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CO-FREE Alternative Test Products for Copper Reduction in Agriculture

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INTRODUCTION

The project CO-FREE (2012-2016) aimed to develop strategies to replace/reduce copper use in organic, integrated and conventional farming. CO-FREE alternative test products (CTPs) were tested and integrated together with decision support systems, disease-tolerant varieties, and innovative breeding goals (ideotypes) into improved management strategies. CO-FREE focused on apple/apple scab (*Venturia inaequalis*), grape/downy mildew (*Plasmopara viticola*), and tomato and potato/late blight (*Phytophthora infestans*).

Starting point of the project were ten CTPs with direct or indirect modes of action including *Trichoderma atroviride* SC1 and protein extract SCNB, *Lysobacter* spp., yeast-based derivatives, *Cladosporium cladosporioides* H39, the oligosaccharidic complex COS-OGA, *Aneurinibacillus migulanus* and *Xenorhabdus bovienii*, sage (*Salvia officinalis*) extract, liquorice (*Glycyrrhiza glabra*) extract, PLEX- and seaweed plant extracts. As the project progressed, further promising CTPs were included by the partners. Field trials were performed in different European countries in 2012-2015 following EPPO standards. In the first years, stand-alone applications of CTPs were tested. In the following years these were integrated into complete strategies. Effects on main and further diseases, on yield and on non-target organisms were assessed. Here, field trial results with CTPs are summarized.

FIELD TRIAL RESULTS IN APPLE / APPLE SCAB

Field trials were carried out in apple orchards in northern Italy and southeastern France from 2013 to 2015. The trials aimed to evaluate the efficacy of CO-FREE test products against the major fungal diseases of apple, primarily apple scab. In some trials carried out in Italy in 2012-2015, single compounds (H39, yeast-based derivatives and a resistance inducer (19-19)) controlled apple scab to an extent comparable to the reference copper treatments. In 2013,

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under high disease pressure, H39 was comparable to copper, in both the primary and the secondary infections in Italy. However, in many cases, the CTPs did not show levels of disease control, which can be considered comparable to the commonly used reference fungicides based on copper, lime sulphur, hydrogen carbonates, and acidified clays. It must be pointed out that not all CTPs that have been tested in laboratory and greenhouse trials, have also been evaluated in open fields due to limited availability at their respective developmental stage. Thus, further field trials will be necessary.

FIELD TRIAL RESULTS IN GRAPEVINE / DOWNY MILDEW

Trials in grapevine against downy mildew were undertaken in southern Greece, northern Italy and France. During 2012 to 2014, single alternative compounds were selected in Italy and Greece, and then they were included in control strategies according to their mechanism of action in 2014 and 2015. Since none of the products tested so far was able to fully control the disease as stand-alone treatments in the first years, a maximum of three treatments with low dosages of copper (during bloom) were built in the strategies with the experimental products.

In France, trials were performed at two trial sites, in southwestern and southeastern France. In control strategies, the CTPs were applied weekly from the beginning of the season. In 2013 and 2014, a low copper dose was applied weekly in association with the alternative products in order to slow down the epidemic. As no high infection risk occurred in 2015, the low copper dose was ommited. In general, only CTPs following the French national criteria, i.e. only products with an ADE permission (Autorisation de Distribution pour Expérimentation delivered by national authorities) could be assessed. As a consequence, only a limited number of CTPs could be included in the field tests.

Results in Italy

In Italy in 2014, the disease pressure was extremely high and some infections happened without the possibility to reapply the treatments due to continuous rain. None of the products, including the copper reference, did adequately control the disease, and the trial was stopped in July. However, the low copper strategy, i.e. treatments with a resistance inducer, followed by 1.5 kg/ha and year of Cu and afterwards use of sage extract ensured a protection efficacy of the vineyard (severity on leaves and bunches) of ca. 40%, comparable to the copper reference (7 kg/ha and year).

In the 2015 season, the disease pressure was not as high as in former years. The tested plant extracts (TRIFCOF-03 and larch extract) and a milk derivative applied with only 1.5 kg/ha and year of Cu showed an effectiveness against downy mildew on leaves and bunches, statistically similar to that of the copper reference (8 kg/ha and year).

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Results in Greece

In 2014, the disease pressure was extremely high. All products tested, including the copper reference (7 kg/ha and year), did not achieve downy mildew reduction at a sufficient level, hence the trial was also stopped in July. Reduction in percentage leaf area infected over the season was 61% for the full copper reference. In a strategy where in addition to alternative compounds a low copper dose was applied (only 0.7 kg/ha and year at high risk), protection of the vineyard was statistically not different from the full copper dose.

