
# Publishable Summary

## Project description

The CO-FREE project aims to develop innovative methods, tools and concepts for the replacement of copper in European organic and low input fruit, grapevine, potato, and tomato production systems. Copper-free production systems will be achieved by (i) providing alternative compounds, (ii) 'smart' application tools and (iii) by integrating these tools into traditional and novel copper-free crop production systems. The copper-free apple, grapevine, potato and tomato production systems will be (iv) evaluated in a multi-criteria assessment with respect to agronomic, ecologic and economic performance. CO-FREE will also develop strategies to develop (v) 'smart' breeding goals by development of crop ideotypes and (vi) foster consumer acceptance of novel disease-resistant cultivars by consumers and retailers. By involving farmers, advisors, plant protection industry, policy makers and researchers as well as the stakeholders of the European organic and low input sector (food supply chain, retailers, producers associations), CO-FREE will ensure a rapid development, dissemination and adoption of the copper replacement strategies.

## Objectives

The objectives of CO-FREE are to develop plant protection products (PPPs) of microbial origin and plant origin including optimization of field application, characterization of the mode of action, identification of the spectrum of activity, development of a suitable formulation and optimization of the production **(objective 1)**. The developed PPPs will be combined with disease control strategies in organic and low input/IPM farming systems to achieve the maximum disease control (**objective 2)**. The novel tools and techniques will be applied to different productions systems (apple, grapevine, tomato, potato). This is addressed in **objectives 3-5**. **Objective 6** focuses on eco-toxicological studies of the newly developed products. Evaluation of the economic impact of novel PPPs and strategies resulting from objectives 1 to 5 and development of a strategy to improve wholesaler and consumer acceptance of novel cultivars is the content of **objective 7. Objective 8** addresses the dissemination of new knowledge and novel practices to representatives, farmers, advisors, retailers, policy makers and researcher.

## Work performed and results

The eight objectives are organized in eleven work packages (WPs). Their content and progress is described in the following.

**WP1** addresses the co-ordination of scientific activities. The consortium met in January 2012 for a kick off meeting **(Del. 1.2)**. This meeting and the following meeting in November 2012

were used for planning of further steps and strategies and for discussing about new developments in the consortium. For both meetings minutes were prepared and provided to the partners **(Del. 1.3)**. Furthermore, a homepage was set-up (**Del. 1.1**), which is available at: www.co-free.eu. All important public information is presented, such as project summary, objectives, events, results and the CO-FREE newsletter. A steering committee and a general assembly were established and a consortium agreement was prepared and signed by all partners.

The work in **WP2** addresses the development of alternative PPPs from microbial origin. This work started in time and made good progress**.** It was shown that *Trichoderma*-induced resistance is strongly affected by the grapevine genotype and by exposure of plants to abiotic stresses. So to improve the efficacy of induced resistance under field conditions responsive cultivars should be preferred and combined abiotic stresses should be prevented (**Task 2.1**). COS-OGA could be linked with systemic acquired resistance (SAR)-like mechanisms of signalling and also formulations could be identified with better effect in defense induction and efficacy. Furthermore, application parameters were optimized (**Task 2.2**). For yeast-based derivatives, formulations and application protocols were improved in indoor efficacy trials and the mode of action was analyzed by gene expression (**Task 2.3**). The mode-of–action of the apple scab antagonist *Cladosporium cladosporioides* H39 was studied *in vitro* and *in planta*. The involvement of several modes-of-action common for antagonists could be excluded (**Task 2.4**). For *Aneurinibacillus migulanus* the production parameters of gramicidin S and a biosurfactant were optimized. In addition, out of four screened isolates of entomopathogenic bacteria, one was identified as highly effective against tomato/ *Phytophthora infestans* in indoor efficacy trials (**Task 2.5**).

