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Pathways to phase-out contentious inputs from organic agriculture in Europe

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Evaluation of alternatives and cost/benefit analysis

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Table of contents

List of Tables

1. Summary

This deliverable evaluates the cost/benefit status of the agroecological alternatives which are based on system approach and novel bio control agents, preparations, fertilisers and soil conditioners of copper, sulphate and mineral oil against Mediterranean horticultural crops' selected diseases. The annual crops are grown largely in greenhouses and the perennials are widely produced fruit trees. The targeted annual varieties were selected mainly from the *Solanaceae* family (potato, aubergine, tomato) and the permanent plants are olives and citrus.

INRAE and MAF aimed to demonstrate advantages in "disease resistance" of both the bred varieties (for potato late blight caused by *Phytophthora infestans* at INRAE) and the landraces (for aubergine early blight caused by *Alternaria solani* at MAF). The reactions of the resistant plants to the diseases were found apparently successful to demonstrate their benefits. However, although potato resistant variety use is important, it has to be combined with other strategies. In a dry and hot year no *Phytophthora infestans* control was required and the potato crop suffered from other environmental stresses. In tomato greenhouses, UTH and IFAPA investigated innovative digital monitoring of a Decision Support System (DSS) developed by University of Thessaly that predicts *Botrytis* disease. The DSS system paved the way for producers to predict the steps of *Botrytis* management.

UNICT in citrus (*Colletotrichum gloeosporioides*, *Alternaria alternata*, and *Pseudomonas syringae*) and IFAPA, MAF, UTH in olive leaf spot disease *(Spilocae oleaginaea)* found cost effective and disease efficient bio control agents, preparations, fertilisers and soil conditioners (home-made and commercial) in replicated trials and collaboration with organic grower NGO's as well as adviser and technical dissemination representatives. The tested alternatives' efficacy was found to be up to 50% as compared with the control (copper oxychloride), which attracted the organic sector community. The costs of these alternative preparations changed depending on the country and location.

However, in Turkey it could not be predicted exactly because of the fast fluctuating prices. Furthermore, highly effective alternatives such as a mixture of Ca and Si, kelp extract and amino acids (foliar), potassium bicarbonate, potassium silicate ($KSiO_3$) and vermicompost tea were found acceptable in terms of their costs for organic farmers.

In citrus, commercial products (fertilisers with low or no-copper content, essential oils, vegetable extracts, plant defence stimulators, basic products and biological control agents) were found to be promising.

2. Potatoes system approach

2.1 Results from alternatives evaluation in France-INRAE

2.1.1 Alternatives evaluated in lab and field trials

At INRAE, the field demonstration trials set up in 2021, and again in 2022, were based on the use of potato cultivars with different features contributing to their resistance to late blight (caused by *Phytophthora infestans*), which is the main target for copper application in organic potato production. Among these features are constitutive resistance genes (essentially major resistance genes, causing hypersensitivity reactions upon challenge by a virulent *P. infestans* strains) and unfavourable plant and canopy architecture (taller, more open foliage to limit the length of foliage wetness). These trials involved large plots (0.5 ha in tilled or no-till conditions), close to farming practice.

In earlier trials, we also tried other combinations of control means linked to the plant, notably quantitative host resistance, architecture, intercropping and biocontrol products. The results of these trials have already been published (Menil et al., 2019)

Trials in Denmark and Sweden also mobilised other control means besides resistance, namely the use of a Decision Support System (Blight Manager) and some applications of biocontrol products of natural origins (please see Deliverable 3.8).

2.1.2 Results of alternatives evaluation

In 2021, despite climatic conditions very favourable to the disease, late blight was observed only late in the season in the two resistant cultivars used in the French trial (see please see Deliverable 3.8 for details).

No pesticide was applied to control late blight, which resulted in some tuber infections in both cultivars. However, infected tubers were discarded directly during harvest. The marketable yields obtained from both cultivars were close to 50 t/ha (tonnes per hectare).

The same trial is conducted again in 2022, with the same design and cultivars. The climate was highly unfavourable to late blight development, with a summer unusually hot (max temperatures reaching over 35 °C in July and August) and dry (no rainfall during July and the first half of August). Therefore, no late blight attacks were observed, and no spray was applied against this disease.

2.1.3 Cost/benefit analysis of the alternatives evaluated

We used standard certified seed potatoes of the recently registered cultivars ‘Cephora’ and ‘Azilis’ for the trial. The cost of the seed was therefore comparable to that incurred when using certified seed of any recent, protected cultivar. The fact that these two cultivars were resistant to late blight did not generate a premium price.

The high level of performance of the resistance in our trial site meant that we were able to conduct the whole cropping process without any fungicide application. This saved both the costs of the copper normally applied to susceptible stands, the costs of the application (tractor/sprayer time and energy) and the time needed to make these applications. As the trial was a demonstration, we had no ‘control’ plots to directly compare to. We do, however estimate that given the highly conducive climatic conditions and high resulting blight pressure in 2021, susceptible stands would have needed full copper coverage (4 kg ha^{-1}, usually split into about 8 applications) to produce a crop. Each application costing *ca*. 60-80 € ha^{-1}, the savings in pesticides can thus be estimated at about 500 to 650 € ha^{-1}.

There were no extra costs involved, the grading/sorting of damaged tubers having been done directly during harvest. Although tubers were stored for several weeks before a new disease scoring, no significant losses to late blight during storage were recorded. The 2022 trials are not harvested yet, so no information on grading/sorting and yield are available at the time of writing. Final result of this year and previous will be published in a peer-reviewed scientific publication.

The former trials were not well suited to assess economic performances, as they were carried out on small plots and with dedicated equipment. It is thus difficult to extrapolate from such trials a cost/benefit analysis relevant to farm operation.

2.1.4 Concluding remarks

Switching from a late-blight susceptible to a late-blight resistant cultivar proved **suitable to avoid copper use** in the demonstration trial carried out in Le Rheu in 2021, despite the yearly climate being favourable to the disease. This switch did **not generate additional production costs** compared to a system using certified seed of a susceptible, protected cultivar. In fact, cost savings in pesticides are estimated at about 500 € ha^{-1}, however this is an annual result, data from a second trial in 2022 will be useful to validate these conclusions.

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3. Greenhouse crops

3.1 Results from field evaluation in Greece-UTH

3.1.1 Alternatives evaluated in lab and field trials

UTH developed and assessed a preventive farm management practice for the control of *Botrytis* disease based on a Decision Support System (DSS) that predicts in advance the risk of development of the disease *Botrytis* and assists growers in deciding when to proceed to a certain action in order to modify the conditions that favour the development of the disease.

The validation of the DSS was performed at the greenhouse facilities of UTH, Thessaly, Greece on greenhouse hydroponic tomato crops (non-organic) and at the greenhouse facilities of the IFAPA – Centro La Mojonera, Almeria, Spain, in a typical organic soil-based greenhouse cultivation system. Both systems were using the same identical DSS system developed by the University of Thessaly.

The DSS is based on disease models already available in the literature that correlate the rate of disease development to crop microclimate conditions and cultivation practices. The DSS makes use of the outside climate forecast for the region to predict the greenhouse microclimate conditions during a set of days that will follow by means of the greenhouse energy and vapour balance, the greenhouse control concept and methodology, the climate control equipment and the greenhouse climate set points. Then, based on the predicted microclimate inside the greenhouse, the *Botrytis* disease development models are used to assess the potential risk of disease development in a greenhouse tomato crop in the subsequent days. The last part of the DSS contains a tactical and strategic tool, that according to the risk assessment, suggests climate control actions to prevent fungi development for preventive and optimal disease control.

