüner Veltliner’ and ‘Zweigelt’) showed rather promising results. In these trials, rape oil was applied two times, 30 and 15 days before bud break with a concentration of 10%. A delay of 8-15 days could be achieved. Shoot development and leaf area was significantly reduced on treated plants,but these differences were minimal by flowering.. There was no effect on yield or quality observed in these trials. On the other hand, field experiments in Germany with other varieties of grapevine (‘Silvaner’) showed a delay of more than 30 days with one treatment, in some treatment variation, bud break was suppressed completely.

The advantages of the method are the simple application of a natural substance, which can be realised individually on field or variety scale. The timing of the application is vital for the success of delaying bloom andremains the biggest challenge of this method. Therefore, the date of bud break must be estimated closely. In addition to the expert knowledge of farmers and advisors, phenological models could be helpful in this context. For Austria, until now, no satisfactorymodel for the cultivars in question is available and applications in field trials were therefore timed according to historical data.

[1] Forneck , Astrid et al. (2018): Austriebsverzögerung als Spätfrost-Prävention. Der Winzer 1/2018

funded by European Commission

Possible restrictions and uncertainties are:

- Varying reaction of different cultivars of grapevines
- Reaction depends on general vitality and vigour of grapevines
- Effect of weather conditions after the application of oil
- Damage on beneficial organisms (predatory mites), due to the high concentration of oil

Side effects (mainly because of an overdose or late application) reduced yield or phytotoxic reactions of the vines were observed in some field trials and should therefore be investigated for several cultivars and under different conditions. Field trials in Austria in 2017 showed neither yield depression nor phytotoxic reactions on 'Grüner Veltliner' and 'Zweigelt'.

Phytohormones

Phytohormones – plant regulators affect plant growth and development. As signal molecules, they act in very low concentrations. According to their chemical nature, function and mode of action, the phytohormones are:

- Auxins
  Apical dominance - stimulation of top bud growing, inhibition of lateral buds, inhibition of root growing, stimulation of lateral root formation
- Cytokines
  Stimulation of shoot growing, inhibition of root growing, delaying of senescence, suppression of apical dominance
- Gibberellins
  Intensive elongation growth, releasing dormancy in seeds and buds, regulation of flowering, increase the number and size of flowers
- Abscisic acid
  Antagonist against auxins, cytokines and gibberellins, onset, induction and duration of dormancy of seeds and buds, stress reaction, senescence and abscission of leaves (through ethylene induction).
- Ethylene
  Accelerates maturing of fruits, shortens dormancy of seeds and buds
- Jasmonic acid, Brassinoids, oligosaccharides, polyamines – play a role in stress response to pathogens

Abscisic Acid (ABA) plays an important role in processes of dormancy of grapevines. Experiments in USA, Canada and Brazil showed an effect of application on leaves after harvest on date of budbreak (delay) and enhancement of frost hardiness. However, the same study pointed out the variability of effect for different cultivars and regions. The application of alpha naphthaleneacetic acid (NAA) or Gibberellin on dormant buds during vegetation period both had a retarding effect on budbreak. As the use of these phytohormones is not tested for European conditions or cultivars, a use in practice cannot be recommended at the moment.[2]

Several trials were carried out to delay bloom of fruit trees. Applications with Gibberellin resulted in a retardation, but repeated treatments are necessary. Ethephon, sprayed in early autumn, had a delaying effect of 4-7 days. However, the determination of the suitable dose is difficult: effective doses usually also have stronger negative effect on budbreak (forever sleeping buds).

Products exist which can be applied to plant tissue for the purpose of delaying bloom. Growth regulators (Ethephon or Ethrel) have been used in studies on blueberries, which acts by releasing ethylene on contact with plant tissues if applied to crops the previous autumn. Effective results in bloom delay as well as delayed fruit ripening have been recorded in blueberries without any negative impact on berry weight. Additional applications have also been shown to increase flower bud density in one study.[3]

---

[2] Forneck, Astrid (2017): Möglichkeiten der Austriebsverzögerung. Der Winzer 03/2017

[3] Krewer et al., 2005

Substances to help coping with frost (pre or post treatments)

Several substances are known to help plants cope with negative temperatures (e.g. by lowering critical temperature) or to increase recover after a frost event, hence reducing damage and yield depression.

