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# I. Executive summary

The overall aim of WPI is the development of novel copper alternatives and integration in crop protection strategies to replace copper (no copper strategies) or to limit the use of copper (low copper strategies) in organic plant production. The specific objectives related to this Deliverable are to develop copper alternatives according to the companies involved in the project and based on this, to develop strategies to reduce copper use under field conditions on crops with high relevance, in particular grapevine, apple and glasshouse crops (e.g. tomato, cucurbits) as well as on minor crops such as roses and others. Four copper alternatives (test product, TP) were provided by the RELACS partners (named TPI, TP2, TP3 and TP4), and a product from outside RELACS was tested against apple scab (TP5).

## Low/no copper strategies in grape vine:

Results showed that a significant reduction of copper in grapevine is possible and best results were obtained when copper and TPs are applied in tank mixture at the most susceptible phenological phases, where high infection risks prevail. Thus, this strategy (TP+Cu strategy) will be further evaluated on farm in order to maximise product efficacy and minimise the use of copper.

#### Low/no copper strategies in apple:

Results showed that a significant reduction of copper in apple is possible and best when copper is used only in primary season of apple growing. During that time, the application of copper can be optimally timed by strictly following infection forecast from decision support systems, therefore only treating when necessary.

TPs should be applied in tank mixture at the most susceptible phenological phases, where high infection risks prevail. Thus, this strategy (TP+Cu strategy) will be further evaluated on farm in order to maximise product efficacy and minimise the use of copper.

## Low/no copper strategies in vegetables:

For cucumber, mainly TP2 was selected for on-farm trials as stand-alone treatment. For tomato, TP2 and TP3 were selected. In on-farm trials the potential of these TPs should be evaluated in order to reveal their copper replacement potential under practice conditions.

## Low/no copper strategies in minor use specialty crops:

For roses results were 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.

For raspberries final conclusions cannot yet be drawn.

#### 2. Introduction

The overall aim of WPI is the development of novel copper alternatives and integration in crop protection strategies to replace copper (no copper strategies) or to limit the use of copper (low copper strategies) in organic plant production. The specific objectives related to this Deliverable are to develop copper alternatives according to the companies involved in the project and based on this, to develop



strategies to reduce copper use under field conditions on crops with high relevance, in particular grapevine, apple and glasshouse crops (e.g. tomato, cucurbits) as well as on minor crops such as roses and others. Four copper alternatives (test product, TP) were provided by the RELACS partners (named TPI, 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 I.2). These alternatives were then applied under field conditions (Tasks I.3) and application strategies were optimised, in order to minimise the use of copper according to phenological stages and infection pressure. Field trials were carried out in all crops in two consecutive seasons by the RELACS partners FIBL, FEM/UNITN, JKI and Bioselena. Field experiments were carried out on grape/downy mildew, apple/apple scab, cucumber/downy mildew, tomato/late blight, roses/rust and black leaf spot and raspberries/Didymella and anthracnosis (Task I.3), in order to develop at least one efficient low/no copper strategy for each pathosystem to be further validated on-farm in Task I.4.

# 3. Low/no copper strategies for grapevine

#### 3. I Material and Methods

Field trial was carried out by FEM/UNITN on grapevine in order to evaluate four copper alternatives. During the 2019 season (Figure 1A), each copper alternative (test product, 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 (Thuerig et al., 2018). As control, strategies were compared to 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 with high infection risks (UTC\_Cu\_UTC strategy, 1.3 kg/ha of copper ions per year).

During the 2020 growing season, strategies were slightly optimised according to the results of the 2019 seasons and they were further validated under field conditions (Figure 1B). In particular, each TP was tested as no copper strategy (stand-alone TP strategy, 0 kg/ha of copper ions per year) or alternated with copper (TP\_Cu\_TP strategy, 1.5 kg/ha of copper ions) and in tank mixture with copper (TP+Cu strategy, 1.0 kg/ha of copper ions per year) in correspondence of the most susceptible phenological phases with high infection risks. As control, 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 were used (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).