3.1.2 Results of alternatives evaluation

The validation of the DSS tool was based on the comparison of the predicted and actual microclimate and crop data observed outside and inside the greenhouse.

The validation of the outside weather forecast showed that the forecast predictions were in strong agreement both at level and time course with the measured air temperature as well as air relative humidity values outside of the greenhouse. The largest difference between forecasted and measured air temperature and relative humidity values outside the greenhouse, 1.7°C and 18%, respectively, were observed mainly during periods with rapid change of the climate variables (early morning and late afternoon). Concerning the solar radiation, the forecast values were in very good agreement with those measured, with few differences observed during the midday of partially cloudy days. In relation to the wind speed values, the forecast was also able to predict the time course of measured wind speed, but the level of the forecasted values was frequently much higher than the measured one. This could be due to the fact that the predicted values correspond to the level of 10 m height and no adjustment was made to the level of the greenhouse microclimate station. In addition, the forecast cannot currently not take into account the detailed morphology of the location, while the observed values may be affected by the surrounding trees and buildings. Further research might be able to adjust for this, too.

The validation of the greenhouse microclimate model showed that the model can fairly well simulate both the level and time course of the air and crop temperature as well as the air relative humidity values inside the greenhouse. The greatest differences between the simulated and measured values were observed during the night.

The values estimated for the three disease risk indices (‘*spore production density*’, ‘*Botrytis Development Rate’* and ‘*Probability of infection*’) seemed to have a clear correlation with the greenhouse microclimate data measured or estimated inside the greenhouse. The predicted length of the period that each of the crop and microclimate parameters inside the greenhouse remain under the different risk levels during the upcoming five days gives the possibility to the grower to decide when to act while the DSS offers a set of solutions among which a grower can choose in order to avoid the risk of potential disease development.

The DSS was used in Greece to assist greenhouse management during two cultivation periods between 2020-2021. During both periods the climatic conditions inside the greenhouse were not so favourable for the development of the disease. Thus, only a **few applications were made in the control** greenhouse compartments while **the use of the DSS led to the elimination of plant protection products** applications for *Botrytis* control. The marketable yield was similar in both greenhouses, not affected by the preventive farm management methods suggested by the DSS.

3.1.3 Cost/benefit analysis of the alternatives evaluated

In the conventional greenhouse compartment, 3 spraying applications related to *Botrytis* control were performed from February to April 2021, and 2 from October to December 2021. At the greenhouse compartment where the alternative solution was tested, the prevention of *Botrytis* disease was based on the greenhouse management actions suggested by the DSS. Actions included the reduction of greenhouse air relative humidity by means of (a) ventilation, (b) dehumidification system or c) by increasing the temperature of the heating pipes. On some occasions, the DSS proposed actions to avoid free water formation on plant tissues, such as the use of energy screens to prevent loss of heat through radiation from the plants during the night, heating after sunrise when cold nights were forecasted and heating early in the morning before dawn – with a step wise increase of air temperature of not more than 1^{o}C per hour.

The cost of spraying applications (plant protection product + labour) was estimated about 0.17 € m^{-2} that is in total about 0.5 € m^{-2}. The total cost of the preventive climate management (dehumidification by parallel heating and ventilation, increase of air temp set point) suggested by the DSS was estimated to be **about 0.45 € m^{-2}** . This was slightly lower than the total cost of spraying applications. Nevertheless, although the DSS application could not reduce the production cost, it aided to eliminate all the plant protection products applications. The trials were performed in greenhouse compartments of about 250 m^2 each that means that were relatively small and not well suited to assess economic performances. It is thus difficult to generalise the results from such trials and perform a wider cost/benefit analysis. The fact that all plant protection products could be eliminated with no extra cost is however relevant for organic certification (the trial was done in a non-organic greenhouse). Such a certification system rewards zero plant protection with the benefit of a potentially higher price (the organic premium) for the grower and therefore the cost/benefit could increase if a premium price is achieved, which is not always the case.

3.1.4 Concluding remarks

The Decision Support System (DSS) developed under T3.4 was evaluated in a tomato and cucumber greenhouse in Greece (UTH) and in a tomato greenhouse in Spain (IFAPA). In Greece, two greenhouse compartments were used: in the one, used as a control treatment, the greenhouse management practices were similar to those commonly applied by the growers of the region, while in the second one, the greenhouse management was supported by the DSS.

Based on the risk predicted by the DSS, the *Botrytis* disease development models were used to assess the potential risk for disease development in the greenhouse. The efficiency of the model to predict the greenhouse microclimate conditions related to the development of the disease was fairly good validated. The validation of the system was further supported by evaluating its reliability in predicting the occurrence the Risk Levels of the greenhouse microclimate parameters related to *Botrytis* epidemiology and the prediction rate observed was above 80% for most of the parameters studied. Although the disease pressure during the period of measurements was low, it was found that the use of the DSS could eliminate the need for *Botrytis* related plant protection products.

3.2 Results from field evaluation in Spain-IFAPA

3.2.1 Alternatives evaluated in lab and field trials

Available formulations of alternative substitutes for copper were the subject of study in this research.

For the *in vitro* tests, eleven products were evaluated. The products were differentiated in 4 groups according to their nature:

1. Mineral fertilisers with low or no-copper content (3 products): Copper gluconate 5%, Zinc 6%, Silicon 23%,
2. Basic substances (3 products): *Equisetum* (2 formulations), Chytosan,
3. Plant extracts (4 products): *Cinnamon* (2 formulations), *Mimosa*, *Camelia,*
4. Registered Fungicides (2 products): Potassium hydrogen carbonate (85%) (PHC), Lime sulphur (18.5%).

The efficacy of products was tested at 2 concentrations (minimum and maximum recommended by the distributor) against two isolates of *Botrytis cinerea* (vegetative and sporulative isolates).

Based on their efficacy in the *in vitro* tests, two alternatives were taken into consideration for the scenario evaluated in the field trials, and were compared with the copper reference:

1-Copper oxychloride: applied at 400 g/hL

2-Cinnammon extract formulation 2 (Cinna, HORTALAN): applied at 200 mL/hL

3-Potassium hydrogen carbonate 85% (Armicarb, CERTIS): applied at 300 g/hL

In the field trials, two different tomato types (‘Valenciano’ type and ‘Branched’ type) were evaluated for their susceptibility to *B. cinerea*, and two different techniques for pruning were assessed (petioles cuttings with a length of 3-10 cm, vs flush cuttings).

3.2.2 Results of alternatives evaluation

In the *in vitro* trials, the products showing fungicidal effect were PHC (Potassium hydrogen carbonate) and Cinnamon extracts. The control practice used as reference (copper oxychloride 50%) did not show fungicidal effects to *Botrytis*, which means that PHC and Cinnamon extracts were better at controlling (*in vitro*) the pathogen. Of the other products tested, Chytosan, and Equisetum extracts were not good *Botrytis* inhibitors; this is not strange, as they are supposed to be plant defence stimulators, but at least we could conclude that they are not fungicide nor good fungistatic. A similar conclusion can be obtained from the other plant extracts tested (Mimosa and Camelia). Only one mineral fertiliser showed interesting efficacies for *B. cinerea*: Gluconic copper, this is a good alternative to higher-content copper formulations, but it does still contain copper. The Zinc product did not show an effect on *Botrytis*, and the Silicon product showed only partial fungistatic effects at the maximum dose. Lime sulphur expressed a very good fungistatic (but not fungicide) action, better than copper oxychloride, for *B. cinerea*.