Phytohormones and growth regulators

Gibberellin applications are known to reduce yield depressions of pears after spring frost incidents at the beginning and during blossom[4]. GA_3 1.6% (60-120 cc/Hl) applied in the 48 hours after the frost event is highly effective on pears to overcome fruit loss. However, it has to be considered, that Gibberellin application on pears can lead to deformation of some fruits ('flute' or elongated shapes), lower quality (firmness, sugar content) and is therefore not suitable for all production systems.. Furthermore, reactions of different varieties are not equal, for example in pears, Abbe Fetel, Blanquilla, Conference, General Leclerc and Guyot show a high response to GA_3 applications after a spring frost and normal yields can be obtained. In these varieties, GA_3 dose must be precisely adjusted, as the application may result in oversetting, leading to high thinning requirements and induce alternate bearing. However, Comice, Coscia, Beurre hardy, Passa Crassana and Moretini, among others, have a low response and GA_3 applications are generally ineffective. Although rather effective on pears, this strategy cannot be adopted for stone fruit due to the completely different morphology of the flowers: petals impede the contact of sprayed Gibberellin with the ovary.[5]

Field trials in Austria following frost events in 2002 and 2012 resulted in higher yields after post frost applications of Auxine and Acylcycleohexadione on apples (Braeburn). After the spring frost in 2016, trials with two varieties and several substances were carried out. For Elstar, only Promalin showed a positive effect on yield, for Braeburn two applications of Prohexadion Ca and one application of Prohexadion, followed by one application of GA_{4+7} resulted a yield stabilisation. The second version led to best results regarding fruit quality[6].

Application of small doses of Regalis (0,5-1 kg/ha) stops vegetative growth temporarily. Due to this effect, synthesis of ethylene in plants is limited and consequently, without ethylene, June drop is reduced. For overcoming this "critical period", one or two applications are necessary. Conditions on the leaf surface for uptake and temperature should ideally be temperature above 18°C (at least 15°C) for a longer period. Most probably, the effect is particularly significant on strongly vigorous crops, where June drop is normally very strong.

Dioxygenase inhibitor Prohexadione-Ca induces several changes in their flavonoid composition. The most pronounced change is an accumulation of flavanones, which are further metabolized through an unusual pathway[7]

Gibberellin GA_{4+7} is used either as pre or post treatment to reduce frost sensitivity or damages, respectively. The applications before spring frost should be performed several days before expected frost nights with doses of 0,2 to 0,5 l (usually added to regular sprayings or if needed, as an extra application). Conditions for GA_{4+7} should be temperatures some degrees above zero, ideally more than 10 degrees and on plants, which have at least some leaves for uptake. As post frost treatments, GA_{4+7} is used to apples and pears with a view to avoid quality damages on apples (cell destruction by frost causing skin russeting) and to increasing leaf surface. The effect should be an increased elasticity of cell-walls and stimulation of cell division. Higher doses causes "nosy fruits" (deformations).

---

[4] Wurm, Lothar: Erfolgreicher Obstbau.

[5] Steinbauer, Leonhard: personal communication

[6] Lafer, Gottfried: Versuche zur Fruchtansatzförderung nach den Frostschäden. Haidegger Perspektiven, 1/2017, p18-20

[7] Roemmelt, S., Zimmermann, N., Rademacher, W., Treutter, D. 2003. Formation of novel flavonoids in apple (*Malus × domestica*) treated with the 2-oxoglutarate-dependent dioxygenase inhibitor prohexadione-Ca. Phytochemistry 64, 709– 716.

funded by European Commission

Phenolic substances, Nutrients, Aminoacids

Phenolic substances are supposed to be able to "recover" partly damaged cells. Commercial products are available (e.g. N-fenolmix), but scientific proofs are missing at the moment.

Products like amino acids or Boron (B) are supposed to recover partly damaged cells, but the impact is questionable; in addition, amino acids might be very expensive. The accumulation of certain sugars and amino acids in plants can act as anti-freeze by lowering the freezing point of some cell contents. Amino acids such as proline and threonine act as natural antifreeze and are thought to reduce the freezing point of some plant cells which could limit damage caused by frost events. Products such as "AminoA Plus" are available in the UK and are reported to prevent freezing of cell cytoplasm in strawberries if applied as a foliar spray (3.5kg ha in 500L water) prior to moderate frost (-3-4ºC). Arginine and glycine have also been reported to play a part in plant protection from freezing injury and reducing damage sustained by abiotic stress.