The work in **WP3** addresses the development of PPPs from plant origin. This work started in time and progressed well. **Del 3.3** was achieved, i.e. first formulations were developed to increase important aspects such as efficacy, UV stability, rain fastness and shelf-life. Different sage extract formulations were produced and examined for improved rain fastness. Besides that, the chemical composition of common sage extract was characterized and efficacy against late blight on tomato was proven (**Task 3.1**). Licorice extract formulations were produced, and the chemical composition was analyzed. Further improvement of the formulation is in progress. New possible sources for the purchase of licorice leaves were identified. In addition the mode of action was further analyzed (**Task 3.2**). The saponine work (**Task 3.3**) was terminated and the respective resources will be re-allocated as agreed by the general assembly. For PLEX highly active fractions could be identified. Formulations were produced and examined for better rain fastness, UV stability and shelf life. Results are indeed promising, confirming the high fungicidal activity of PLEX. Field trials were started **(Task 3.4)**. The work on seaweed (**Task 3.5**) was temporarily discontinued but will be carried on. In **Task 3.6** efficacy of several CO-FREE PPPs was tested against *Phytophthora infestans* in potato indoor trials with promising results.

The work in **WP4** addresses the optimization of use of the developed PPPs and management tools. WP4 started on time. A database consisting of novel and established copper alternatives was compiled and is continually updated (**Task 4.1**). In accordance with the working plan the work of **Task 4.2** has not started yet. In **Task 4.3** assays were optimized for testing efficacy on fungicide resistant *P. infestans* isolates after FRAC. A number of data sets to develop a

Decision Support System (DSS) for the management of downy mildew on grapes were collected. These data sets have been processed and evaluated and were compared to a simulation. On basis of these observations the model was further adapted (**Task 4.4**). Besides that a plant growth model for organic potato production is under development. A 3-factorial field trial (copper, variety, fertilisation) with potatoes at a site with usually moderate late blight pressure was carried out. Collected data will be used to improve the current DSS Öko-SIMPHYT with respect to determination of the necessity of copper sprays. The model will be further adapted to timing of application of alternative PPPs (**Task 4.5**).

In **WP5** work was started to evaluate novel techniques and technologies in the context of traditional, low input and copper-free apple production systems. Field trials in traditional apple production systems were conducted in Italy and France. In apple cultivar Golden Delicious, PPPs from CO-FREE showed some effect on primary infection of leaves with apple scab in Northern Italy when applied as stop treatment. Also with respect to secondary infection, one compound showed to be highly effective when applied as stop treatment. Field trials for 2013 were set up in France and Italy according to the workplan **(Task 5.1)**. The potential and limits of 'very low input' and 'no PPP input' production systems are explored by comparing current commercial certified IPM and organic farming systems with an established advanced very low input production systems trial **(Task 5.2**). This system includes the consequent use of preventive techniques that go far beyond current state-of-the-art IPM and organic apple production schemes under central European conditions by including all proven preventive tools and sanitation measures. The climate in 2012 was extremely difficult and demonstrates the limits of preventive strategies: In 2012, a first major outbreak of virulent *Venturia inaequalis* populations has been observed. Control strategies were subsequently adapted in order to limit the further established virulent populations in 2013ff. In contrast, pests did not cause economic losses although direct pest control was limited to treatments against codling moth (*Cydia pomonella*) with granulo virus (CpGV) whereas aphids (e.g. rosy aphid) and other insect pests were controlled only by beneficials. The advanced production system excelled the standard IPM and organic systems consistently with respect to biodiversity (flora/fauna) indicators. In the evaluation of agroforestry systems **(Task 5.3),** total apple yields were higher than those from a local organic orchard: The yields from the agroforestry compared favorably and apple scab incidence in the agroforestry apples was less than half of those in the orchard apples. Insect pest damage levels caused by sawflies and capsid bugs were slightly higher in the agroforestry system. Solitary and bumblebee abundance was higher in the orchard, while predatory and parasitic wasps were more abundant in agroforestry.

