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I. Executive summary

This deliverable includes an overview on, and validation of novel low- or no- copper strategies for grapevine, apple and glasshouse crops carried out under on-farm conditions using commercial production systems. Novel low- or no- copper strategies were optimised and then validated under semicommercial and commercial conditions in different countries and by different partners involved in the project. Close interaction between farmer/advisor groups and research/industry teams providing alternative products have been carried out for a rapid know-how transfer. These results provide a realistic view and perspective for further reduction of copper in each crop.

In grapevine, no-copper strategies are possible under low disease pressure using stand-alone treatments with the TPs. However, strategies with low copper dosages in tank mixture with one of the three TPs are suggested at the most susceptible phenological phases and/or where high infection risks prevail.

In apple, one TP has shown very good efficacy and would be a valuable addition to the currently available product portfolio in organic apple production.

In greenhouse crops (tomato, cucumber), the validated TPs developed in RELACS are well suited for use in practice and could contribute to the reduction of copper use.

In organic cultivation of roses, different strategies for replacement or reduction of copper use can be developed on the basis of the results obtained in the project.

The test products in this report are presented under their code names as their registration is ongoing and should not be interfered with in this report.

This deliverable is part of an ongoing manuscript for a scientific peer-reviewed publication. It will be submitted to a scientific journal soon (likely submission date is 2023), and, once published (gold open access), will be linked to this deliverable. The details contained in this study are still confidential until online publication by the journal.

2. Introduction

The overall aim of WPI in RELACS is the development of novel copper alternatives and their integration in crop protection strategies either to replace copper (i.e. no copper strategies) or to limit the use of copper (i.e. low copper strategies) in organic plant production. The specific objectives related to this deliverable are to validate strategies for the proper application of copper alternatives under commercial conditions (on-farm) on crops with high relevance, in particular grapevine, apple, glasshouse crops (e.g. tomato, cucurbits) and roses. Four copper alternatives (test products, TPs) were provided by the RELACS partners (named TP1, TP2, TP3 and TP4), and a product from outside RELACS was tested against apple scab (TP5).

In WPI, the efficacy of alternatives against target pathogens and compatibility with products registered for organic farming were assessed under controlled conditions (Tasks 1.2). Alternatives were then applied under field conditions (Tasks 1.3) and application strategies were optimised. Field trials were carried out in all crops in two consecutive seasons by the RELACS partners using methodologies typically applied by growers in the fields/greenhouses for commercial production, in order to validate on-farm at least one efficient low/no copper strategy for each of the following pathosystems: grape/downy mildew, apple/apple scab, cucumber/downy mildew, tomato/late blight and the minor use specialty crop roses/rust and black leaf spot (Task 1.4).

In this Deliverable, the investigated and validated low/no copper strategies are reported.

3. Novel low-or no-copper strategies for grapevine

3.1 Copper alternatives and optimized low- or no- copper strategies for grapevine

Field trials were carried out by the RELACS partners on grapevine in order to evaluate four copper alternatives (test products, TPs). Each TP was tested as no copper strategy (stand-alone TP strategy, 0 kg/ha of copper ions per year) or alternated with copper, which was applied at the most susceptible phenological phases with high infection risks (TP_Cu_TP strategy) for a total of 1.3 kg/ha of copper ions per year. In a second season, strategies were further optimised under field conditions and each TP was tested as no copper strategy (stand-alone TP strategy, 0 kg/ha of copper ions per year), alternated with copper (TP_Cu_TP strategy, 1.5 kg/ha of copper ions) or in tank mixture with copper (TP+Cu strategy, 1.0 kg/ha of copper ions per year) in correspondence with the most susceptible phenological phases with high infection risks. Three controls were used: the standard copper treatment (Cu, 4.1 kg/ha of copper ions per year), untreated control plants (UTC) and untreated plants treated with copper only at the most susceptible phenological phases (UTC_Cu_UTC strategy, 1.5 kg/ha of copper ions per year; UTC+Cu strategy, 1.0 kg/ha of copper ions per year).