Vitamins

Hołubowicz et al. (2004)[8] reported a protective effect of spraying with HELP (mixture of vitamins and glycerol) against low temperature damages of flower buds, flowers and fruitlets.

The sprayings were applied 24, 42 or 44 hours before the frosts occurred. The treatment increased the survival of flowers and fruitlets in 6-36% in peach trees and 2-31% in apple trees in comparison to the control samples. This study relies only on laboratory tests and is not confirmed under field conditions. Wölfel and Noga (1998)[9] also indicated positive protective effect of α-tocopherol and glycerol mixture. The commercial solution is Frost Protect (Compo Company).

Substances to help coping with frost (sprays)

The role of sprays that could be used to cope with freezing events can be divided into few categories. The first category are substances changing the freezing point of the plant tissue or water. The second category is the reduction of ice nucleating bacteria on the plant surface to lower the ice formation from the exogenous nuclei. The third category are substances affecting isolation of plant tissues and/or preventing ice nucleation from exogenous nuclei. The fourth category, where plants are stimulated to produce cryoprotective substances in their tissues.

Cryoprotective substances

There are many substances of cryoprotective effect known which lower the melting point of plant solutions and consequently also the ice nucleation temperature of plant tissues. The majority belongs to saccharides, polyols, urea and modified polymer compounds. The limited ability of existing cryoprotective sprays to protect frost sensitive flowering plants at temperatures much below their freezing point may be due to the need of very high solute concentrations required to significantly improve the thermodynamic characteristics. Such concentrations can be toxic to cells, causing a detachment of the cell membrane from the cell wall and cell death. Therefore the approach of combination of different cryoprotectants and interacting compounds is studied. An example of

[8] Hołubowicz T., Basak A., Pacholak E. 2004. Effectiveness of HELP application on the protection of fruit plant flowers against frost. Folia Hort. Ann. 16/2, 2004, 65-69.

[9] Wölfel., Noga G., 1998. Minderung frostbedingter Blutenschäden an Apfeltrieben durch Vitamin E (α-Tocopherol) in Kombination mit Glycerol oder Ethylenglykol. Erwerbsobstbau 40: 34-38.

funded by European Commission

such an approach is the mixture tested in Franko et al. (2011)[10]. A report by the developer indicated that up to 5 °C protection was provided to flowers, leaves, and fruit. In contrary, Anderson et al. (2012)[11] did not find any frost protection effect on detached tomato leaves, pepper seedlings, celosia seedlings and other plant material.

Bacteria control

The most common ice nucleating source in nature are ice nucleation active bacteria (Pseudomonas, Erwinia, Xanthomonas, etc.). The well known snow inducer for making artificial snow Snowmax®, is made from the bacterium Pseudomonas syringae strain collected from nature. Their presence at plant surface increases the temperature of ice crystallisation a few degrees above the melting point to plant tissues. Consequentially the absence of these bacteria is supposed to prevent ice nucleation and therefore frost damage. Plants are treated with antibacterial sprays, such as bactericides, antibiotics, copper salts, etc. Furthermore, as sprayings are washed away or diluted quickly and bacteria multiplication is expected to be quick, treatments would have to be repeated. Sprays including antagonistic bacteria against ice nucleation supporting bacteria can have prolonged effects. The sprayed antagonistic bacterial strains can be predominant bacteria on plants for up to 45 days after the spray[12]. But the range of the antibacterial treatment is limited to the level of the endogenous ice nucleation levels of selected plant species. The positive effect can be achieved during specific frost conditions when flower tissues have lower temperature than branches.

Preventing compounds

Cellulose nanocrystals[13] pretreatment

Electrostatic application, a spraying technique covering entire surface of sprayed material, of cellulose nanocrystals (CNC) dispersion to fruit buds forms a thermal insulation layer with low thermal conductivity. The benefits of the electrostatic application is creating a uniform layer on plant surfaces, and saving spray material. The dispersion was applied to dormant grapevine (*Vitis vinifera*) buds, and hardiness was evaluated by differential thermal analysis (DTA). CNC-treated buds were more resistant to freezing temperatures than untreated buds. Cold hardiness was improved by 2°C with CNC treatment. The hardiness of sweet cherry (*Prunus avium* L.) reproductive buds at the 'first white' stage of development was also tested comparing CNC-treated (2% mass) and non-treated clusters. In general, CNC treatment improved cold hardiness of grape and sweet cherry buds by about 2-4°C. The effect of CNC spray may be of constituting a barrier to ice propagation in the buds under freezing conditions and lowering the emissivity of the bud, thereby reducing thermal emittance.