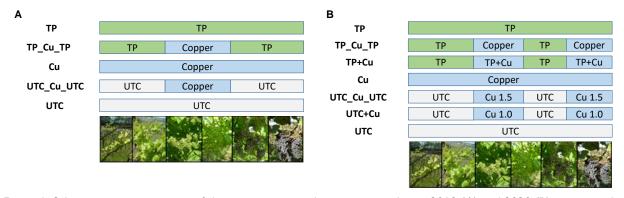


Figure 1. Schematic representation of the strategies tested on grapevine during 2019 (A) and 2020 (B) season with test products (TP) as copper alternatives applied as no copper strategy (stand-alone strategy; TP) or low copper strategy: alternated with copper (TP\_Cu\_TP) or in tank mixture with copper (TP+Cu).

Treatments were applied 3-24 h before the probable infections of *Plasmopara viticola*, according to weather forecast and DSS RIMpro-Plasmopara prediction model (http://www.rimpro.be/PlasmoparaWeb/) and plants were re-treated in case of wash-off (rain > 25-30 mm). Products were sprayed with a motorised backpack mistblower. Downy mildew severity and incidence on leaves and bunches were assessed visually every 7-12 days according to the guidelines of the European and Mediterranean Plant Protection Organization (EPPO 2001). The efficacy against *P. viticola* of the different products (%) and the Area Under the Disease Progress Curve (AUDPC) values on leaves over time were calculated.

The side effects on the number of predatory mites, grape quality, must vinification and phytotoxicity on leaves and bunches (EPPO 2014) were monitored.

# 3.2 Results

In the 2019 season, grapevine downy mildew showed low infestations, while in the 2020 season caused serious problems due to the high infection pressure at FEM/UNITN.

In both seasons, the AUDPC on grapevine leaves was significantly reduced by the low copper strategies for three products (TP2, TP3, TP4). The low copper strategies (TP\_Cu\_TP and TP+Cu) showed better results compared to their respective controls (UTC\_Cu\_UTC and UTC+Cu) with a disease reduction comparable to the standard copper treatment (Cu), particularly in the case of TP2, TP3 and TP4. Interestingly, the no copper strategies (stand-alone TP strategy) sufficiently reduced the AUDPC on leaves in the case of TP3 and TP4 in both seasons with an efficacy comparable to Cu.

At the last assessment of the 2019 season at FEM/UNITN, the disease severity on bunches and leaves was reduced by the no copper strategy (stand-alone TP strategy) and low copper strategy (TP\_Cu\_TP) of TP2, TP3 and TP4 with an efficacy comparable to the standard copper treatment (Cu). Although the 2020 season was characterised by high infection pressure, the disease severity on bunches and leaves was reduced by the low copper strategies (TP\_Cu\_TP and TP+Cu) of TP2, TP3 and TP4 with an efficacy comparable to the standard copper treatment (Cu). The no copper strategy (stand-alone TP strategy) of TP2, TP3 and TP4 provided a sufficient protection, particularly on bunches.



TP treatments generally presented no severe phytotoxic effect, but some TP tended to partially remove natural berry's waxes or to cause slight phytotoxicity on berries. However, the low copper strategies attenuated the phytotoxic effects of all the TPs. The shoot growth, number of *Phytoseiidae* mites, grape quality and must fermentation were not negatively affected by the TPs and data of the low/no copper strategies were comparable to the Cu strategy in both seasons.

Compared to the full copper application (4.1 kg/ha of copper ions per year) the use of TPs allowed a consistent reduction of copper use while giving good grapevine protection against downy mildew. Reductions down to 1.0 and 1.5 kg/ha of copper ions per year were achieved when copper was applied in tank mixture (TP+Cu strategy) and as alternated strategy (TP\_Cu\_TP strategy) with TPs, sprayed at the most susceptible phenological phases, respectively. Moreover, a sufficient grapevine protection was even obtained with the no copper strategy of TP2, TP3 and TP4 (stand-alone TP strategy).

