Open-field trials for the control of apple scab conducted within the FP 7 Project CO-FREE in Italy and France

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Abstract

Within the FP 7 Project CO-FREE, several open-field trials were carried out on apple in Italy and France from 2013 to 2015. The trials aimed at evaluating the efficacy against the major fungal diseases of apple of novel plant protection products based on plant extracts, algae, and microorganisms, and their crop safety. None of the new products tested showed levels of disease control, which can be considered comparable to those of the commonly used reference fungicides based on copper, lime sulphur, hydrogen carbonates, and acid clays. This emphasizes the importance of supporting the former products in the ongoing approval and registration procedures on both a European and national level, in order to have them available for crop protection without any additional restrictions. Nevertheless, it must be pointed out that not all the novel products that have been tested by other project partners in laboratory and greenhouse trials, have been evaluated also in the open field. Therefore, hopefully, one or more innovative organic plant protection products will be developed in the future.

Keywords: apple scab, Marssonina blotch, Alternaria blotch, Gloeosporium rot, Co-free, alternatives to copper

Introduction

At the moment, copper-based plant protection products are still of sound importance for effective fungal disease control in organic fruit production systems (Kiem *et al*., 2015). However, the European Union requires to promote sustainable and quality-oriented agriculture, and copper compounds have been included in the list of candidates for substitution due to the risk of soil contamination by copper. The development and evaluation of plant protection strategies, which allow to limit or avoid the use of copper-based plant protection products in organic farming systems, is therefore essential. Within the EU Project CO-FREE (www.co-free.eu), several innovative products of botanical and microbial origin have been tested since 2013 as possible tools for the reduction or even replacement of copper in organic farming. An international research consortium including 21 partners from 11 European countries, has been working on this topic in laboratory and open field trials from 2013 to 2015.

Herein we provide a summary of the results obtained in the open-field trials conducted by the Research Centre Laimburg (Italy), the Technical Institute for Organic Farming (ITAB, France), and the Research Group for Organic Farming (GRAB, France). The trials aimed at evaluating novel plant protection products for the control of apple scab (*Venturia inaequalis*), Gloeosporium rot (*Neofabraea alba*), Alternaria blotch (*Alternaria* spp.*),* and Marssonina blotch (*Marssonina coronaria*) in the open field. All tested products, developed within the CO-FREE project, were evaluated in comparison to reference plant protection products (Zemmer *et al*., 2002; Kelderer *et al*., 2006; Kunze *et al*., 2014) based on copper,

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hydrogen carbonates, lime sulphur, and acid clays. Furthermore, also potential new crop protection strategies were tested.

Material and Methods

The open-field trials were carried out from 2013 to 2015 in several apple orchards located in Italy and France. A detailed description of the study orchards is provided in Table 1.

Table 1: characteristics of the Italian and French study orchards.

Experimental orchards									
Partner	**Year**	**Disease**	**Location**	**Experimental design**	**Cultivar**	**Rootstock**	**Plant spacing in m**	**Canopy height in m**	**Planting year**
RC Laimburg Italy	2013	Primary scab	Pfatten, Block 64/2	4 blocks / 10 trees	Golden Del.	M 9	3.0 x 0.8	3	1993
		Secondary scab	Pfatten, Block 71	4 blocks / 10 trees	Golden Del.	M 9	3.2 x 0.9	3.5	2001
		Gleosporium	Tramin, (privat orchard)	4 blocks / 6 trees	Pinova	M 9	3.0 x 0.9	3	2005
		Marsssonina Alternaria	Auer, (privat orchard)	2 blocks / 10 trees	Gold Rush	M 9	3.2 x 1.0	3.5	2003
	2014	Primary scab	Pfatten, Block 52	4 blocks / 10 trees	Golden Del.	M 9	3.1 x 1.0	3	1997
		Secondary scab	Pfatten, Block 51	4 blocks / 10 trees	Golden Del.	M 9	3.2 x 0.8	3	2009
	2015	Primary scab	Pfatten, Block 103	4 blocks / 10 trees	Fuji	M 9	3.2 x 1.4	3	2007
		Secondary scab	Pfatten, Block 92	4 blocks / 10 trees	Cripps Pink	M 9	3.0 x 1.0	3	2005
Itab / Grab France	2013	Primary scab	Renage	4 blocks / 12 trees	Golden Del.	Pajam 2	2.5 x 5.0	3	1988
	2014	Scab (prim+sec)	Renage	4 blocks / 12 trees	Golden Del.	Pajam 2	2.5 x 5.0	3	1988
	2015	Scab (prim+sec)	Saint Marcel les Valence	2 Blocks / 10 trees	Golden Del.	M 111	1.5 x 5.0	2	2014
		Scab (prim+sec)	Saint Marcel les Valence	2 Blocks / 10 trees	Fuji	M 111	1.5 x 5.0	2	2014