Hydrophobic particle films[14]

Recently some chemical compounds have been marketed on the basis of giving frost protection by covering the leaf surfaces with an inert layer. Application of hydrophobic particle film as a hydrophobic kaolin particle film led to less damage of potato, grapevine and lemon leaves. The hydrophobic kaolin film acted as a barrier to spreading the exogenously triggered ice nucleation, the ice from outside the plant surfaces, into plant tissues and causing lethal crystallisation. In contrast, the acrylic polymer capable of forming an elastic coating

---

[10] Franko, D.A., K.G. Wilson, Q.Q. Li, and M.A. Equiza. 2011. A topical spray to enhance plant resistance to cold injury and mortality. HortTechnology 21:109–118.

[11] Anderson, J. A. 2012. Does FreezePruf Topical Spray Increase Plant Resistance to Freezing Stress?. HortTechnology, 22(4), 542-546.

[12] Lindow , S.E. 1983. Methods of Preventing Frost Injury Caused by Epiphytic Ice-Nucleation-Active Bacteria. Plant Dis. 67:327.

[13] Alhamid J., Mo C., Zhang X., Wang P., Whiting M., Zhang Q. 2018. Cellulose nanocrystals reduce cold damage to reproductive buds in fruit crops. Biosystemsenineering 172: 124-133.

and: https://fruitgrowersnews.com/news/wsu-researchers-test-nanocrystals-to-prevent-frost-damage/

[14] Fuller, M. P., Hamed, F., Wisniewski, M., & Glenn, D. M. 2003. Protection of plants from frost using hydrophobic particle film and acrylic polymer. Annals of applied biology 143(1): 93-98.

funded by European Commission

on the leaves of plants used in the same study did not clearly demonstrate a consistent frost protection effect and was unable to delay the penetration of ice in potatoes and grapevine leaves although there was some evidence of a delay in lemon. The hydrophobic particle film was capable of giving some protection against freezing in a range of frost susceptible plant tissues. The protection effect was due to prevention of the ice nucleation of leaves by the exogenous nuclei. The ice penetration was delayed on average by up to 2 h.

Stimulation of plant frost resistance

An example of stimulating mechanism is the use of nitric oxide (NO) [15]. The NO treatment significantly reduced freezing injury of apricot flowers when exposed to low temperature (-3°C). It enhanced the cold hardiness of the flower by increasing the sugar and proline contents. It was found that spray application of NO in pink tip stage was more efficient compared to the green tip stage. Min et al. (2018) [16] Salicylic acid-fertigation with cold acclimation improved freezing tolerance of spinach seedlings. Treated plants with 0.5 mM salicylic acid by sub-fertigation were more freeze-tolerant (visual estimates and ion-leakage test) than untreated plants.

Discussion

As a result, the following important points can be established:

- Applications are rather costly, especially when several treatments with phytohormones are needed
- Timing of application can be tricky and environmental conditions should be monitored (e.g. temperature)
- Effect of phytohormones depend on various factors, such as vigour of plants, and concentration of phytohormones (higher concentrations do not necessarily have a better effect). Undesirable side effects are likely.
- Several substances were tested only in laboratory tests or results are based on single trials. Results are therefore not implicitly valid under field conditions.
- Chemical substances for plant treatments need to go through the procedures for authorising plant protection products. As this is a substantial and expensive process, but a small target market, even successful products might fail authorization.
- Environmental issues of PGR usage. In some cases, instead of frequent exogeneous PGR application the better solution is internal, (genetic mode) shift of hormones' balance (e.g. rootstock which delays the vegetation start permanently). However, it is not a solution for early cultivars.

Conclusions

Further research needs should focus on substances, which are affordable in practice and for which an authorization seems attainable. Delaying is judged a very promising method, especially for sensitive species with short dormancy phases (apricots, sweet cherries or grapevine). However, the attained delay might not be effective enough (depending on situation and timing of spring frost) and active protecting methods may still be required. Regarding possible methods for fruit trees, few information is available. The effect of substances that

---

[15] Pakkish Z., Tabatabaienia MS. 2016. The use and mechanism of NO to prevent frost damage to flower of apricot. Scientia horticulturae 198, 318-325.