The work of **WP6** on development of copper-free grapevine production systems has started. In 2012 and 2013, trials in Italy, Greece and France were conducted. In trials conducted in Italy in 2012, a high pressure of grapevine downy mildew was observed which highlighted the limits of the pilot PPPs in their current form, if used as stand-alone treatments. However, the improvement of PPPs and refined application strategies are currently developed. In South Greece a trial was performed on the cultivar Agiorgitiko, in a vineyard in the Prefecture of Korinthos. Disease incidence was low to moderate and none of the PPPs controlled the disease on leaves under these disease conditions. The effects of the test compounds on the

crop itself were also recorded. Trials showed that regular treatments every 7 to 10 days with CO-FREE alternative products from beginning of May to beginning of September had no adverse affects on yield nor on quality of grapes, which e.g. in the case of resistance-inducing compounds is a crucial aspect and cannot be taken for granted. All 2012 compounds were included in the 2013 trial. In France in 2013 a high pressure of grapevine downy mildew was observed which limited the efficacy of the tested PPP (**Task 6.1**). Preparations for **Task 6.2**, which is scheduled for 2014, started.

In **WP7** the development of copper-free annual crop (potato, tomato) production systems was started according to the work plan. In field trials conducted in Germany (**Task 7.1**), the application of new PPPs increased yield of cultivar Ditta by up to 4 t / ha (35%). The difference was, however, not statistically significant. In order to further improve yield stability and late blight control, spraying regimes are modified in 2013 and low doses of copper are combined with the PPPs. Field trials in Poland were conducted on small plots with the two potato varieties, Ditta and Jelly. Small differences in yields of both varieties with respect to treatments and cultivar were noted. As expected, the most important factor in control of *P. infestans* was the plant cultivar. The obtained results were used to improve treatment methods in field trials in 2013. In field trials conducted in France, alternative PPPs tested so far provided little if any protection against potato late blight (*P. infestans*) on susceptible cultivars in field trials under oceanic conditions (**Task 7.1**). This again stresses the importance of combining such preparations with suitable potato genotypes.

Resistance to late blight is a major feature of cultivar ideotypes adapted to organic and low-input production, according to a questionnaire targeted to identify key traits in the different types of organic potato crops (**Task 7.2**). In south west France four alternative products were tested in 2012 on *P. infestans* in an organic tomato field. None of the applied treatments proved to be phytotoxic to the tested host cultivar. The tested products showed no significant effect on diseases. As the copper based reference also hardly controlled late blight in 2012, due to a sudden development of *P. infestans*, results at the end of the season were difficult to analyse and trials have to be repeated in the coming years. In Greece trials with open field industrial tomatoes *c.v* Vulcan were conducted. Three novel PPPs were tested. None of the pilot PPPs controlled *P. infestans* on leaves under low disease pressure conditions. Improvement of PPPs and refined application strategies are currently developed. Further trials are done in 2013. (**Task 7.3**).

**WP8** addressed the horizontal activity 1: Ecological impact assessment of novel copper-free techniques and production systems. In line with this, in Greece initial toxicity bioassays with the beneficial arthropod *Coccinella septempunctata* to evaluate four formulated alternative compounds were set up (**Task 8.1**). Literature review and exchange of opinions with European experts, concerning the experimental protocols to be followed for testing of microbial pesticides on aquatic indicator organisms was completed. Preliminary tests with internal control samples have been conducted. In addition the $EC_{50}$ values for *Selenastrum capricornum* and *Daphnia magna* will be determined for the first four available alternative compounds and gradually for the rest of the PPPs of the project (**Task 8.2).** The toxicity tests on the earthworms *Eisenia foetida* (compost worm), and *E. albitus* (white potworms) according to Guidelines of OECD 207, 1984 and OECD 220, 2004 as non-target soil indicator