Except for the trials conducted in 2015 in France, in all trials the tested treatments were applied using motorized spraying equipment, very similar to that commonly used by growers. Details on the application equipment and techniques used are reported in Table 2.

Table 2: Description of the application techniques used in the field trials conducted in Italy and France.

Application technique								
Partner	**Year**	**Type of sprayer**	**speed in km/h**	**eingine turns in rpm**	**pressure in bar**	**ventilator speed**	**valves**	**water volume/ha**
RC Laimburg Italy	2013	Waibl (trasverse current blower)	3.8	1300	6	low speed	Albuz green	1hl/m canopy height
	2014	Waibl (trasverse current blower)	3.8	1300	6	low speed	Albuz green	1hl/m canopy height
	2015	Waibl (trasverse current blower)	3.8	1300	6	low speed	Albuz green	1hl/m canopy height
Itab / Grab France	2013	KWH (Mistral 2X90)	4.0	1500	1.5	high speed	KWH standard	8 hl
	2014	KWH (Mistral 2X90)	4.0	1500	1.5	high speed	KWH standard	8 hl
	2015	Manual air sprayer	-	-	-	no	no	10 hl

The products, crop protection strategies, and application rates tested in the trials against primary and secondary infections of apple scab in Italy and France from 2013 to 2015 are listed in Tables 3-6. Depending on their mode of action, the products developed within the CO-FREE project were applied weekly (plant strengtheners) or just before rainfall (preventative fungicides), as recommended by the manufacturers. Those products, which were supposed to have also a certain curative action, were applied curatively during the period of spore germination of apple scab, determined by using the forecast models scab stop or RimPro 300. All CO-FREE products were tested in comparison to reference fungicides commonly used in organic farming systems for apple scab control (i.e. lime sulphur, copper compounds, hydrogen carbonates). The reference products were applied either according to label instructions or at lower rates, in case reduced label rates are a common and well-established practice in organic farming.

The products, strategies, and application rates tested against Gloeosporium rot on the cultivar Pinova, and against Marssonina and Alternaria blotch on the cultivar GoldRush® in 2013 in Italy are listed in Table 7 and 8.

Table 3: description of the products, crop protection strategies, and application rates tested against primary apple scab infections in Italy from 2013 to 2015.

Treatments applied against scab primary apple scab infections				
Partner	**Year**	**Product**	**Strategy**	**Dose/hl**
RC Laimburg Italy	2013	CoFree A2	weekly	32 g
		CoFree A2 + Lime sulphur	weekly + scab stop at RimPro 300	32 g + 1500 g
		CoFree A2	weekly + 500 - 600 dh./curativ on dry leaf	32 g
		CoFree 24 + Sulphur	2-3 days before rain	75 g + 300 g
		CoFree 24-2 + Nu-Film-P	2-3 days before rain	75 g + 30g
		CoFree 24	2-3 days before rain	75 g
		CoFree 52A73	weekly	400 ml
		CoFree 6715A	weekly	400 ml
		CoFree EN02-Y + Nu-Film-P	weekly	100 g + 30 g
		Sulphur	2-3 days before rain	300 g
		Poltiglia disperss	2-3 days before rain	4 g metallic Cu
		Untreated	-	-
	2014	Poltiglia dispress	2-3 days before rain	10 g metallic Cu
		$NaHCO_3$ + Lime sulphur	scab stop - weak infection scab stop - strong infection	1000 g 1500 g
		CoFree 19/01(4x) - and after CoFree 19/04 + $NaHCO_3$ + Lime sulphur	weekly scab stop - weak infection scab stop - strong infection	19/01 (166 ml) 19/04 (66,6 ml) 1000 g 1500 g
		$NaHCO_3$ + $NaHCO_3$	scab stop - weak infection scab stop - strong infection	1000 g 1000 g
		CoFree 19/01(4x) - and after CoFree 19/04 + $NaHCO_3$ + $NaHCO_3$	weekly scab stop - weak infection scab stop - strong infection	19/01 (166 ml) 19/04 (66,6 ml) 1000 g 1000 g
		$NaHCO_3$ + $NaHCO_3$ + CoFree A2	scab stop - weak infection scab stop - strong infection curative	1000 g 1000 g 39 g
		CoFree 19/01(4x) - and after CoFree 19/04 + $NaHCO_3$ + $NaHCO_3$ + CoFree A2	weekly scab stop - weak infection scab stop - strong infection curative	19/01 (166 ml) 19/04 (66,6 ml) 1000 g 1000 g 32 g
		CoFree 19/03	weekly	166 ml
		CoFree 19/05	weekly	166 ml
		Untreated	-	-
	2015	Poltiglia dispress	2-3 days before rain	5 g metallic Cu
		CoFree 19/16	weekly	500 ml
		CoFree 19/19	weekly	500 ml
		CoFree 19/21	weekly	500 ml
		CoFree A2	curative on dry leaf	25 g
		Lime sulphur + CoFree A2	2-3 days before rain curative on dry leaf	1500 g 25 g
		Lime sulphur	2-3 days before rain	1500 g
		Lime sulphur	300 dh./curative on dry leaf	1500 g
		Untreated	-	-