[16] Min K., Showman L., Perera, A., Arora, R. 2018. Salicylic acid-induced freezing tolerance in spinach (Spinacia oleracea L.) leaves explored through metabolite profiling. *Environmental and Experimental Botany*. 10.1016/j.envexpbot.2018.09.011.

funded by European Commission

enhance frost resistance of plants is estimated to be rather limited – and consequently not sufficient for severe or long lasting spring frost events. Therefore, the risk of damages in spite of (costly) measures is too high.

- Methods tested already in field trials and practice are:
  - GA3 saves the harvest in some pear cultivars (which may help to regulate bloom and vegetative growth), but inferior fruit quality - as in the natural parthenocarpic behaviour - must be taken into account. The pronounced effect is evident only in respect of parthenocarpic fruits.
  - Applications of Prohexadione-Ca (Regalis), potentially combined with GA_{4+7}, as post treatment reduce yield losses on apples.
  - Sprayings with Gibberellin GA_{4+7} are used either as pre or post treatment on apples and pears to reduce frost sensitivity or damages, respectively. Effects on fruit quality are to be expected at higher doses.
  - Rape oil for delaying budbreak of grapevines is judged a hopeful method, but cultivar specific reactions and other uncertainties are still to investigate.
- Promising further approaches seem to be:
  - Melatonine
  - Seaweed extracts (like *Ascophyllum nodosum* = Gömar)[17]
  - 5-aminolevulinic acid: applications with 5-ALA increased frost resistance of tree apple cultivars[18]
  - Substances, which enhance frost resistance by affecting osmotic pressure or cell elasticity.
  - Agents that eliminate water crystallisation or work as insulation
- Less promising appear:
  - Auxins (NAA, etc.) are revealing side effects (like fruit softening) and are therefore not recommended, especially not for pome fruits.
  - Bacteria control: repeated treatments are necessary, effect doubtful

For all mentioned methods, it has to be considered that attained effects and unwanted side effects are highly depending on various inner (e.g. cultivar, vigour) and outer factors (e.g. conditions at/after application) and the timing of applications is vital for success.

Research needs

- The relation of the application of chemical substances and the phenophase/physiological condition of plants should be investigated. The effect of treatments as well as the occurrence of side effects depend on various factors such as stage, water status or vigour. There are numerous knowledge gaps, which have to be closed to allow a practical implementation in fruit production.
- Influence of weather conditions and timing of the application of chemical substances have to be evaluated in detail.
- Combination of sprinkling and chemical substances may be interesting to save water or enhance efficiency, respectively.
- Could water status or other parameters serve as indicators for phenological stage and susceptibility to frost?

[17] Burchett S, Fuller MP Jellings AJ (1998) Application of seaweed extract improves winter hardiness of winter barley cv Igri. The Society for Experimental Biology, Annual Meeting, The York University, March 22–27, 1998. Experimental Biology Online.Springer ISSN 1430-34-8

[18] Lysiak, G.P., Kurlus, R., Michalska, A. (2016): Increasing frost resistance of “Golden Delicious”, “Gala” and “Sampion” apple cultivars. Folia Hort. 28/2 (2016): 152-135

funded by European Commission

Ideas for operational groups and other innovative projects

- Field experiments to assess the efficiency of treatments, which showed an effect in lab tests. The effect and side effects of treatments should be tested under different field conditions. Weather conditions and timing in relation to phenology have to be considered.
- Further field trials on different varieties and on other oils / combinations of different oils to delay bud break of vines.
- Assess the possibility to delay bloom in other species or crops than vines, by the use of oils (presumably not feasible) or other treatments.
- Improving efficiency of treatments with oils or phytohormones by determining the most appropriate plant phenological phase and develop the phenological models that better mimic the developmental response to weather conditions.
- Search for physiological markers to determine phenological stages (compare MP 5B)
- Development of new measuring devices, which evaluate physiological or phenological parameters.
- Experiments to possible combinations of chemical substances with other methods (e.g. frost irrigation)

Further research needs coming from practice, ideas for EIP AGRI operational groups and other proposals for innovation can be found at the final report of the focus group, available at the FG webpage **https://ec.europa.eu/eip/agriculture/en/focus-groups/protecting-fruit-production-frost-damage**