Concerning the field trials, focusing on the parameters linked with the dissemination of *Botrytis*, despite the absence of significant differences in three of four trials arranged, there was a trend that was repeated in all of them: a lower efficacy of the cinnamon extract to control the expansion of *Botrytis* in the greenhouses. This result was more evident in the first campaign for one of the greenhouses of the trials (GH3). In this trial, the best treatment for controlling the disease was PHC, improving the results of copper oxychloride. In view of our results in the first campaign, we decided to apply cinnamon extract at a higher dose: 3 mL/L, but after a first application we returned to the lower concentration, as we found spotty phytotoxic symptoms on leaves where cinnamon extract was applied at 3 mL/L. Concerning PHC, we used the lowest recommended dose all the time, for economy reasons and also to avoid phytotoxicity. With this dose, the results in the field trials were similar or better than the control with copper oxychloride.

With regards to the plant material, the selection of the tomato cultivar has shown a high impact on the distribution of the disease, showing the pathogen has a clear preference for infesting the fruits of tomato 'Valenciano'. This could be due to the shape of these fruits with indentations and cavities where the humidity is preserved for longer periods, which may have facilitated *Botrytis* infection and growth, although other physiological features can be part of these differences.

A particular observation was made for the effect of pruning on the damage caused by *Botrytis*. The results were quite evident and expectable as *Botrytis cinerea* has necrotrophic behaviour and wounds are a common site for infections. In the second campaign (flush cuttings) there were no stem infections, whereas in the first campaign (long petiole cuttings), stem infections were the reason for a plant mortality that reached 40% at the end of the crop for plants from the plot of cinnamon extract applied.

3.2.3 Cost/benefit analysis of the alternatives evaluated

In terms of costs, the real price paid by the farmer for all the products tested *in vitro* is summarised in Table 1.

Table 1. Real costs per unit of treatment (ha) for the products evaluated. A volume application of 1,000 L/ha has been assumed. A maximum and minimum dose is shown where applicable. PHC = Potassium Hydrogen Carbonate.

			Maximum dose			Minimum dose		
Active ingredient	**Price**	**Unit**	**Dose**	**Product L/ha or kg/ha**	**Price €/ha**	**Dose**	**Product L/ha or kg/ha**	**Price €/ha**
Gluconic Copper	5.9	€/L	400 mL/hL	4	23.60	200 mL/hL	2	11.80
Zinc 6%	15.63	€/L	250 mL/hL	2.5	39.08	200 mL/hL	2	31.26
Silicon	12.26	€/5L	400 mL/hL	4	9.80	200 mL/hL	2	4.90
Equisetum 1	9.35	€/L	500 mL/hL	5	46.75	200 mL/hL	2	18.70
Equisetum 2	6.54	€/L	500 mL/hL	5	32.70	300 mL/hL	3	19.62
Chytosan	10.61	€/L	300 mL/hL	3	31.83	100 mL/hL	1	10.61
Cinnamon 1	50	€/L	150 mL/hL	1.5	75.00	100 mL/hL	1	50.00
Cinnamon 2	26.13	€/L	300 mL/hL	3	78.38	150 mL/hL	1.5	39.19
Mimosa	18.65	€/L	300 mL/hL	3	55.94	200 mL/hL	2	37.29
PHC	103	€/5kg	500 g/hL	5	103.00	300 g/hL	3	61.80
Lime sulphur	75	€/25L	10 L/hL	100	300.00	n.a.		
Copper oxychloride (Cu 50%)	6	€/kg	400 g/hL	4	**24.00**	200 g/hL	2	**12.00**

Taking into consideration the *in vitro* efficacy of the products and the relatively low cost of copper oxychloride, only **Gluconic copper** is in the same price range per application, and could be a good alternative based on its efficacy *in vitro*. However, this product still contains copper. Looking among the non-copper effective alternatives, the cheapest alternatives are the minimum doses of Cinnamon products. In this case, the cost per application would be double that of copper oxychloride and 162% for Cinnamon-formulation 1 and Cinnamon-formulation 2, respectively. More expensive are PHC (258% of copper oxychloride price for the minimum dose) and Lime sulphur.

If we analyse the field trial results, the option is the application of PHC, what implies a cost 2.58 times higher than copper oxychloride. This difference is high, however, taking into account that in the year with the highest number of applications the number was 5, this implies a cost of 309 €/ha for PHC vs. 120 €/ha for copper oxychloride. The difference is 189 €/ha/year, an amount very affordable for a greenhouse winter crop with produces high yields at good prices per hectare.

Regarding the selection of a *less-susceptible* cultivars of tomato, the wide range of cultivars available makes it difficult to estimate the cost. The benefits derived from using one or another cultivar will be linked to the agronomic features of each cultivar. In other words, because of the very affordable higher cost of alternative treatments to copper a less-susceptible cultivar will only make economic sense if it has the same or better agronomic and taste features.

The cost of pruning is the same regardless the system used, so it is very recommendable to do it in the right way (flush cut) to avoid dangerous *Botrytis* infections.

3.2.4 Concluding remarks

In terms of product substitutions for copper, a couple of them have been accurately tested in field conditions and one of them (PHC, Potassium hydrogen carbonate, potassium bicarbonate) has shown efficacy results similar or even better than copper oxychloride. The efficacy, together with the relatively affordable price of the product, show potassium bicarbonate as one feasible alternative among the several available alternatives to copper that are available on the market.

However, only the smart integrated management of the different factors mentioned, can lead to one scenario suitable for the phase-out of copper form organic greenhouse production. The existence of different crop systems, climates, greenhouses and market conditioning, makes it necessary to increase the number of cases studied, with the aim of generating realistic knowledge that is useful to, and will be accepted by growers.

3.3 Results from field trials in Turkey-MAF

3.3.1 Alternatives evaluated in lab and field trials

The main target of this study is to find ecologically and economically available system-solutions involving plant diversity. For this reason, a study of how increased genetic diversity of local aubergine landraces of Turkey can reduce or totally remove the need for copper fungicides in *Aternaria solani* disease, was performed. It is important to note that the availability of organic aubergine seed is improving all over the world, but could be further enhanced from the wide and diverse aubergine landrace gene pools which are described. The current study investigated the surveying, collection, morphological characterisation and disease resistance to *Alternaria solani* of aubergine (*Solanum melongena* L.) landraces across Turkey under organic management to present ready-to-use knowledge. Aubergine landraces were surveyed and collected from aubergine growing locations of Turkey. A randomised block design trial was conducted with 1,300 plants at the organic experimental farm of the Institution. The 65 accessions were morphologically identified using ECPGR (European Cooperative Programme of Plant Genetic Resources) Descriptor (ECPRG, 2008) for a minimum set of important traits. Using the Main Component Analysis, the variation was determined (Sneath and Sokal, 1973; Clifford and Stephenson, 1975; Tan, 1983). Among the 65 landraces of aubergine that were identified, it was then tested how strong resistance was to the commonly contracted fungi. The testing was performed by removing plant leaves and putting agar discs (3-4 mm) containing an *Alternaria solani* fungal colony on them. As a control, water–agar plugs were placed on some of the leaves with 10 repetitions for each landrace. A 0-5 scale was used to score disease severity. Values were calculated by the Townsend–Heuberger Formula (Townsend and Heuberger 1943). 0% disease severity was used to define resistant and 2% to define tolerant.