Table 4: description of the products, crop protection strategies, and application rates tested against primary apple scab infections in France from 2013 to 2015.

Treatments applied against scab primary apple scab infections				
Partner	**Year**	**Product**	**Strategy**	**Dose/hl**
Itab / Grab France	2013	Farmer reference: Copper sulfate Sulfur Lime sulphur	2-3 days before rain	1500 g 1000 g 1800 g
		Farmer reference + 6715A	2-3 days before rain + weekly	250 ml
		52A73	weekly	250 ml
		A5215	weekly	250 ml
		928B7	weekly	250 ml
	2014	Farmer reference: Copper sulfate KHCO3 Lime sulphur	2-3 days before rain weak infection strong infection	300 g metallic Cu 375 g 2250 g
		CoFree 19/03 alone (x3) and then associated with Farmer reference strategy	weekly and according to infection risk level	188 ml
		CoFree 19/01 alone (x3) and then associated with Farmer reference strategy	weekly and according to infection risk level	188 ml
		KHCO3 alone (x3) and then CoFree 19/03 + KHCO3 or Lime sulphur	weekly and according to infection risk level	188 ml
		KHCO3 and then Farmer reference strategy	2-3 days before rain and according to infection risk level	375 g
	2015	Reference: Copper sulfate KHCO3 Lime sulphur	2-3 days before rain weak infection strong infection	250 g metallic Cu 375 g 1250 g
		CoFree 19/16D + KHCO3+Sulphur or Lime sulphur	weekly weak infection strong infection	1000 ml 375 g + 360 g 1250 g
		CoFree 19/21 + KHCO3+Sulphur or Lime sulphur	weekly weak infection strong infection	1000 ml 375 g + 360 g 1250 g

Table 5: description of the products, crop protection strategies, and application rates tested against secondary apple scab infections in Italy from 2013 to 2015.

Treatments applied against scab secondary apple scab infections				
Partner	**Year**	**Product**	**Strategy**	**Dose/hl**
RC Laimburg Italy	2013	CoFree A2-1	weekly	32 g
		CoFree A2-2	after rain, if Rim shows infestation on fruits	32 g
		CoFree 24-2	2-3 days before rain	75 g + 30 g NuFilm
		CoFree 52A73	weekly	400 ml
		CoFree 6715A	weekly	400 ml
		CoFree EN02-Y	weekly	100 g + 30 g NuFilm
		Poltiglia disperss	2-3 days before rain	10 g metallic Cu
		Vitisan	after rain, if Rim shows infestation on fruits	1000 g
		Limesulphur	after rain, if Rim shows infestation on fruits	1000 g
		Untreated	-	-
	2014	Poltiglia disperss	2-3 days before rain	10 g metallic Cu
		$NaHCO_3$	after rain, if Rim shows infestation on fruits	1000 g
		$NaHCO_3$	after rain, if Rim shows infestation on fruits	500 g
		Armicarb 85	after rain, if Rim shows infestation on fruits	500 g
		Vitisan	after rain, if Rim shows infestation on fruits	500 g
		CoFree 19/05 + $NaHCO_3$	weekly + after rain, if Rim shows infestation on fruits	200 ml 500 g
		Untreated	-	-
	2015	Poltiglia disperss	weekly	10 g metallic Cu
		CoFree 19/21B + Poltiglia disperss	weekly	500 g 10 g
		CoFree A2	after rain, if Rim shows infestation on fruits	32 g
		Untreated	-	-

Table 6: description of the products, crop protection strategies, and application rates tested against secondary apple scab infections in France in 2014 to 2015.