In 2015, the disease pressure was lower than in former years also in Greece. The larch extract, the milk derivative and a preparation of *Lysobacter capsici* AZ78 showed a good effectiveness against downy mildew, statistically similar to the copper reference. Thus, these alternative compounds in combination with 0.7 kg/ha and year of Cu were as effective as 7.7 kg/ha and year applied in the copper reference plots.

Results in France

In 2013, the disease level was high at both trial sites in France, while in 2014 disease level was moderate. In both years, the best efficacy was observed for copper-based treatments. At the southwestern site, the same level of efficacy was observed in both years for the low copper dose (0.6 kg/ha in 2013 and 0.9 kg/ha in 2014 per year) and the full copper reference (1.6 kg/ha in 2013 and 3.6 kg/ha in 2014 per year). In 2014, one of the CTPs seemed to slow down the epidemic (observed in mid-August). However, no statistically significant effect was demonstrated.

In 2015 at the southeastern site, the disease level was initially low, but reached 63% in August. The CTPS tested as stand-alone or with low doses of copper had no effect in controlling neither downy nor powdery mildew. At the southwestern site, infection occurred after inoculation and fogging. The best efficacy was observed for copper-based treatments. Nevertheless, an interesting effect of low copper strategies with two CTPs was observed at the end of July on leaves, where disease severity was significantly lower than in the untreated control, but higher than in the full copper reference. However, no control of the disease was observed on grape bunches.

FIELD TRIAL RESULTS IN POTATO/LATE BLIGHT

Trials in potato against late blight were carried out in Poland, France and in Germany. In the majority of trials, at least two varieties with different susceptibility to late blight were used, and the effects of CTPs as stand-alone applications or in combination with other CTPs or with low doses of copper were assessed. Again, in France only CTPs with ADE permission could be assessed.

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Results in Poland

In 2012 and in 2013, no significant differences in leaf infection between any of the treatments were noted in the susceptible cv. `Ditta´. However, in 2013, the highest yield (25 t/ha) was obtained after treatments with an elicitor applied together with a half dose of copper (3.0 kg/ha and year). None of the CTPs had negative influence on the presence of ladybirds.

In 2014, a potato cv. with medium resistance to late blight (`Sante´) was treated with CTPs (CO-FREE strategy) and compared for disease control to a susceptible cv. (`Ditta´) treated with 3.5 kg/ha and year Cu (reference strategy). No copper reference was included in `Sante´. Disease pressure was high, and the highest total yields in `Sante´ were obtained in the CO-FREE strategy based on stand-alone treatments with either a bacterial preparation of *Aneurinibacillus migulanus* or an elicitor (6715B). In total, 34.3 and 34.1 t/ha, equalling ca. 40% yield increase over the controls were achieved for the treatments, respectively.

In 2015, CTPs as stand-alone treatments or followed by low doses of copper were compared for control of late blight in the cv. `Ditta´ and the highly susceptible cv. `Lord´. In `Ditta´, combined treatments with *A. migulanus* and 6715B reduced leaf infection by the pathogen, as well as the programme in which the elicitor was followed by three copper sprays at 0.25 kg/ha and year, each. The highest yield in `Ditta´ (20.5 t/ha) was obtained for the elicitor in combination with low copper doses. In cv. `Lord´ treatments with the elicitors 6715B or 19-21E combined with *A. migulanus* did not reduce disease severity. However, the treatments increased the yield compared to the untreated control by almost 20%, to 16.8 and 17.1 t/ha, respectively.

Results in France

CTPs were tested for their efficacy against late blight in 2012-2015 under high disease pressure in the oceanic environment of western France. The trials involved several potato cultivars with different levels of field resistance to the disease. The results showed that the CTPs tested here performed significantly lower than the copper reference and were not able to significantly reduce the disease spread under such conditions. They also showed that, contrary to initial expectations, cultivars responded in different ways and with different intensities to defense stimulation by potential elicitors. This differential response was independent of the field resistance level in untreated plots. This suggests that genotypic responses to elicitation should be part of designing new strategies for field control of the disease.

Results in Germany

Results from Brandenburg

In the field trials in Brandenburg (northeastern Germany), three different potato varieties (`Ditta´, `Jelly´ and `Allians´) were tested from 2012-2015. To indicate the spray start, simulations with the decision support system Öko-SIMPHYT were used. The alternative compounds were preventively applied 4 to 6 times at intervals of 7 to 10 days.