3.3.2 Results of alternatives evaluation

Results showed that 14 accessions of the total 65 aubergine landraces are resistant (defined as 0% disease severity) to Early Blight (*Alternaria solani)* and a further 7 accessions had a high tolerance (defined as 2% disease severity). This is a very promising result linking genetic diversity directly to potentially lower cupper fungicide input requirements. It is also found that a high diversity of at least 65 aubergine landraces exist in Turkey.

3.3.3 Cost/benefit analysis of the alternatives evaluated

The study shows that ‘system-solutions’ like more agricultural biodiversity in the genetic resources used, including the use of landraces, could contribute to reducing the need of any copper-based fungicides. Therefore, the results could help making both conventional and organic production less input reliant and more sustainable. As discussed earlier, resistant varieties have wider cost/benefits also including their agronomic and taste performance, therefore a narrow cost/benefit calculation as with input substitution is not possible.

3.3.4 Concluding remarks

To reach overall disease tolerant cultivars, further research on possible disease resistance and molecular characterisation are needed to better integrate aubergine landrace diversity and disease resistance in organic food and farming systems. In addition to current disease screening studies under controlled conditions, field trials under disease pressure should also be carried out. The characterised set of landraces can be a valuable resource for organic aubergine breeding programmes. In addition, organic aubergine seeds bred using these landraces may contribute to the phase-out of copper fungicides. The potential of possible disease tolerant production systems with respect to environmental impact and ecosystem services should also be explored for possible diseases of the aubergines.

One of the novel tools and techniques to control *Alternaria solani* pathogen without copper in traditional susceptible aubergines depends on the availability of the bred organic seeds. To reach a successful disease resistant propagation material such as organic aubergine seeds in organic agriculture, there is a need to better integrate the landraces into breeding programmes in the management of organic farming systems. The overall effect of the resistant and/or at least tolerant seeds used in organic production systems could result in much lower copper fungicide application. Establishing a cost-benefit model using plant genetic resources to identify their unseen additional/system may promote a copper-free designed plant production model.

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4. Perennial crops

4.1 Results from field trials in Spain-IFAPA

4.1.1 Alternatives evaluated in lab and field trials

Available formulations of alternative substitutes to copper were the subject of study in this research.

For the *in vitro* tests, eleven products were evaluated. The products were differentiated in 4 groups attending to their nature:

1. Mineral fertilisers with low or no-copper content (3 products): Copper gluconate 5%, Zinc 6%, Silicon 23%,
2. Basic substances (3 products): *Equisetum* (2 formulations), Chytosan,
3. Plant extracts (4 products): *Cinnamon* (2 formulations), *Mimosa*, *Camelia,*
4. Registered Fungicides as such (2 products): Potassium hydrogen carbonate (85%) (PHC), Lime sulphur (18.5%).

The efficacy of products was tested at 2 concentrations (minimum and maximum recommended by the distributor) against two isolates of *Colletotrichum gloeosporioides* (vegetative and sporulative isolates).

Two alternatives were selected for the field trials, based on their efficacy in the *in vitro* tests: Copper gluconate (5%) (300 mL/L) (Cobregluc, BIOERA) and the formulation 2 of the *Cinnamon* extract (200 mL/L) (Cinna, HORTALAN).

The field trials involved the evaluation of the alternatives in three groves with three different olive cultivars: 'Picual', 'Picudo', 'Hojiblanca'.

4.1.2 Results of alternatives evaluation

In the *in vitro* trials, the products showing fungicidal effects were PHC and Cinnamon extracts. The control practice used as reference (copper oxychloride 50%) did not show any fungicidal effect to *Colletotrichum*, what means that PHC and Cinnamon extracts were better at controlling *in vitro* the pathogen. Of the other products tested, Chytosan, Lime sulphur, and Equisetum-formulation 2 were not good *Colletotrichum* inhibitors. A similar conclusion can be obtained from the other plant extracts tested (Mimosa and Camelia). Regarding mineral fertilisers, Gluconic copper is revealed as a good alternative to higher-content copper formulations, but it does still contain copper. The Zinc product efficacy was slightly lower than copper oxychloride, and Silicon did not show any inhibitory effect on *C. gloeosporiodes*.

Concerning the field trials, in this work, three different olive cultivars have been used, at the same location and during the same period. In the first season, the presence of symptoms due to fungal and bacterial diseases was not recorded. Climatic conditions could be the reason for these results, as the weather was different in the second season, and so the occurrence was seen of at least one disease: leaf spot disease.

The results observed for the cultivar 'Picual' showed significant differences between the treatment with copper oxychloride against the alternative products, which were less effective at controlling the fungus. It could be concluded that the products evaluated, at the doses used (minimum commercial doses), do not allow a control in field. Using the highest doses recommended, could perhaps increase their efficacy, and this could be an option as phytotoxicity was not observed. However, the cost of the applications would be higher. Although no significant differences were found between Copper gluconate and Cinnamon extract formulation-2, the plant extract presented lower values of expression of the disease, with a difference between both products of 20% for the case of incidence and 30% for average severity.

The results for cultivars 'Picudo' and 'Hojiblanca' did not show significant differences among treatments, which may indicate that perhaps the presence of disease and its severity was not enough in the field, even in the second season. The treatment with copper oxychloride reflected the lowest incidence and severity values of all the samples, although no significant differences were found with the alternative products. Again, and this is something repeated for the three cultivars, the Cinnamon extract presented the lowest values of expression of the disease, with a difference in the cultivar 'Picudo' trial between the two alternatives of 33% for the case of incidence and 54% for the average severity. However, the results for these cultivars are not conclusive.

4.1.3 Cost/benefit analysis of the alternatives evaluated

The highest cost of application in the field took place for the cultivar 'Picual' (whose trees showed a higher volume), being of 700 L/ha, applied 4 times, these are 2,800 L/ha/year. Taking into consideration the prices indicated in Table 1 (section 3.2.3.), this means a cost of 50.4 €/ha for the treatments with copper oxychloride and a similar cost the copper gluconate (49.6 €/ha), but a cost of 146 €/ha for the cinnamon extract formulation-2. This is more than 3 times the cost of copper treatments. In view of the lack of better results for the plant extract products, the benefit for the substitution is nearly zero. However, in a year with low disease pressure the plant extract products were found to be not significantly worse than copper and under those conditions could replace copper, however with higher cost. In a year with higher disease pressure and need for copper the zero copper amount saved could then be added as the regulation has 4 kg/ha per year on average but 28 kg/ha in total over 7 years, so a year without copper could be beneficial for the next with high disease pressure.

4.1.4 Concluding remarks

In view of the results observed, there is a need to carry out new experiments using doses and application strategies different from those tested, and to continue evaluating their possible effects on the disease. The influence of the climatic conditions has proven to be a key factor for the onset and development of disease, which has been very low. The type of cultivars and type of cropping system (organic) are factors that can also reduce the impact of the diseases. Differences between cultivars were clearly expressed, showing the cultivar 'Picual' has a higher susceptibility to leaf spot disease. As the alternatives tested in field did not improve the efficacy of copper, they are not good alternatives in terms of efficacy, but also in terms of price and further research is needed for perennial crops like olives.