3.2 On-farm validation of novel low- or no- copper strategies for grapevine

Low/no copper strategies for downy mildew control in grapevine were validated on grapevine in five countries, six different conditions (environmental and production) and in two seasons (Table I).

 Table 1. Overview of the on-farm validation of novel low- or no- copper strategies for grapevine.

| Country | RELACS partner | Prevailing conditions in season I | Prevailing conditions in season 2 |
|------------------------|------------------|--|--|
| Italy | FEM/UNITN | high disease pressure | medium disease pressure |
| (North, wine grape) | | | |
| Switzerland | FIBL | high disease pressure | extremely high disease |
| (wine grape) | | | pressure |
| Italy | IAMB/Federbio | medium disease pressure | no on farm validation was |
| (South, table grape) | | | carried out due to extremely low disease pressure and environmental conditions that precluded <i>P. viticola</i> infections |
| Germany | Naturland | high disease pressure | extremely high disease |
| (wine grape) | | | pressure |
| Hungary | ÖMKI | medium disease pressure | high disease pressure |
| (wine grape) | | | |
| United Kingdom | Soil Association | severe frost damages | severe frost damages |
| (wine grape) | | occurred and the sporadic survivor of plants precluded the planned trials | occurred and the sporadic survivor of plants precluded the planned trials |

Season 1 and season 2 correspond to 2020 and 2021 seasons for all partners with the exception of ÖMKI, which carried out the on-farm validation in 2019 and 2020.

Plant treatments were applied 3-24 h before the probable infections of *Plasmopara. viticola*, predicted according to the weather forecast and a decision supporting system (DSS RIMpro-Plasmopara prediction model). In case of wash-off (rain > 25-30 mm), plants were re-treated. Products were sprayed using the standard procedures and equipment used by growers under commercial production in the respective areas where the trials were carried out. Downy mildew severity and incidence on leaves and bunches were assessed visually. The efficacy against *P. viticola* of the different strategies was compared with the

typical program for organic management applied in the respective country, such as copper strategy with 4 kg/ha of copper ions per year.

All the TPs showed good miscibility and high compatibility in tank mixture with copper and other products for organic grapevine protection. Thus, all TPs can be easily applied with standard procedures and equipment used by growers for commercial production of wine and table grape.

TPs showed no phytotoxic symptoms and no negative effects on grape quality, must fermentation, wine quality and predatory mites, indicating high suitability for grape production. In addition, one TP showed good efficacy against powdery mildew and allowed a significant reduction of sulphur treatments under field conditions, as an added value of the product for organic farming.

Under low disease pressure, three TPs can protect grapevine plants with good efficacy against downy mildew using a stand-alone strategy. However, low dosages of copper should be applied under medium/high disease pressure in order to obtain a good protection level against the disease. More specifically, a strategy with low copper dosages (100-300 g/ha metal ion for each treatment) in tank mixture is suggested under environmental conditions with high disease pressure, in phenological phases with high susceptibility and in the last two treatments of the season. However, proper estimation of the disease pressure could avoid these treatments reducing the amount of copper. Thus, the use of Decision Support Systems is crucial in order to predict the infection pressure and then apply low dosages of copper only when they are really needed.

3.3 Summary of novel low- or no- copper strategies validated for grapevine protection

On-farm validation results showed that a significant reduction of copper in grapevine is possible in different EU countries under different environmental conditions, infection pressure and production practices. No-copper strategies are possible under low disease pressure using stand-alone treatments with the TPs. Moreover, strategies with low copper dosages in tank mixture with one of the three TPs are suggested at the most susceptible phenological phases and/or where high infection risks prevail.

4. Novel low-or no-copper strategies for apple

4.1 Copper alternatives and optimized low- or no- copper strategies for apple

Field trials were carried out by the RELACS partners on apple in order to evaluate four copper alternatives (test products, TPs). Each TP was tested as no copper strategy (stand-alone TP strategy, 0 kg/ha of copper ions per year) or in combination with copper or other fungicides used in organic apple production (strategies). The various low or no copper strategies in apple were described in RELACS Del1.3 and summarized below in table 2.