Treatments applied against scab secondary apple scab infections				
Partner	**Year**	**Product**	**Strategy**	**Dose/hl**
Itab / Grab France	2014 Fuji	Reference: Copper sulfate KHCO3 Lime sulphur	2-3 days before rain weak infection strong infection	300 g metallic Cu 375 g 2250 g
		CoFree 19/03 alone (x3) and then associated with Farmer reference strategy	weekly and according to infection risk level	188 ml
		CoFree 19/01 alone (x3) and then associated with Farmer reference strategy	weekly and according to infection risk level	188 ml
		KHCO3 alone (x3) and then CoFree 19/03 + KHCO3 or Lime sulphur	weekly and according to infection risk level	188 ml
		KHCO3 and then Farmer reference strategy	2-3 days before rain	375 g
	2015 Golden	Reference: Copper sulfate KHCO3 Lime sulphur	2-3 days before rain weak infection strong infection	250 g metallic Cu 375 g 1250 g
		CoFree 19/16D + KHCO3+Sulphur or Lime sulphur	weekly weak infection strong infection	1000 ml 375 g + 360 g 1250 g
		CoFree 19/21 + KHCO3+Sulphur or Lime sulphur	weekly weak infection strong infection	1000 ml 375 g + 360 g 1250 g

Table 7: description of the products, strategies, and application rates tested against Gloeosporium rot on the cultivar Pinova in 2013 in Italy.

Treatments Gloeosporium 2013				
Partner	**Year**	**Product**	**Strategy**	**Dose/hl**
RC Laimburg	2013	Ulmasud	weekly	1000 g
		CoFree A 2	weekly	32 g
		Untreated	-	-

Table 8: description of the products, strategies, and application rates tested against Marssonina and Alternaria blotch on the cultivar Gold Rush® in 2013 in Italy.

Treatments Marssonina/Alternaria 2013				
Partner	**Year**	**Product**	**Strategy**	**Dose/hl**
RC Laimburg	2013	Poltiglia disperss	weekly	10 g metallic Cu
		Lime sulphur	weekly	800 g
		Ulmasud	weekly	1000 g
		Armicarb	weekly	300 g
		CoFree A2	weekly	170 g
		CoFree 24-2	weekly	75 g
		CoFree 6715A	weekly	400 g
		CoFree EN02-Y	weekly	100 g
		Untreated	-	-

All the assessments were done on a representative number of leaves and fruits per plot at the end of the period of occurrence of primary infections in the trials against primary scab infections, at harvest in the trials against secondary scab infections, Marssonina and Alternaria and after 6 months of storage at commercial storage conditions, commonly used in South Tyrol in the trials against Gloeosporium.

Statistical analysis

In each trial, data were compared across treatments using 1-way ANOVAs, followed by Tukey's HSD test for post-hoc comparisons of means ($P<0.05$). All analyses were performed with the statistics software IBM SPSS 20 Statistics.

Results

Mean values of percent apple scab incidence on leaves and fruits and phytotoxicity on leaves and fruits (scale: 0-10), recorded for the different treatments in the trials conducted in Italy against primary apple scab infections from 2013 to 2015, are reported in Table 9.

In 2013, disease pressure was extremely high. In addition, the severity of one late-season primary infection had been underestimated, and therefore apple scab incidence and severity was very high in all treatments. Only lime sulphur resulted in a significant reduction of disease incidence on both fruits and leaves. Also the copper-based treatment Poltiglia disperss applied at a reduced application rate (4 g/hl metallic copper) and CoFree A2, when applied weekly and curatively (and not just weekly) significantly reduced scab incidence on leaves in comparison to all the other treatments tested, but not on fruits. In 2014, the products developed within the CO-FREE project applied by themselves did not show satisfactory efficacy in reducing apple scab incidence on leaves and fruits. A significant reduction in disease incidence in comparison to the untreated control was only achieved with CO-FREE products used in a strategy, i.e. in combination with lime sulphur and/or $NaHCO_3$. The levels of apple scab control on leaves and fruits of these strategies were similar to those of Poltiglia disperss applied at 10 g/hl metallic copper. The phytotoxic