4.2 Results from field trials in Turkey-MAF

4.2.1 Alternatives evaluated in lab and field trials

MAF has conducted climate chamber and organic olive orchard trials (transition period) with 11 treatments which are alternatives to copper (Cu) to observe their effects against olive leaf spot disease (*Spilocae oleaginaea)* and explore the effectiveness of different preparations.

MAF has completed **climate room tests** between November-2019 and November-2020. In controlled conditions according to the timetable, an artificial inoculation was performed. Incidence of the disease observed was weak on olive sapling-leaves. For this reason, the performance of different inoculation techniques in a hydroponic system using fresh olive leaves and little branches were investigated to obtain disease incidence but disease could not be observed to perform a test of alternative Biological Control Agents (BCAs) substances. So that second inoculation of the disease spores onto olive saplings stage has performed in the following period.

At **farm scale**, in an olive orchard (that had been converted from conventional to organic production) a trial conducted in 44 plots (4 replications + 11 treatments + 2 olive trees). Application of alternative Biological Control Agents (BCAs) substances in the open field area was made following climate DSS system existing in the case farm, previously to observe olive leaf spot disease in the case farm in Akhisar, Manisa Province of Turkey. The 11 treatments including *Glomus intradices*, KSiO3, *Bacillus subtilis* EU007, vermicompost tea enriched with *Platanus orientalis* leaves, Maxicrop and mouldy bread pieces were applied to the plots between November-2019 and May-2021. On overview of treatments is shown in

Table 2.

Table 2. Alternative Biological Control Agents (BCAs) treatments to copper tested in a climate chamber and in on-farm olive orchard trials

1	Copper oxychloride (Control 1)	Total applied copper 6 kg/ha/year, WG, 4 g. 20 lt^{-1} water, [700gr/ Litre metallic copper equivalent commercial product, appl. dosage is 150 cc/ 100 Litre water] , applied after early warning system alarms with "leaf spray" application system (within 48 hours after signal occurred).
2	*Bacillus subtulis* EU 007 WP	$1x10^{4-6}$ CFU, applied after early warning system alarms with "leaf spray" application system (within 48 hours after signal occurred).
3	*Platanus orientalis* (extract of leaves)	Extracted in boiling water, 25 leaf. 20 lt^{-1} water, homemade, applied after early warning system alarms with "leaf spray" application system (within 48 hours after signal occurred).
4	Vermicompost tea	Liquid, 30 cc. 20 lt^{-1} water, commercial product, applied after early warning system alarms with "leaf spray" application system (within 48 hours after signal occurred).
5	Mycorrhiza mix	WP, 3.2 gr. 20 lt^{-1} water, commercial product, applied "drench from soil" application system. Application made according to the phenologic stage of tree. *(a-1 month before the autumn leaves are seen, b-1 month before the spring leaves are seen, c- before the flower buds are seen)*
6	Seaweed	WP, 14 gr. 20 lt^{-1} water, commercial product, applied after early warning system alarms with "leaf spray" application system (within 48 hours after signal occurred).
7	*Trichoderma citrinoviride* TR1	$1X10^6$ CFU, applied "drench from soil" application system. Application made according to the phenologic stage of tree. *(a-1 month before the autumn leaves are seen, b-1 month before the spring leaves are seen, c- before the flower buds are seen)*
8	Non-used (Control 2)	-
9	Potassium silicate ($KSiO_3$)	Liquid, 250 cc. 20 lt^{-1} water, commercial product, "drench from soil" application system approx. 80-100cc KSİO3 per tree. Application made according to the phenologic stage of tree. *(a-1 month before the autumn leaves are seen, b-1 month before the spring leaves are seen, c- before the flower buds are seen)*
10	Vermicompost tea + *Platanus orientalis* (extract of leaves)	Liquid, 50% of each, applied after early warning system alarms with "leaf spray" application system.
11	*Penicillium*	Mouldy bread pieces, 4 kg per tree "mixed into soil 0-20 cm depth" application system Application made according to the phenologic stage of tree. *(a-1 month before the autumn leaves are seen, b-1 month before the spring leaves are seen, c- before the flower buds are seen).*

4.2.2 Results of alternatives evaluation

<u>Climate Room Trial Results:</u>

The lowest germination rates were observed in Vermicompost tea (Treatment 4), Platanus orientalis (Treatment 3) and Platanus orientalis + Vermicompost tea (Treatment 10) and Seaweed (Treatment 6) treated spores. However, interestingly, Spilocae oleaginaea spores did not germinate in water-agar including Copper oxychloride (Control 1) (Treatment 1) and Potassium silicate ($KSiO_3$) (Treatment 9). Therefore, Copper oxychloride (Control 1) (Treatment 1) and Potassium silicate ($KSiO_3$) (Treatment 9) prevented germination of S. oleagineae spores. Potassium silicate ($KSiO_3$) (soil application), Penicillium (soil application), Platanus orientalis (extract of leaves), Bacillus subtilis EU 007 WP, Trichoderma citrinoviride TR1 (soil application), and Vermicompost tea showed up to 50% efficiency against S. oleaginea incidence on olive saplings. In the climate room conditions trial, among these treatments, Potassium silicate ($KSiO_3$) (soil application) (Treatment 9) was found the most promising alternative as 77,63% efficiency for the olive spot disease (

Table 3). On the other hand, the Mycorrhiza mix WP (Soil application), seaweed, and Vermicompost tea + *Platanus orientalis* (extract of leaves) efficiencies were found to be below 50%. In addition, mycelium lengths of spores were decreased by all treatments (except of Copper oxychloride and Potassium silicate ($KSiO_3$) treatments). In the trial, we observed that *Spilocae oleaginea* spores did not germinate in Copper oxychloride and Potassium silicate ($KSiO_3$) applied to olive sapling plots.

We concluded that for all applications (foliar and soil) that we applied, olive spot disease incidence percentage were found to be below the non-used (Control 2) (untreated) treatments of the tested olive saplings.

Table 3. The effect of BCAs on the percentage efficiency and disease severity caused by the inoculated S. oleaginea on olive seedlings in climate room conditions

Applications	%Efficiency	% Disease incidence
Mycorrhiza mix WP (Soil application)	18.74	16.56±1.80b
Potassium silicate ($KSiO_3$) (soil application)	77.63	4.53
Penicillium (soil application)	55.13	8.91
Platanus orientalis (extract of leaves)	57.95	8.59
Copper oxychloride (Control 1)	100.00	0.00
Sea weed	24.24	16.25
Bacillus subtilis EU 007 WP	54.97	9.22
Trichoderma citrinoviride TR1 (soil application)	50.12	10.00
Vermicompost tea	56.70	8.75
Vermicompost tea + *Platanus orientalis* (extract of leaves)	43.58	11.25
Non-used (Control 2)		20.46±2.72a

<u>Olive Orchard Trial Results:</u>

During the 2020 and 2021 production season of the olive orchard *(transition period from conventional to organic management)* (Domat variety) *in Akhisar Province/Manisa-Turkey.* Potassium silicate ($KSiO_3$) (soil application) (69.82%) and Vermicompost tea (56.14%) showed higher efficiency than the other applications under %20-21 disease conditions while Copper oxychloride (Control 1) showed 90.06% efficiency (Table 4).