4.2 Validation of novel low- or no- copper strategies for apple

Low/no copper strategies for apple scab control were validated on different apple cultivars in four countries under varying conditions (environmental and production) and in two seasons (Table 3).

| | | primary season | | | | secondary season | | n | |
|-------------------------|----------|----------------|--------------|-----------|------------|------------------|------------|-----|-------------|
| | pre-b | loom | bloom | | post-bloom | | Summer | | pre-harvest |
| | prev. | cur | prev. | cur | prev. | cur | prev. | cur | prev. |
| stand alone | TP | TP | TP | ТР | TP | TP | TP | TP | TP |
| TP preventive | ТР | cur | TP | cur | TP | cur | TP | cur | ТР |
| TP curative | Cu | TP | S | TP | Cu | TP | Cu | TP | TP |
| TP & lowCu | TP&lowCu | cur | TP (+ S) | cur | TP&lowCu | cur | TP&lowCu | cur | TP&lowCu |
| post-bloom stand alone | Cu | cur | S | cur | TP | TP | TP | TP | TP |
| post-bloom combined | Cu | cur | S | cur | TP | cur | TP | cur | TP |
| post-bloom comb + lowCu | Cu | cur | S | cur | TP&lowCu | cur | P&lowCu | cur | TP&lowCu |
| | | | | | | | | | |
| | | curative | Potassiumbio | carbonate | | | preventive | Cu | Copper |
| | | | Sulphur | | | | | | |
| | | | Lime sulphu | r | | | | | |

Table 2: Application strategies for fungicides in organic apple production

Table 3. Overview of the on-farm validation of novel low- or no- copper strategies for apple.

| Country | RELACS partner | Prevailing conditions in season 2020 | Prevailing conditions in season 2021 |
|--|-----------------------|---|--|
| Germany (cv. Wellant) | ÖON | low/medium disease pressure | |
| Belgium (cv. Novajo) | PCfruit / Bioforum | low/medium | high disease pressure |
| Switzerland (cv. Pinova 2021, Gala 2020) | FIBL | medium/high disease pressure | high disease pressure |
| United Kingdom (cv. Kidd's Orange) | Soil Association | very high disease pressure | |

Test-products were applied preventively before the probable infections of *Venturia inaequalis* predicted according to the weather forecast and a decision supporting system (DSS RIMpro-apple scab model).

For curative applications of test and reference products, plants were re-treated during the germination phase of the scab spores, as indicated by the DSS.

Products were sprayed using the standard procedures and equipment in the respective areas where the trials were carried out. Severity and incidence of scab on leaves and fruit were assessed visually. The efficacy of the different strategies was compared with the typical program for organic management applied in the respective country.

4.3 Summary of novel low- or no- copper strategies validated for apple protection

Within the candidates to replace copper products, TP5 has shown best performance against apple scab in preventive, curative and combined use in different regions and under various conditions. It would be a valuable and welcome addition to the currently available product portfolio in organic apple production. However, new crop protection products alone are not sufficient to avoid or critically reduce copper in organic apple production. Further components are needed, such as intelligent strategies (combinations with other crop protection products), infection forecast for optimized timing of applications as well as cultivars resistant to scab and other diseases.

Nevertheless, results show that a significant reduction of copper in apple is possible and can be achieved in various ways:

Optimised use of copper during primary season for early infections: following the scab model and infection forecast results by treating only when necessary and in combination with curative stop-scab treatments should result in optimal control of scab at the end of the primary season. This reduces the need for further use of copper during the secondary summer season.

Some of the TPs showing slightly lower activity than copper can be applied in tank mixture at the most susceptible phenological phases, where high infection risks prevail. Thus, this strategy allows to maximize product efficacy and minimize the use of copper. However, under high disease pressure and on susceptible cultivars this strategy does not reach the efficacy of standard copper strategies. But even these copper strategies may give only limited efficacy under such extreme conditions.

Scab resistant cultivars are important in an overall scab prevention and copper reduction strategy. They allow a reduction of the number of scab treatments over the complete season. Alternative products developed and evaluated in the current project may further reduce the need for copper use against other apple diseases on these cultivars.