Results show that a significant reduction of copper in grapevine is possible and best results were obtained when copper and TPs are applied in tank mixture at the most susceptible phenological phases, where high infection risks prevail. Thus, this strategy (TP+Cu strategy) will be further evaluated on farm in order to maximise product efficacy and minimise the use of copper.

# 4. Low/no copper strategies for apple

#### 4.1 Introduction

Efficient crop protection strategies to prevent from apple scab and other leaf and fruit diseases in organic apple production relay mainly on three actives, namely copper, sulphur and potassium bicarbonate. In apple, copper is usually used early in the season to prevent from apple scab and during summer, against scab and other leaf and fruit diseases. National limits for copper use per hectare and year vary between zero and six kg.

Crop protection in apple can be divided into three phases: primary (scab) season, secondary (scab) season and storage period. During the primary season, starting with bud break in early spring, apple scab is of highest importance, during bloom time fire blight may additionally cause serious problems. Besides apple scab, further leaf diseases may cause damage during secondary (summer) season, but the main focus then is on fruit health. Fruit scab, bitter rot and sooty blotch are the challenging diseases then. During storage, Neofabrea and other fruit rots may cause loss of harvest.

During the primary season of apple scab, starting with bud break in early spring up to May, infections are mainly caused by overwintering ascospores of apple scab, which are actively ejected during rainy periods. Prevention of these early infections is the key issue for a season long control of scab in an orchard. Once scab has established on the leaves it produces conidia for further multiplication during the rest of the season.

Successful fungicide strategies in organic production comprise protective applications of copper, sulphur or clay mineral products or timely targeted applications of sulphur or potassium bicarbonate products during the germination phase of the scab spores ("curative" or "RIMstop" treatments", optimally 5 to 8 hours after a rain event with ascospore discharge) or a combination of preventive and curative. While



copper is very efficient when applied preventively, potassium bicarbonates or lime sulphur show excellent efficacy when used curatively.

Within these strategies, copper is used early in the season and during summer as a preventive treatment in organic apple production. For a replacement of copper during the primary season, products with good preventive activity are needed. For a replacement of copper during the summer season, products with activity against scab as well as against further summer and storage diseases are needed.

## 4.2 Proposals for low/no copper strategies in apple (to be tested in Task 1.4)

Based on the above general framework, it is therefore reasonable / recommended to test the RELACS products first during the primary season in combination with curative treatments and in a second step to test them during the summer season to assess their effect against further diseases.

As there is limited data available about the efficacy of TPI and TP4 against scab and other diseases under field conditions, stand-alone variants need to be included in field trials of the RELACS project. The following table gives an overview on combined use strategies for the new products.

primary season secondary season pre-bloom post-bloom bloom Summer pre-harvest prev prev cur prev prev prev stand alone TP ΤP TP TP ΤP TP TP TP TP TP preventive ΤP TP TP TP TP cur cur cur cur TP curative ΤP S ΤP ΤP ΤP ΤP Cu Cu Cu TP&lowCu TP & lowCu TP&lowCu TP (+ S) TP&lowCu TP&lowCu cur cur cur cur S ΤP ΤP ΤP ΤP ΤP post-bloom stand alone Cu cur cur post-bloom combined S TP ΤP TP Cu cur cur cur cur post-bloom comb + lowCu S Cu cur cur ΓΡ&lowCu cur TP&lowCu cur TP&lowCu preventive Cu curative Potassiumbicarbonate Copper

Table 1: Application strategies for fungicides in organic apple production

Results show that a significant reduction of copper in apple is possible and best when copper is used only in primary season of apple growing. During that time, the application of copper can be optimally timed by strictly following infection forecast from decision support systems, therefore only treating when necessary.