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In 2012, no difference in leaf infection with late blight between the CTPs COFREE24 and COFREE25 were found in the cv. `Ditta´. Considering the leaf area diseased by *Alternaria spp*., CO-FREE25 showed a significant reduction by 14%, about 50% of the efficacy after copper treatment (26%). The more resistant cv. `Jelly´ showed similar, however statistically not significant results at a lower disease level. Copper applications and the CTPs delayed late blight infestation for about 6 days and 3 days, respectively. The surplus in yield for copper treatments compared to the untreated control was 6 t/ha for `Ditta´ ($\alpha = 0.1$). The tested CTPs gave yield increases of up to 4 t/ha (statistically not significant). Field trials with cv. `Jelly´ showed negative yield effects compared to the untreated control after both, copper or CTP treatments.

In 2014, the mean yields were appropriately high (untreated control (untreated): cv. `Ditta´ 39.3 t/ha, cv. `Allians´ 37.4 t/ha). The test product CO-FREE24 showed no statistically significant influence on *Alternaria spp*. and late blight.

The year 2015 was dry and resulted in lower yields than in 2014 (untreated: cv. `Ditta´ 25.7 t/ha, cv. `Allians´ 28.9 t/ha). No late blight occurred in the trials and leaf losses were due to *Alternaria spp*. infections. The copper treatment as well as the combination of an elicitor (19-21E) + *A. migulanus* showed a non-significant reduction of infections.

Results from Hessen

A two-factorial field trial was performed in 2014 at an experimental farm in central Germany on two varieties (cv. `Vitabella´ resistant; cv. `Allians´ tolerant) for disease control. The elicitor F6715B was tested as CTP.

Phytophtora infestans developed moderately during the season. The first incidence of late blight was observed in early July. The strongest effect on the disease progress was related to the variety. Cv. `Vitabella´ was almost without disease symptoms throughout July, irrespective of any treatment, while the disease could develop in cv. `Allians´ to almost 100% in the control plots by the month's end. Copper treatments (in total 1 kg/ha and year) delayed the disease progress by approximately 3 days. The elicitor slightly delayed the disease progression between July 3rd and 14th. The disease reduction did not result in a significant yield increase in any of the treatments. However, the yield increase in copper treated plots was 5% in cv. `Allians´, while in cv. `Vitabella´ also F6715B showed a stimulating effect and increased potato yield by 6% compared to the untreated control.

In conclusion, it was shown that the resistant variety had the strongest impact on both disease and yield, including a slight yield increase with the elicitor F6715B. Copper was best performing in the more susceptible variety.

FIELD TRIAL RESULTS IN TOMATO/LATE BLIGHT

Field trials in tomato were performed under conditions conducive to high disease development in organic tomato production systems in mediterranean France from 2012-2015 and in IPM

tomato production systems in southern Greece from 2012-2014. In 2013-2015, novel strategies proposed under CO-FREE or inspired by a producer's survey and the results of experiments with stand-alone applications of CTPs were evaluated.

In France, the CTPs tested alone gave no disease control. No additional effect of the CTPs combined with a low copper dose was observed in 2012-2015. Interesting trends occurred in 2013 and 2014 with one alternative compound, suggesting the need of further investigation.

The most promising among the tested CTPs was the plant extract TRIFCOF-03 (tested in Greece in 2014). Disease pressure was extremely high and control plots showed 100% crop damage in early July. Even in this 'worst case scenario' treatments with this plant extract without copper or in a low copper strategy (0.9 kg/ha and year) still resulted in more than 50% disease control on leaves (yield was not assessed). The reduction was statistically similar to that of the full copper dose (8.1 kg/ha and year). Considering the efficacy achieved under such conditions, there are promising indications that copper use in tomato could be reduced with the use of such novel CTPs.

6. CONCLUSIONS

Substantial progress has been made in the development of copper reduction and replacement strategies, elucidating both, the potential and the limits of individual techniques. In CO-FREE, a variety of candidate compounds and low copper strategies with comparable effectiveness as full copper doses were identified. Since only a limited number of CTPs was tested in each crop in the field and since optimization with respect to formulation, application technique etc. in most cases was not yet finalized, further improvement is to be expected. However, copper remains difficult to replace, not only due to the techniques but also due to legal limitations and costs involved. From a present perspective, immediate and general phasing out of copper would create unbearable risks and costs (e.g. for tools or risk compensation). However, in the four pathosystems under investigation in CO-FREE, further reduction is achievable in most crops, and possibilities are seen highest in potato, followed by apple, tomato and grapevine. The key to the reduction/replacement of copper is the use of smart management strategies, which comprise a variety of approaches. Already farmers are looking more for disease resistant varieties, and combine them with reduced copper applications. CO-FREE is contributing to further copper reduction by having identified a number of CTP candidate compounds, by improved decision support systems (data not shown) and by having investigated strategies to be used in the crops.

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