organisms were set up (**Task 8.3**). In line with the work planned for **Task 8.4** a handbook for ecological impact assessments on arthropods in field trials was prepared (**Del. 8.9**). First assessments on the ecological impact of novel PPPs, strategies and production systems in the field were conducted. These assessments led to the observations that in tomato there is a positive effect (increased number) on Collembola, which is a biodiversity index insect group. The main insect pests recorded were whiteflies, aphids (*Aphis* sp., *Macrosiphum* sp.), *Liriomyza* sp., thrips as well as Heteroptera bugs, the cotton ballworm (*Helicoverpa armigera)* and the tomato leafminer (*Tuta absoluta)* in the second sampling. In grape vine, spiders and predatory mites of leafhoppers from the family Erythreidae were the most abundant among the beneficial arthropods. Leafhoppers were the major pest species. In 2013, ecotoxicological field assessments were conducted for predatory mites in vine and for beneficial insects in tomato in Greece.

**WP9** addressed the horizontal activity 2: Socio-economic impact assessment of copper-free production systems and strategies. In line with this, a common protocol was designed (**Del 9.1.**) to describe production system parameters and cost/benefit analyses based on standard quantitative approaches. A report (**Task 9.2, Del 9.3**) has been published on literature review and expert consultation to identify key-obstacles and promising strategies to introduce new sustainable food products in different supply chains. The **Del. 9.4** on pilot studies to introduce new varieties into the supply chains is in progress.

In **WP10** the overall aim is to disseminate new knowledge and novel practices (horizontal activity 3) which was successfully implemented. The CO-FREE homepage was established to spread results and information concerning the project. On national and international conferences and furthermore in a meeting with Israel´s Minister of Agriculture and with representatives of the Chinese Academy of Agricultural Sciences, the work and aims were communicated by partners. At the Swiss organic fruit and grapevine grower's reunions CO-FREE activities and objectives were reported. At an expert discussion on copper strategies in Germany, the project was introduced. In addition FiBL takes part in DG Sanco's "Botanicals and plant extracts" expert group that currently develops a guidance document for improved registration procedures for botanicals. The frame and venue of a workshop in the second year was discussed with the ABIM steering committee.

The consortium and technical management addressed in **WP11** was successfully implemented by JKI. Controlling the progress of the CO-FREE project was guaranteed by regularly delivered 3 months reports of the partners.

## The expected final results and their potential impact and use

The overall aim of the CO-FREE Project is to develop copper free organic and low input production systems while maintaining yield and quality of crops and reducing negative environmental impacts. During the first 18 months a good progress was made. It was shown that by using PPPs in combination with different cultivars (e.g. potato) and different production systems, the yield could be positively influenced. Due to unfavourable weather conditions the field trials were not easy to evaluate and so the formulations of the PPPs and strategies will be further optimized addressing these obstacles.

In the end promising novel PPPs engineered under WPs 2 and 3 together with an optimized application strategy will be developed in close collaboration between SMEs, researchers, advisors and farmers (WP4). In WPs 5-7 a range of component strategies adapted to specific crops and pedo-climatic conditions will be adopted, taking into account regional and cultural differences as well as the economic realities and the local legal framework. The development of innovative generic strategies to increase/support acceptance of innovative systems by policy makers, advisors, farmers, wholesalers, consumers and the wider public will be provided as a key component by CO-FREE through horizontal activities organised under WPs 8-10. With these activities, it is anticipated to decrease the overall dependency on copper use in organic and low input farming systems in EU/EFTA countries substantially. This will have a positive impact on the environment and will create a substantial growth of organic and low input farming systems in regions where organic and low input farming was limited due to high disease-related production risks. This increased demand will also strengthen the competitiveness of European biopesticides manufacturers. CO-FREE will strongly contribute to the market introduction of up to 10 novel products of plant or microbial origin, will develop/refine smart tools for apple, grapevine, potato and tomato disease control, and will explore advanced apple and grapevine production systems with reduced dependency on pesticide inputs and will contribute to open the market for disease-tolerant cultivars. This will have wide socioeconomic impact on the use of copper.
To follow the CO-FREE project, see http://www.co-free.eu/.