Sulphur Lime sulphur

TPs are applied in tank mixture at the most susceptible phenological phases, where high infection risks prevail. Thus, this strategy (TP+Cu strategy) will be further evaluated on farm in order to maximise product efficacy and minimise the use of copper.



# 5. Low/no copper strategies for greenhouse crops

#### 5.1 Material and Methods

In cooperation with the State Horticultural College and Research Institute (LVG) in Heidelberg, Germany, greenhouse trials of cucumbers and tomatoes took place with JKI in 2018 to 2020 in which the performance of the 4 TPs was tested.

The cvs. used were `DeeLite´ FI (rootstock `Cobalt ´FI) for cucumber and `Roterno´ FI (rootstock `Emperador´ FI) for tomato. In 2018 and 2019, cultivations of cucumbers were carried out, while trials in tomato took place in 2019 and 2020. The greenhouse trials were performed following modified protocols by Scherf et al. (2010). Figure 2 shows the trials sites for tomato and cucumber 2019.



Figure 2: Greenhouse with a) tomato cultivation and

b) cucumber cultivation

Each trial consisted of 4 replicates of every treatment variant with 10-12 plants per replicate in a double row, distributed in a randomized design. A border row was planted on both sides of the trial and border plants were situated between the plots. An untreated variant was used as control and for comparison one variant was treated with copper (Cuprozin progress, in both tomato trials and 2019 in cucumber) or Elot-Vis (plant strengthener, 2018 in cucumber). In 2020 in tomato, defoliation was included as second control as this is a common measure in commercial tomato cultivation. In 2020, the TPs I to 3 were investigated. Artificial inoculation was performed when plants had reached a height of about 2m. In both years, one leaf of each plant was infected in ca. 70cm height with sporangia suspensions of *Pseudoperonospora cubensis*. and *Phytophthora infestans* with at least IxI0<sup>4</sup>-I,5xI0<sup>4</sup> sp./ml. In every trial artificial inoculations took place I day after the first treatment with TPs. In 2020, due to high temperatures, further inoculations took place bi-weekly, each time on a different leaf.

Treatments with the TPs were carried out as stand-alone treatments in order to evaluate their potential to control the diseases in comparison to copper. As no forecast models exist for greenhouse crops, applications were performed weekly with a motorised backpack sprayer with the recommended concentrations.



In 2018, border rows were kept untreated to facilitate spread of infection. Due to the fact that as a result in the cucumber trial 2018 the neighbouring rows proved to be more infested than rows in the middle of the site, in 2019 and 2020, treatments of the cucumber and tomato border rows with Cuprozin progress were performed weekly along with the other treatments,

Originating from the infected leaf, 7 leaves upwards were evaluated in both crops. Ratings of disease progression were made weekly (cucumber) or two-weekly (tomato) and began with appearance of first symptoms of downy mildew or late blight (I-2 weeks after inoculation). Table 2 gives the general trial design in the greenhouse.

Table 2: Number (=n) of rated plants (4 replications/plots per variant) in greenhouse trials 2018, 2019 and 2020

	Cucumber 2018	Cucumber 2019	Tomato 2019	Tomato 2020
Plants per plot	10	12	12	10
Rated plants per plot	6	6	8	6
Total # plants rated per variant	24	24	32	24

During the cultivation periods, cucumbers and tomatoes were treated with parasitoids and NeemAzal against pests and Kumar against powdery mildew as needed. All treatments were done separately to the TP application. Cucumber cultivations had to be ended because of excessive infestation with downy mildew (2019) and/or spider mites (2018). The tomato cultivation 2019 had to be terminated due to severe infestation with rust mites. In 2020 infestation of tomatoes with rust mites was moderate and the trial ended as planned.

Within these periods, 8 disease ratings were performed in cucumbers, both in 2018 and 2019, and 6 ratings in tomatoes 2019 and 2020. Cucumber and tomato fruits were harvested twice to three times per week throughout the trial periods. Fruits were analysed for number and weight as well as for disease symptoms and deformations due to insect attacks and finally the amount of marketable and non-marketable yield was calculated.