Table 4. The effect of BCAs on the percentage efficiency and disease severity caused by the S. oleaginea on olive trees in the orchard trial

Applications	% Efficiency	% Disease incidence
Mycorrhiza mix WP (Soil application)	13.45	20.38
Potassium silicate ($KSiO_3$) (soil application)	69.82	7.09
Penicillium (soil application)	43.53	13.28
Platanus orientalis (extract of leaves)	43.80	13.22
Copper oxychloride (Control 1)	90.06	2.34
Sea weed	15.30	20.06
Bacillus subtilis EU 007 WP	43.03	13.41
Trichoderma citrinoviride TR1 (soil application)	39.98	14.13
Vermicompost tea	56.14	10.31
Vermicompost tea+ *Platanus orientalis* (extract of leaves)	33.50	15.63
Non-used (Control 2)	-	23.56

According to the yield pre-evaluations of the olive tree plots for unproductive and productive period, *Trichoderma* citrinoviride TR1 (soil application) increased the yield during both periodicity stages. In productive period, yields of the Seaweed, Mycorrhiza mix WP (Soil application), Vermicompost+ *Platanus orientalis* leaf extract, and *Trichoderma* citrinoviride TR1 (soil application) alternative treatment plots were found higher than untreated control plots. However, regarding unproductive period; Copper oxychloride (Control 1), Potassium silicate ($KSiO_3$) (soil application), *Platanus orientalis* leaf extract, and *Trichoderma* citrinoviride TR1 (soil application) treatments resulted in an increase in the yields of table olives (Domat variety) as we compared the Non-used (Control 2).

However, the statistical difference not found in TWSP (Total Water Soluble Phenols) content of the treatments, it was observed lower disease incidence in *Bacillus subtilis*, *Platanus orientalis* leaf extract and vermicompost tea. Regarding this trial, higher content of TWSP in *Bacillus subtilis, chenar* (*Platanus orientalis*) leaf extract and vermicompost treatments which stimulates antioxidant capacity in olive fruit, addressed that olive leaf spot (*Spilocaea oleaginea* (Cast.) Hughe.) disease incidence was found to be lower than in the other treatments. For all of the applications (foliar and soil) the disease percentages were lower than the untreated seedlings. Copper oxychloride, K_2SiO_3, *P. orientalis* leaf tea, vermicompost tea were the most promising applications. Their efficiency was 100%, 77.6%, 66.6% and 57.9%, respectively.

4.2.3 Cost/benefit analysis of the alternatives evaluated

All of the alternative control agents are inexpensive and easily accessible. Fertilisers containing Silicon (*silicium)* and Vermicompost tea are available in plant protection stores and farmers (if they want) can prepare vermicompost tea. Fertilisers containing Silicon are a bit cheaper than vermicompost tea.

The amount of TWSP (Total Water Soluble Phenols) in the plots treated with copper oxychloride is higher than for the other treatments. The total soluble phenol content of the copper application is followed by *Bacillus subtilis* EU 007 WP, *Platanus orientalis* leaf extract and Vermicompost tea treated plots, respectively. We divided the alternative materials to copper into two groups, taking into account the TWSP as well as other measured parameters in the study. As the “yield” results of the olive orchard trials were not found to be constant and were highly variable; yield parameter was not included in this evaluation. The first group consists of copper oxychloride, *Bacillus subtilis* EU 007 WP, *Platanus orientalis* leaf extract and vermicompost tea applications, while the second group is made up of Mycorrhiza, seaweed, *Trichoderma citrinoviride* TR1, Non-used (Control 2), Potassium silicate ($KSiO_3$), Vermicompost tea+*Platanus orientalis* (extract of leaves), and *Penicillium* (Moulded bread pieces). When we compare those two groups related to increased phenol content with second group of the treatments, it can be assumed that these alternatives could be more resistant to olive leaf spot (*S. oleginea*) disease attack. This may be due to the high antioxidant capacity of polyphenols. This means reactive oxygen species formed in *Spiloceae*-induced cells may be swept by the polyphenols. Polyphenol effects are widely known to allow the plant to tolerate stresses such as plant diseases including olive leaf spot.

At the approximately 20% disease incidence level in the years we performed the olive orchard trials, the efficiency of the treatment-potassium silicate ($KSiO_3$) we applied showed very promising observations. This alternative also performed effectively (77%) under controlled conditions. We re-obtained same results under open field conditions.

Table 5. TWSP (Total Water Soluble Phenols) * (mgCAE.100 g^{-1} olive fruit) contents of the trial.

No	Treatment	Average of Replications	Standard Deviation
1	Copper oxychloride (Control 1)	202.78 A	14.33
9	Potassium silicate ($KSiO_3$)	195.31 AB	29.13
4	Vermicompost tea	180.95 B	11.32
5	Mycorrhiza mix	178.77 B	9.94
7	*Trichoderma citrinoviride* TR1	178.31 B	13.16
3	*Platanus orientalis* (extract of leaves)	164.68 C	7.39
8	No application (Control 2)	162.86 C	10.23
10	Vermicompost+*Platanus orientalis* (extract of leaves)	160.85 C	6.50
11	*Penicillium* (Mouldy bread pieces)	156.82 C	7.11
6	Seaweed	139.59 C	11.16
2	*Bacillus subtilis* EU 007 WP	137.87 C	9.75
	CV (coefficient of variation)(%)	2.08	
	p-value ≤ 0.05: *	*	

4.2.4 Concluding remarks

We concluded that after evaluation using 0-5 Scale, K_2SiO_3 soil applications considering the phenologic stage of tree and vermicompost tea foliar applications (electronic warning and forecast system supported) are the most promising alternative applications to copper based fungicide for olive leaf spot disease.

We also conclude that long term trials should be designed to test the treatments against olive leaf spot disease in the future. Additionally, in the next step, eco-toxicological studies of the promising treatments are needed in order to prove and quantify the added ecological value of such systems.

4.3 Results from field trials in Greece-UTH

4.3.1 Alternatives evaluated in lab and field trials

Trials on table olives in Greece

In 2019 and 2020 the following package of alternatives to Cu were used in experimental fields (Table 6):

Table 6. Time schedule of treatments of olive trials in Greece

Month	Activity
October	Lime-Sulphur 5%
March	Mixture of Ca+Si, kelp extract and amino acids foliar
April	Mixture of Ca+Si, kelp extract and amino acids foliar
May	Bloom time, insecticide applied, mixture of Ca+Si, kelp extract and amino acids foliar
June	Mixture of Ca+Si, kelp extract and amino acids foliar
July, early	5% foliar zeolite

4.3.2 Results of alternatives evaluation

During 2019 and 2020, 3 treatments were evaluated in 2 commercial olive farms:

Control, no foliar applications of fungicides applied;

Conventional, Cu products were applied throughout the year;

Alternative, Cu use was eliminated, lime sulphur, Ca-Si nanoparticles, kelp extract, amino acids and zeolite were applied through the year.

In one farm, double intensity pruning was tested compared to farmer's pruning intensity. The following parameters were measured: leaf drop and causes of leaf loss, leaf and fruit characteristics in June and at commercial harvest (September), fruit quality and yield.

Results, showed that the alternatives did not improve leaf characteristics over the summer and were more efficient at reducing leaf loss due to disease in a wet year (2019) compared to Cu applications. The alternatives also had a positive effect on fruit size (better market price), but slightly decreased yield (in a central Greece olive grove). Alternatives had no effect on fruit size or yield (in northern Greece olive groves). Double pruning nullified leaf disease presence and improved fruit size, but slightly reduced yield.