5. Novel low-or no-copper strategies for greenhouse crops

5.1 Copper alternatives and optimized low- or no- copper strategies for greenhouse crops

All four TPs were found to be well compatible with most of the tested registered plant protection products used for cucumber and tomato cultivation. Treatments with the TPs were carried out as no-copper strategies in both crops for evaluation of their potential to control the diseases in comparison to copper.

5.2 On-farm validation of novel low- or no- copper strategies for greenhouse crops

Validation trials for no-copper strategies were performed by three partners in three countries and in two seasons each. In total, six validation trials took place, both in cucumber and tomato. In cucumber, one TP was evaluated in Spain and Germany, while two TPs were evaluated in Italy. In tomato two TPs were assessed in Spain and 3 TPs in Italy and Germany. Table 4 shows the overview of trials performed and the prevailing conditions in the different locations, crops and seasons.

| Country | RELACS | Prevailing conditions in | Prevailing conditions |
|-----------------------------------|---------------|---|--|
| Spain - cucumber - tomato | Ecovalia | very severe disease pressure medium to severe disease pressure | very severe disease pressure medium to severe disease pressure |
| Italy - cucumber - tomato | Federbio/IAMB | low to medium disease pressure low to medium disease pressure | low disease pressure and hot climatic conditions during spring/summer low disease pressure and hot climatic conditions during spring/summer; trial extended to autumn/winter and in progress |
| Germany - cucumber - tomato | Naturland | severe disease pressure but irregular occurrence of <i>P.</i> <i>cubensis</i> in the plots low disease pressure and hot climatic conditions that inhibited infections with <i>P.</i> <i>infestans</i> despite of repeated artificial inoculations | severe disease pressure low disease pressure |

Table 4. Overview of the on-farm validation of novel no- copper strategies for greenhouse crops.

Season I and season 2 correspond to 2020 and 2021 seasons for all partners except for Germany, where in tomato, both trials took place in 2020 (in spring/summer and in autumn).

As no forecast models exist for greenhouse crops, applications were performed weekly with spraying equipment used for greenhouse production of vegetables. Concentrations of the TPs were applied as recommended by the companies providing them.

No phytotoxicity was observed in cucumber in all trials, while in tomato, one TP showed phytotoxic symptoms on leaves in the trial in Germany. However, on different cultivars and under different environmental conditions in the Southern validation sites, no phytotoxicity occurred. The background for the differing observations needs to be further elucidated.

Validation results in tomato

In Germany in tomato, no spread of infestation happened during spring/summer due to extreme hot temperatures. Even repeated artificial inoculations did not result in a spread of late blight in the crop. In a second trial in autumn, the disease reduction of late blight by the validated TP was good, but led however to strong phytotoxic symptoms on leaves.

In Spain in both seasons, under medium to severe disease pressure, both TPs led to a significant reduction of infestation with *P. infestans* on tomato leaves compared to the untreated control. One TP performed as well as copper treatment. Also, both TPs protected tomato fruits from late blight.

In Italy, under low to medium disease pressure, efficacy of two TPs was similar to the efficacy after copper treatment, while one TP was less effective. The trial of season II was extended to continue in autumn and is still in progress, as no disease occurred in the hot and dry spring/summer period.

Validation results in cucumber

In Spain, with extremely severe disease pressure of downy mildew in cucumber, the TP that was tested recorded generally comparable values of severity and efficacy on affected leaf area as did copper treatments (no significant differences) and both variants were significantly different to the untreated control in both seasons.

In Italy, with low to medium disease pressure, only one of the two tested TPs performed better than the untreated control, while the other TP and copper had no effects on disease reduction.

In Germany, in season I the distribution of downy mildew was very irregular and no final conclusions could be drawn for the tested TP. In season II with extremely severe disease pressure, the tested TP performed as good as copper.

5.3 Summary of novel low- or no- copper strategies validated for greenhouse crops

In tomato, two TPs applied as no- copper strategy were able to protect plants from late blight in the same range as copper under medium to severe disease pressure. However, one of these TPs in some cultivars and test sites caused phytotoxic symptoms on leaves. The reasons for this, have to be further clarified.