symptoms on leaves (leaf burn) were due to repeated applications of $NaHCO_3$ at a high rate on wet leaves. In 2015, apple scab pressure was very low, especially on fruits, and therefore differences among treatments in scab incidence on fruits failed significance. Slightly higher disease incidence levels occurred on leaves, and the reference products lime sulphur and Poltiglia disperss showed complete or almost complete disease control on leaves. The results of the trials conducted in France against primary apple scab infections from 2013 to 2015, are reported in Table 10. Since trials took place on private farms, no untreated control treatment had been included in the study design. Therefore, the efficacy of the tested strategies was compared to a Farmer's reference, *i.e.* a common strategy used by farmers in organic apple commercial plots.

In 2013, the „Farmer's reference strategy" showed lowest apple scab incidence levels on both leaves and fruits. In 2014, no statistically significant differences among treatments emerged, and numerically lowest infection levels were again recorded for the „Farmer's reference strategy". In 2015, disease pressure was extremely low, and differences among treatments failed significance.

As far as efficacy trials against secondary apple scab infections are concerned, it must be pointed out that in general the conduct of these trials is difficult for the following reasons: very often disease pressure at the beginning of the period of occurrence of secondary infections is too low for results to be considered conclusive; if, instead, the initial infection level is already very high, the efficacy of the products allowed in organic farming for apple scab control is limited, and comparisons among treatments may become complicated.

The results of the efficacy trials against secondary apple scab infections carried out in Italy from 2013 to 2015 are reported in Table 11.

In 2013, initial disease pressure was extremely high. Disease incidence on leaves was lowest in the untreated control, but this was because of the heavy leaf drop due to extreme disease levels that occurred in this treatment just before the assessment. No phytotoxic symptoms were observed in any of the tested treatments in 2013. Under these conditions, CoFree 24-2 and CoFree 52A73 showed the same disease control potential as Poltiglia disperss at 10g/hl metallic copper. In 2014, differences among treatments failed significance due to too low a disease pressure. In 2015, no conclusive results were obtained for leaves, while apple scab incidence on fruits reached 34 % in the untreated control, and the copper-based product Poltiglia disperss at 10 g/hl metallic copper showed highest efficacy in reducing fruit infections. In 2015, the percentage of fruits affected by phytotoxic symptoms was significantly higher in the Poltiglia disperss-based treatment. However, it must be considered that the product had been applied weekly. Therefore, care should be taken when making repeated and frequent applications of exclusively this product for apple scab control.

The results of the efficacy trials against secondary apple scab infections carried out in France in 2014 and 2015 are summarized in Table 12. As for the trials against primary infections, no significant efficacy of the alternative compounds tested was observed. This can be explained in 2014 by a very high initial apple scab infection, making scab control very challenging on susceptible cultivar. Conversely, scab pressure was very low in 2015 despite irrigation by an overhead sprinkler to enhance scab development. A slight trend of the farmer's reference strategy showing highest efficacy seems to exist. As for the trials against primary infections, also for these trials it is difficult to draw conclusions, because no untreated control treatment had been included in the study design on private farms. In all trials, differences among treatments failed significance. The reasons were too high an initial apple scab infection in 2014, and too low a disease pressure in 2015. A slight trend of the farmer's reference strategy showing highest efficacy seems to exist.

Table 9: Apple scab incidence (%) and phytotoxicity (scale: 0-10) on leaves and fruits in the different treatments and trials conducted in Italy from 2013 to 2015 during the period of primary infections. Different letters within the same column and trial indicate statistically significant differences (Tukey's test: P<0.05).