4.3.3 Cost/benefit analysis of the alternatives evaluated

The combination of biostimulants tested could be used as an alternative to Cu fungicides, but the cost of these products is higher (almost double in total, due to material costs and 2 additional equipment uses for application) than copper products commonly applied today. The Control trees behaved in many respects similarly to conventionally managed trees.

Of course, the control treatment (no use of fungicides and biostimulants) is cheaper than the conventional or biostimulants' based plant protection, but the farmers would not be willing to undertake the risk of not protecting the trees. Nevertheless, the control treatment gave us an insight of what could be an efficient, but risky table olive cultivation.

4.3.4 Concluding remarks

Interestingly, under the experimental conditions, **good air circulation in the canopy** (double pruning) was sufficient to reduce disease problems, but reduced yield. Also, the olive farm with younger plants away from other olive farms showed minor disease damage e**ven in the absence of any disease protection** management, but this is an example unlikely to be followed by farmers (because of the higher risk of losing the crop). The mixture of biostimulants was more expensive, but **efficient in controlling leaf loss and disease pressure**, though it did not increase the yield sufficiently to be economically viable (this is under non-organic growing conditions).

4.4 Results from field trials in Italy-UNICT

4.4.1 Alternatives evaluated in lab and field trials

Alternatives evaluated in the laboratory.

Fertilisers with low or no copper content ((Vitibiosap 458 Plus, Kiram, Kiram AT, Kiram Film, Dentamet), essential oils (Prev-am plus, 18 essential oils locally produced), vegetable extracts (*Cynara cardunculus* leaf extracts), plant defence stimulators ((Bion), basic products (chitosan, equisetum) and biological control agents (Amilo-X, Botector, *Wickerhamomyces anomalus* BS91, Bacillus spp. LIS1), were evaluated for their *in vitro* inhibitory efficacy against *Colletotrichum gloeosporioides*, *Alternaria alternata*, and *Pseudomonas syringae*, citrus pathogens widely present in Sicily, and against *Xanthomonas euvesicatoria* pv. *perforans,* tomato pathogen recently detected in Sicily. Ossiclor 35WG (copper oxychloride) was used as a control.

Alternatives evaluated in the greenhouse and field trials.

The following alternative products were evaluated on citrus and tomato plants.
a) in greenhouse: Vitibiosap 458 Plus, Kiram, Kiram AT, Dentamet, Prev-am Plus, *Origanum vulgare* essential oil (EO), *Cynara cardunculus* leaf extracts, Bion, chitosan, equisetum, Amilo-X, *W. anomalus* BS91.
b) in the field: Vitibiosap 458 Plus, Kiram, Chitosan, Equisetum, Prev-am Plus, Serenade, chitosan+Prev-am plus, chitosan+equisetum.
Ossiclor 35WG (copper ohychloride), tribasic copper sulphate+copper hydroxide (Kop-Twin) and pyrachlostrobin (Cabrio) were used as control.

4.4.2 Results of alternatives evaluation

Alternatives evaluated in the laboratory trials.

Results showed that all products were able to reduce, although with variable efficacy, mycelial growth of *C. gloeosporioides* and *A. alternata*, if compared to untreated control and to copper oxychloride and copper hydroxide. Among fertilisers with low copper content, the best growth-inhibition activity of both fungi (no growth after 20 days of incubation) was reached by Dentamet and Vitibiosap 458 at the highest concentrations. Prev-Am, Kiram, AmyloX and Botector, as well Bion, reduced mycelial growth with intermediate efficacy, whereas chitosan and equisetum were ineffective. Five of the 20 tested EOs completely inhibited mycelial growth of C. *gloeosporioides* and *A. alternata*, maintaining this efficacy twenty days' post inoculation. Moreover, the EOs with the highest inhibitory activity *in vitro* against fungal pathogens were also able to produce VOCs with high inhibitory activity against *C. gloeosporioides* and *A. alternata*. Antagonistic yeast *W. anomalus* BS91, alone and in combination with the *Cynara cardunculus* leaf extracts, showed good results too in the inhibition activity against *P. digitatum*. With regard to antibacterial activity, Vitibiosap 458, Kiram and Dentamet showed *in vitro* the highest inhibitory activity against *P. syringae* and *X. euvesicatoria* pv. *perforans*, with efficacy comparable with copper oxychloride; the efficacy of selected EOs against *X. euvesicatoria* pv. *perforans* was proportional decreasing with the reduced concentration of EOs.

Alternatives evaluated in the greenhouse and field trials.

1) Greenhouse trials

In orange fruit artificially inoculated with *C. gloeosporioides*, Chitosan, Vitibiosap, Kiram, Kiram AT, Prevam-Plus and AmyloX reduced disease incidence and severity with values comparable to copper oxychloride, confirming the *in vitro* antimicrobial activity. Chitosan and equisetum, that *in vitro* showed no efficacy, *in vivo* showed intermediate efficacy in reducing disease incidence and good efficacy in reducing diseases severity. Chitosan and Prev-Am, tested on lemon plants in greenhouse trials, confirmed their good protection activity against *C. gleosporioises*, efficiently reducing disease incidence and severity when compared to the untreated control. Combined treatments based on antagonistic yeast *W. anomalus* BS91and *C. cardunculus* leaf extract were effective in controlling *P. digitatum* green mould on orange and lemon fruits both in contemporary and differed inoculation treatments. In greenhouse trials, Dentamet, Kiram and *O. vulgare* essential oil showed good efficacy in reducing *P. syringae* symptoms development on wounded petioles and leaves.

On tomato plants, Kiram and Dentamet showed excellent results in reducing disease incidence and severity caused by *X. euvesicatoria* pv. *perforans*, whereas Vitibiosap and BCAs showed less efficient control ability.

2) Field trials

The field evaluation of alternative products in reducing natural infections caused by *Colletotrichum* spp. *Alternaria* spp. and *Penicillium* spp. in comparison with copper-based fungicides was conducted from 2018 to 2022 in 4 integrated and organic citrus orchards located in eastern Sicily on orange cultivars “Tarocco Scirè” and “Tarocco Tapi”, and on lemon cultivar “Femminello” 2KR

In Experiment I (Pedagaggi), trials were carried out for 4 consecutive growing seasons (2018 to 2022); in Experiment II (Lentini) trials were carried out for 1 growing season (2019-2020). In experiment III (Mineo) and IV (Agnone), trials were carried out for 2 consecutive growing seasons (2020 and 2021). The number of applications for each product in each field and each growing season (2-7) varied according to the meteorological events and the pressure of the disease. The monitoring to assess the efficacy of the treatments (disease incidence and disease severity) were carried out in the field the first time before the first application and then a variable number of times depending on the farm and on the year of each trial. The last monitoring was at harvest time.

Basic products with plant defence stimulator activity (chitosan and equisetum) and the active substance “sweet orange essential oil”, alone and in mixture, efficiently reduced disease incidence and severity of fruits compared with the untreated controls, and often showed similar or better efficacy than copper compounds. Their efficacy against *C. gloeosporioides* and *A. alternata* varied according to the year of the trial and with the pressure of the disease, but was on the whole comparable or higher than copper treatments. Moreover, field treatments exerted prolonged efficacy after cold storage of fruits in postharvest.