In cucumber, even under severe disease pressure, one TP applied as no- copper strategy was able to reduce the infection with downy mildew to the same degree as copper.

In greenhouses with advanced technique for management of climatic conditions, treatments against the two diseases are usually not required. For tomato, the strategy of defoliation of lower leaves is a standard measure, which further helps to reduce treatments against late blight. In greenhouses without technique for climate management, treatments are necessary in both crops under conducive infection conditions. Here, the validated TPs developed in RELACS together with farmer's practices are well suited for use in practice and contribute to the reduction of copper use.

6. Novel low-or no-copper strategies for roses

6.1 Copper alternatives and optimized low- or no- copper strategies for roses

Oil bearing roses are a valuable essential oil crop because of the great variety of biochemical components of the essential oil: vitamins, sugars, amino acids, enzymes, essential oil, glycosides, and antibacterial agents. The most common fungal diseases that require PPP treatments to be managed are rust and black leaf spot. Copper-containing preparations are authorized for use in organic plant production, their use should be reduced while disease protection and oil content should be improved.

6.2 On-farm validation of novel low- or no- copper strategies for roses

Treatments with the TPs were carried out as no- copper strategies in roses for the evaluation of their potential to control the diseases in comparison to copper. All four TPs were tested in oil-bearing rose in cooperation with a research institute in Bulgaria in three consecutive years from 2019 to 2021. In different years, different preparations in different combinations and concentrations were used. For spraying, standard electric back sprayers (16 litres) was used.

In 2019, the 4 TPs were applied as stand-alone treatments, compared to a copper reference (Funguran).

In 2020, TP4 was not included in the trial, while a combination of TP1 + sulphur (Cumulus) was tested as tank mixture. This combination showed lower efficacy against rust than the copper reference. At the same time this combination and TP3 as stand-alone treatment resulted in the best yield of rose flowers.

In 2021 two products (TP2 and TP3) were assessed, TP3 was tested in two concentrations. Two of the test plots with TP3 were treated alone before the start of petals harvesting (beginning of May) and were followed by treatment with copper and sulphur after harvest.

6.3 Summary of novel low- or no- copper strategies validated for roses

Overall, results from the field experiments on roses are encouraging. All four TPs had a positive effect on rose oil yield in the 3 different years. Different strategies for replacement or reduction of copper use in organic cultivation of roses can be developed on this basis.



7. Related RELACS publications

Corneo, Paola E., et al. "Foliar and root applications of the rare sugar tagatose control powdery mildew in soilless grown cucumbers." *Crop Protection* 149 (2021): 105753.

Corneo, Paola Elisa, et al. "Interactions of tagatose with the sugar metabolism are responsible for *Phytophthora infestans* growth inhibition." *Microbiological Research* 247 (2021): 126724.

Perazzolli, Michele, et al. "Ecological impact of a rare sugar on grapevine phyllosphere microbial communities." *Microbiological research* 232 (2020): 126387.

Tamm, Lucius, Thuerig, Barbara, et al. "Copper use in organic agriculture in twelve European countries." *AgriRxiv* 2021 (2021): 20210504874.

Report on exploration of low/no copper strategies for current commercial grapevine, apple and glasshouse crops: <u>https://relacs-project.eu/wp-</u> <u>content/uploads/2022/01/RELACS_D1.3_publishable_report-_on_exploration_of_low_no-copper-</u> <u>strategies.pdf</u>

This deliverable is part of an ongoing manuscript for a scientific peer-reviewed publication. It will be submitted to a scientific journal soon (likely submission date is 2023), and, once published (gold open access), will be linked to this deliverable. The details contained in this study are still confidential until online publication by the journal.

Practice abstract:

Copper reduction strategies in viticulture (OMKI)

Videos:

Copper replacement experiment in oil rose plantation (Bioselena): <u>https://www.youtube.com/watch?v=RH-YgFLzkkw</u>

New products against mildew in organic viticulture (FiBL): https://www.youtube.com/watch?v=sxV9VRqqSgE

Copper replacement experiment in vineyard (ÖMKi): https://www.youtube.com/watch?v=A7M5Ry2_xHQ