Results apple scab - primary infections						
Partner	Year	Product	Phytotox (0-10)		Incidence in %	
			leafs	fruits	leafs	fruits
RC Laimburg Italy	2013	CoFree A2	0	0	84.7 c	100.0 b
		CoFree A2 + Lime sulphur	0	0	16.3 a	79.5 a
		CoFree A2	0	0	71.7 b	100.0 b
		CoFree 24 + Sulphur	0	0	83.9 c	100.0 b
		CoFree 24-2 + Nu-Film-P	0	0	89.0 cd	100.0 b
		CoFree 24	0	0	83.3 c	100.0 b
		CoFree 52A73	0	0	85.5 c	100.0 b
		CoFree 6715A	0	0	84.4 c	100.0 b
		CoFree EN02-Y + Nu-Film-P	0	0	89.2 cd	100.0 b
		Sulphur	0	0	86.2 c	100.0 b
		Poltiglia disperss	0	0	73.9 b	98.5 b
		Untreated	0	0	94.3 d	100.0 b
	2014	Poltiglia disperss	0 a	3 b	6.4 b	3.8 a
		$NaHCO_3$ + Lime sulphur	8 b	1 a	3.6 ab	7.5 a
		CoFree 19/01(4x) - and after CoFree 19/04 + $NaHCO_3$ + Lime sulphur	8 b	2 ab	1.5 a	7.5 a
		$NaHCO_3$ + $NaHCO_3$	8 b	2 ab	1.3 a	7.5 a
		CoFree 19/01(4x) - and after CoFree 19/04 + $NaHCO_3$ + $NaHCO_3$	10 c	2 ab	1.0 a	11.9 a
		$NaHCO_3$ + $NaHCO_3$ + CoFree A2	9 c	2 ab	0.7 a	10.6 a
		CoFree 19/01(4x) - and after CoFree 19/04 + $NaHCO_3$ + $NaHCO_3$ + CoFree A2	10 c	2 ab	1.6 a	3.1 a
		CoFree 19/03	0 a	2 ab	42.2 c	31.3 b
		CoFree 19/05	0 a	2 ab	40.0 c	20.6 ab
		Untreated	0 a	2 ab	42.9 c	37.5 b
	2015	Poltiglia disperss	0 a	1 a	1.0 a	0.3 a
		CoFree 19/16	0 a	1 a	25.9 c	2.4 a
		CoFree 19/19	0 a	1 a	15.3 bc	0.0 a
		CoFree 19/21	0 a	1 a	11.1 ab	1.3 a
		CoFree A2	0 a	1 a	9.0 ab	1.3 a
		Lime sulphur + CoFree A2	2 b	1 a	0.0 a	0.0 a
		Lime sulphur	1 b	1 a	0.0 a	0.0 a
		Lime sulphur	1 b	1 a	0.0 a	0.0 a
		Untreated	0.0 a	1 a	11.8 ab	3.7 a

Table 10: Apple scab incidence (%) and phytotoxicity (scale: 0-10) on leaves and fruits in the different treatments and trials conducted in France from 2013 to 2015 during the period of primary infections. Different letters within the same column and trial indicate statistically significant differences (Tukey's test: P<0.05).

Results apple scab - primary infections						
Partner	Year	Product	Phytotox in %		Incidence in %	
			leafs	fruits	leafs	fruits
Itab / Grab France	2013	Farmer's reference: Copper sulfate Sulfur Lime sulphur	0	0	3.4 a	16.2 a
		Farmer's reference: + 6715A	0	0	17.7 b	54.7 b
		52A73	0	0	49.7 d	73.1 c
		A5215	0	0	46.2 d	81.3 c
		928B7	0	0	34.5 b	74.2 c
	2014	Farmer's reference:	0	0	3.9 a	4.7 a
		CoFree 19/03 alone (x3) and after associated with Farmer reference strategy	0	0	7.9 a	12.5 a
		CoFree 19/01 alone (x3) and after associated with Farmer reference strategy	0	0	8.9 a	7.0 a
		KHCO3 alone (x3) and after CoFree 19/03 + KHCO3 or Lime sulphur	0	0	11.3 a	9.8 a
		KHCO3 and after Farmer reference strategy	0	0	5.3 a	0.4 a
	2015 Golden	Reference: Copper sulfate KHCO3 Lime sulphur	0	0	0.4 a	-
		CoFree 19/16D + KHCO3+Sulphur or Lime sulphur	0	0	1.6 a	-
		CoFree 19/21 + KHCO3+Sulphur or Lime sulphur	0	0	1.7 a	-
	2015 Fuji	Reference: Copper sulfate KHCO3 Lime sulphur	0	0	0.1 a	-
		CoFree 19/16D + KHCO3+Sulphur or Lime sulphur	0	0	1.5 a	-
		CoFree 19/21 + KHCO3+Sulphur or Lime sulphur	0	0	1.1 a	-

Table 11: Apple scab incidence (%) and percent phytotoxicity (%) on leaves and fruits in the different treatments and trials conducted in Italy from 2013 to 2015 during the period of secondary infections. Different letters within the same column and trial indicate statistically significant differences (Tukey's test: P<0.05).