#### 5.2 Results

#### 5.2.1 Cucumbers

In 2018, the infestation of the cucumber plants with downy mildew was very low. Statistical analysis did not show significant differences among the treatments and pointed to a slightly higher impact of localisation within the greenhouse. This result can be explained by the extraordinary dry and hot season of 2018 with very bad conditions for growth of *P. cubensis*. Infestation and spreading of spider mites and aphids affected the variants to a high degree.

In 2019 the cucumber plants showed severe infestation with downy mildew according to the more humid weather conditions. Best protective action was found for treatments with TP2, while the other three pilot products performed less good. Disease development in Cuprozin progress treated plots was similar



to that in control plots. This was most likely due to the relatively low amount of copper applied, in total I.8 kg/ha metal copper. Statistical analysis showed only for TP2 a significant protective effect.

Based on the results obtained here, TP2 was the main TP selected for on farm trials as stand-alone treatment. On farm trials were used to evaluate the potential of different concentrations of TP2 in order to reveal the copper replacement potential of this alternative under practice conditions.

#### 5.2.2 Tomatoes

In 2019, the disease severity of late blight in the tomato cultivation was low. The reasons were too warm and dry weather conditions for effective development of *P. infestans*. Because of the overall low infection, treatments with the pilot products did not show significantly distinguishable results but pointed to a slightly better efficacy for TP2, TP4 and TP1, while TP3 showed phytotoxic effects on the given cultivar.

Statistical analysis for the disease severity and the tomato yield showed no significant differences in the treatment variants.

In 2020 the efficacies of three alternative products (TPI, TP2, TP3) were evaluated. In spite of repeated inoculations, infestation with late blight did not develop well due to extremely hot and dry weather conditions. Late blight mostly occurred in inoculated leaves. The progressions of the disease did not differ significantly between the test product variants and the controls. Treatment with TP3 again resulted in phytotoxic symptoms on the cultivar used.

Analysis of the tomato yield also showed no significant differences between treatment variants. The different treatments had no negative impact on the crop yield.

As results from these trials were not pointing to a clear preference for a best performing TP, for on-farm trials, TPs were chosen according to the companies' preferences (TP2 and TP3). TP3 was of special interest, as in a first on-farm trial with a different tomato cultivar, it did not show any phytotoxic symptoms. Both TPs used as stand-alone performed well under strong disease pressure under practice conditions tested in Task 1.4.

# 6. Low/no copper strategies for roses and raspberries

#### 6.1 Introduction

All four TPs were tested in two minor crops - raspberries and oil-bearing rose in cooperation with two research institutes in Bulgaria. In the case of raspberries, the experiments were conducted in two consecutive years: 2019 and 2020. In the case of the oil-bearing rose - in three consecutive years from 2019 to 2021. In different years and in the different crops different preparations in different combinations and concentrations were used. For spraying standard electric back sprayers (16 litres) was used.

#### 6.2 Raspberries

The experiments with raspberries were conducted at the Institute of Mountain Agriculture and Animal Husbandry - Troyan. In 2019 three TPs (TP2, 3 and 4) were tested. In 2020 TPI to TP3 were tested. In



both years TPs were applied as stand-alone treatments, using copper (Funguran) as reference. As organic fertilizer vermicompost was applied. Other PPP like insecticides were not needed.

Results on raspberries gained in the two years were not enough to draw final conclusions. It would be good to continue the testing with other raspberry varieties (remontant raspberry) and to include different strategies besides the stand-alone treatments.

#### 6.3 Roses

In roses, in 2019 the 4 TPs were applied as stand-alone treatments, with 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. In 2021 two products (TP2 and TP3) were assessed, as 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. In the field trials several times organic fertiliser (foliar application) was applied and once an organic insecticide (Neem Azal) against aphids. All the treatments were made alone (not as tank-mix with the TPs). There was no negative effect observed on the plants or on the performance of the TPs.