During the course of the trials, the orange and lemon crops developed normally and no symptoms of phytotoxicity were observed on fruit treated with the alternative products and with the standard product (copper treatment). With regard to the handling of products, no negative aspect has arisen during the weighing and preparation of the solution. Furthermore, all alternative products are authorised for use in organic citrus plants and were applied according to the manufacturer’s instructions.

4.4.3 Cost/benefit analysis of the alternatives evaluated

The analysis of the costs of the tested alternative solutions and the benefits that the alternative solutions offered was carried out using the following parameters: average production (mean production) equal to 20,000 kg / hectare (20t/ha); average production value equal to 0.35 Euro / kg; volume of water (spray volume) equal to 2000 litres / hectare.

For each product (alternative solution) the following items were ascertained: the cost/kg, the doses used (ml / hl) and the number of applications in each filed trial. The following items were then calculated: net value (Euro / ha) for each treatment (i.e. value minus the cost of each alternative product, applied alone or in mixture), and the net value increase (%) versus untreated control.

We analysed 4 field trials, representative of those carried out during the project, characterised by different disease pressure, different number of treatments and different phytopathogen.

Table 7. Analysis of the net values of the production and the net value increase vs untreated control per unit of treatment (ha) for the treatments evaluated. A volume application of 2,000 L/ha has been assumed.

Citrus farm	Treatment	Disease incidence (%)	Net value of the production (Euro/ha)	Net value increase (%) vs control
Pedagaggi 20/21	Control	69.25	2,153	
treatments =5	Chitosan	32.25	3,758	74.6
	Sweet orange essential oil	26.5	4,105	90.7
	Equisetum	38.5	3,425	59.1
	Chitosan + Sweet orange essential oil	25	3,225	49.8
	Chitosan + Equisetum	28.25	3,158	15.8
	Copper	59.75	2,398	11.4
Pedagaggi 21-22	Control	34.0	4,620	
treatments =3	Chitosan	14.5	5,394	16.8
	Sweet orange essential oil	11.3	5,589	21.0
	Equisetum	14.5	5,457	18.1
	Chitosan + Sweet orange essential oil	10.5	5,050	9.3
	Chitosan + Equisetum	17.8	4,639	0.4
	Copper	14.0	5,768	24.8
Mineo 2021	Control	34.5	4,585	
treatments=3	Chitosan	12.2	5,557	21.2
	Sweet orange essential oil	18.5	5,081	10.8
	Equisetum	29.5	4,407	-3.9
	Chitosan + Sweet orange essential oil	14.2	4,793	4.5
	Chitosan + Equisetum	20.8	4,423	-3.5
	Copper	30.8	4,590	0,1
Agnone 2021	Control	11.67	6,183	
treatments=3	Chitosan	5.00	6,059	-2.0
	Sweet orange essential oil	2.08	6,230	0.8
	Equisetum	3.75	6,210	0.4
	Chitosan + Sweet orange essential oil	0.83	5,727	-7.4
	Chitosan + Equisetum	3.75	5,619	-9.1
	Copper	5.42	6,369	3.0

In conditions of high pressure of the disease (Pedagaggi 2020/21, DI = 69.25%), all the anti-*Colletotrichum* spp. treatments, applied individually and in mixture, obtained a **net value increase (%), variable from 11.4% (copper) to 90.7% (sweet orange essential oil).**

Their effectiveness and convenience were thus demonstrated: the high number of applications (5) and the relatively high cost of the treatments were largely offset by the increased production and by the net value increase of the production compared to the untreated control.

Even in intermediate disease pressure conditions (Pedagaggi 2021/22, DI = 34%), treatments with chitosan, equisetum and sweet orange essential oil, applied individually, registered a net value increase (%), in this case slightly lower than that obtained with copper, the cost of which is however much lower.

Also in Mineo (2021), with intermediate brown spot pressures caused by *Alternaria* spp. (DI = 34,5%), chitosan and sweet orange essential oils showed a good level of protection, a good net value of the production, and a net values increase (%) compared to the untreated control. In this field test, the low efficacy of copper against *Alternaria* spp. was confirmed, highlighting the greater effectiveness and economic convenience of alternative products compared to copper.

Finally, in low pressure conditions of the disease caused by *Alternaria* spp. (Agnone 2021, DI 11.67%,), the significant reduction of DI registered in all theses with alternative products did not turn into an increase of net values of the production (Euro/ha), as the cost of the treatments was equal to, or greater than the benefits recorded.

4.4.4 Concluding remarks

Overall, the good efficacy and economic net value increase performances of these commercial products encourages further large-scale testing in citrus groves with innovative farmers, to study them in more depth and validate their performances as alternatives to copper also under commercial conditions.

The results indicate that under certain conditions and in some years, alternatives can already have a higher economic net value than the copper treatment used as control. Even with copper still available those treatments are interesting for farmers to maximise their economic and yield performance, especially if growing under organic conditions. It is also noted that there is large variation in the efficacy of treatments in one year and between different years. Treatments which showed consistently the relatively most promising net value increase are ‘Chitosan’, ‘Sweet orange essential oil’ and ‘Equisetum’, however other treatments and combination of treatments also have an effect.

5. General conclusions

Results indicated several relatively cheap and profitable actions (treatments, decision support systems -DSS, variety and genotype choice) as alternatives to copper use or to reduce its current usage. Various crops were studied, potatoes, tomatoes, cucumber, aubergine, olives and citrus, and a certain variation found.

INRAE found **resistant potato variety** usage in demonstration trials did not generate additional production costs compared to a system using certified seed of a susceptible, protected cultivar. In some years no control was required, also not with copper alternatives. In Greece (UTH) and Spain (IFAPA) greenhouse cases the **decision support systems** (DSS) used was able to control tomato *Botrytis* disease. Although the disease pressure during the period of measurements was low, it was found that the use of the DSS could eliminate the need for *Botrytis* related plant protection products, at least in greenhouses. IFAPA reported that **potassium bicarbonate** has shown efficacy results similar or even better than copper oxychloride. The efficacy, together with the relatively affordable price of the product, show potassium bicarbonate as one feasible alternative among the several available alternatives to copper that can be found on the market. MAF reported 14 **resistant aubergine landraces** which could possibly be used in organic production systems and result in much lower copper fungicide application. UNICT, UTH, IFAPA and MAF addressed cost effective products with efficacy levels of up to 50% for citrus and olive production.

The cost/benefit assessment of the management of organic production, considering resistance capacity of diverse plant genetic resources, technologically developed digital monitoring systems and direct application of novel products (ready to use, commercial, and/or on-farm produced) was found to be **remarkably productive in the studies**. These utility-based innovations not only have a capacity to effect circular economy if further research supports to improve findings, but also to attract community supported farmers, and certified organic growers' attention in demonstrative cooperation. Although the majority of our studies focus on copper reduction, also called copper minimisation from currently 4 kg/ha/year to 2 kg/ha/year this evaluation and interactions with growers and NGO's have shown that the organic sector's expectations still centre on some copper being available in their production for plant health applications and risk management in difficult years. Thus it is interesting to note that our trials indicate that reduced cost in many successful copper replacement treatment or avoidance strategies (DSS) brought other advantages, which were determined satisfactory.

A world of organic and conventional agriculture without any copper additions to the soil (beside the levels needed as micronutrients), would result in the end of copper pollution in the soil - pollution defined as more copper then needed or naturally present for the soil type. A healthier, pollution-free soil can then also be better used for its current primary function with is carbon storage and carbon drawdown to mitigate the climate and biodiversity extinction crises.