Results apple scab - secondary infections						
Partner	Year	Product	Phytotox in %		Incidence in %	
			leafs	fruits	leafs	fruits
RC Laimburg Italy	2013	CoFree A2-1	0	0	18.9 ab	9.3 a
		CoFree A2-2	0	0	14.3 ab	4.3 a
		CoFree 24-2	0	0	24.7 b	4.1 a
		CoFree 52A73	0	0	26.3 b	7.4 a
		CoFree 6715A	0	0	20.9 ab	5.6 a
		CoFree EN02-Y	0	0	17.9 ab	5.5 a
		Poltiglia disperss	0	0	26.4 b	8.2 a
		Vitisan	0	0	24.5 ab	8.3 a
		Lime sulphur	0	0	20.8 ab	6.1 a
		Untreated	0	0	12.5 a	5.7 a
	2014	Poltiglia disperss	27.5 b	17.1 a	0.3 a	0.0 a
		$NaHCO_3$	11.3 a	14.7 a	0.0 a	0.0 a
		$NaHCO_3$	7.5 a	16.2 a	0.3 a	0.0 a
		Armicarb 85	5.0 a	12.4 a	0.3 a	0.0 a
		Vitisan	5.0 a	15.7 a	0.1 a	0.0 a
		CoFree 19/05 + $NaHCO_3$	7.5 a	15.6 a	0.3 a	0.0 a
		Untreated	5.0 a	13.3 a	0.6 a	0.0 a
	2015	Poltiglia disperss	55.0 a	40.0 b	29.2 ab	8.1 a
		CoFree 19/21B + Poltiglia dispress	42.5 a	10.4 a	33.1 b	13.5 ab
		CoFree A2	43.8 a	9.3 a	25.8 ab	37.2 c
		Untreated	58.8 a	0.9 a	24.9 a	33.6 bc

Table 12: Apple scab incidence (%) and percent phytotoxicity (%) on leaves and fruits in the different treatments and trials conducted in France in 2014 and 2015 during the period of secondary infections. Different letters within the same column and trial indicate statistically significant differences (Tukey's test: P<0.05).

Results apple scab - secondary infections						
Partner	Year	Product	Phytotox in %		Incidence in %	
			leafs	fruits	leafs	fruits
Itab / Grab France	2014	Ref. strat: Copper sulf., KHCO3, Lime sulphur	0	0	22.8 a	88.3 a
		CoFree 19/03 alone (x3) then associated w. ref. strat.	0	0	42.5 a	96.9 a
		CoFree 19/01 alone (x3) then associated w. ref. strat.	0	0	37.8 a	94.5 a
		KHCO3 alone (x3) then CoFree 19/03 + KHCO3 or LS	0	0	34.8 a	93.8 a
		KHCO3 then associated w. ref. strat.	0	0	22.0 a	85.2 a
	2015 Golden	Ref. Strat: Copper sulf. KHCO3 Limesulphur	0	0	0.8 a	-
		CoFree 19/16D + KHCO3+Sulphur or Lime sulphur	0	0	5.2 a	-
		CoFree 19/21 + KHCO3 + Sulphur or Lime sulphur	0	0	3.5 a	-
	2015 Fuji	Ref. strat: Copper sulf., KHCO3, Lime sulphur	0	0	0.1 a	-
		CoFree 19/16D + KHCO3+Sulphur or Lime sulphur	0	0	1.2 a	-
		CoFree 19/21 + KHCO3 + Sulphur or Lime sulphur	0	0	3.5 a	-

After 6 months of commercial storage, Gloeosporium rot incidence on fruits of the cultivar Pinova was significantly lower in fruits treated with the acid clay-based product Ulmasud than in untreated control fruits and in fruits treated with the product developed within the CO-FREE project (Table 13). Gloeosporium rot incidence on fruits treated with the Co-Free product was comparable to that on untreated control fruits.

The studies for the efficacy evaluation of different treatments against Marssonina blotch and Alternaria blotch were both conducted in 2013 in Italy on the cultivar Gold Rush®, and results are reported in Table 14 and 15, respectively. Marssonina blotch incidence on fruits was very low, and no statistically significant differences among treatments emerged. A statistical comparison across treatments for Marssonina blotch incidence on leaves was not possible, because data assessments were made in only 2 replicates per treatment. However, numerically lowest leaf infection levels were recorded for the commonly used reference products Ulmasud, Poltiglia disperss and lime sulphur.