The combination TPI + sulphur was applied in 2020 as a tank mixture, which showed lower efficacy against rust than the copper reference. At the same time this combination and TP3 as stand-alone treatment showed best yield of rose flowers.

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.



## 4 Dissemination activities related with the Deliverable

- I. Discussion and workshop with stakeholders on Replacement of Copper in Viticulture at the annual event 'Strategie innovative per la riduzione del rame in viticoltura' 'Strategie innovative per la riduzione del rame in viticoltura' (Italian), Giovannini O., Nadalini S., Pertot I., Perazzolli M., 22 January 2020, San Michele all'Adige, Trento, Italy (https://www.fmach.it/CRI/info-generali/comunicazione/eventi-CRI/Controllo-di-insetti-e-microrganismi-dannosi-e-fertilizzazione-studi).
- 2. Discussion and workshop on Replacement of Copper in Viticulture at the annual event 'Presentazione prove sperimentali in viticoltura biologica 2020' (Italian), Giovannini O., Nadalini S., Pertot I., Perazzolli M., 6 August 2020, San Michele all'Adige, Trento, Italy (https://www.youtube.com/watch?v=qL\_3nrn8IRQ).
- 3. Dissemination event on Replacement of Copper in Viticulture at open day of Fondazione Mach 'Presentazione prove sperimentali in viticoltura' (Italian), Giovannini O., Nadalini S., Pertot I., Perazzolli M., 10 November 2019, San Michele all'Adige, Trento, Italy (https://www.fmach.it/Comunicazione/Eventi/Porte-aperte-per-il-145-FEM).
- 4. Technical presentation at RELACS National Workshop of copper alternative organized by FEDERBIO 'Workshop progetto RELACS Alternative al Rame' (Italian). Webinar. Giovannini O., 20 May 2021
- 5. (22 July 2020) Raspberries field research Bioselena website





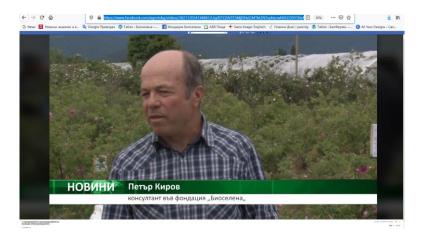
#### **Facebook**

6. (25 April 2020) – <u>Facebook post</u>



#### **MEDIA**

7. (9 June 2020) Agro TV





8. (24 July 2020) Agrovestnik





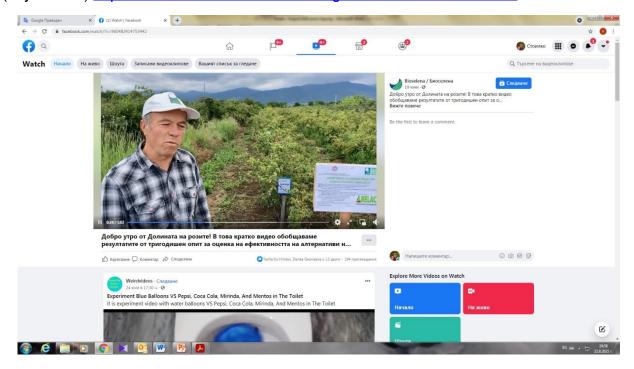
# 9. (22 July 2020) NivaBG

 $\frac{https://nivabg.com/alternativni-preparati-za-otglezhdane-na-malini-predstaviat-ot-bioselena-na-demonstrativno-pole/\\$ 





10. (19 June 2021) https://www.facebook.com/bioselena.bulgaria/videos/960482414753442



## 11. (24 August 2021) Bioselena Facebook

https://www.facebook.com/bioselena.bulgaria/videos/528932241747640



12 Field day and workshop with wine growers within project 'ProBio' supported by Bio Suisse. 'Strategien für die Kupferreduktion im Bioweinbau.' (Strategies for copper reduction in organic wine growing). Hansjakob Schärer. 20 August 2021, Landquart, Switzerland



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