Differences among treatments in Alternaria blotch incidence on fruits failed significance, but also in this trial numerically lowest mean values of disease incidence were recorded for the commonly used reference products.

Table 13: Gloeosporium rot incidence (%) on fruits of the cultivar Pinova in the different treatments tested in 2013 in Italy. Different letters indicate statistically significant differences (Tukey's test: P<0.05).

Results Gloeosporium 2013			
Partner	**Year**	**Product**	**Incidence in %**
RC Laimburg	2013	Ulmasud	10.3 a
		CoFree A 2	55.2 b
		Untreated	57.6 b

Table 14: Marssonina blotch incidence (%) on leaves and fruits of the cultivar GoldRush® in the different treatments in 2013 in Italy. Different letters indicate statistically significant differences (Tukey's test: P<0.05).

Results Marssonina				
Partner	**Year**	**Product**	**Incidence on fruits in %**	**Incidence on leafs in % ***
RC Laimburg	2013	Poltiglia disperss	0.0 a	15.7
		Lime sulphur	1.0 a	48.2
		Ulmasud	0.0 a	14.9
		Armicarb 85	4.1 a	86.1
		CoFree A2	0.0 a	100.0
		CoFree 24-2	0.0 a	99.3
		CoFree 6715A	2.0 a	99.4
		CoFree EN02-Y	0.0 a	99.6
		Untreated	2.0 a	99.4

Table 15: Alternaria blotch incidence (%) on fruits of the cultivar GoldRush® in the different treatments in 2013 in Italy. Different letters indicate statistically significant differences (Tukey's test: P<0.05).

Results Alternaria			
Partner	**Year**	**Product**	**Incidence in %**
RC Laimburg	2013	Poltiglia disperss	4.1 a
		Lime sulphur	5.1 a
		Ulmasud	3.1 a
		Armicarb 85	10.2 a
		CoFree A2	21.4 a
		CoFree 24-2	20.4 a
		CoFree 6715A	16.3 a
		CoFree EN02-Y	20.4 a
		Untreated	12.2 a

Discussion

The results of trials conducted under open field conditions depend on several factors. Especially climatic conditions can strongly influence occurrence and severity of fungal diseases, and disease levels may not be adequate for results to be considered reliable and conclusive.

In 2013, apple scab pressure was extremely high in both Italy and France, in part also due to a misjudgement of primary infections by well-established forecast models, which occurred towards the end of the period of primary infections. High apple scab pressure was present also in the study orchards. Even though the commonly used reference products did not provide adequate disease control, there was one CO-FREE product comparable in efficacy to Poltiglia disperss, both in primary and secondary season (Co-Free A2). In 2013, some of these new experimental products were tested also against Marssonina blotch, Alternaria blotch and Gloeosporium rot, but none of them showed acceptable efficacy levels, comparable to those achieved with the commonly used reference products based on copper, lime sulphur, and acid clays.

In 2014, medium levels of primary apple scab infections occurred in the study orchards. The tested CO-FREE products, used by themselves or applied in combination with conventional plant protection products, did not enhance the performance of the reference control strategies. During summer 2014, 2 additional trials were carried out for the control of secondary apple scab infections, 1 in Italy and 1 in France. In the Italian trial, disease pressure was too low for results to be considered conclusive. In the French trial, instead, disease pressure was extremely high, and the novel products, tested in combination with reference products, did not seem to provide any improvement of the effects of the control strategies.

In 2015, primary infections of apple scab resulted in very limited disease pressure in both Italy and France. Under these trial conditions, the reference products provided adequate disease control, while on leaves the CO-FREE products again failed to do so, both when used by themselves. On fruits, even though no statistical differences were found for any treatment, disease was kept at 0 % by Co-Free 19/19, which was the same as for the standard treatments. Due to low infection pressure during the period of secondary apple scab infections, no conclusions were possible in the studies conducted in France. In Italy, only one CO-FREE product was evaluated for its efficacy against secondary apple scab infections, but no satisfactory results were achieved on fruits. On leaves, both, the standard treatment and the test product did not show any reduction of scab incidence.

However, the study was conducted on the cultivar Cripps Pink, and it is well-known that in organic farming, on this cultivar, apple scab control is extremely challenging.

It must be pointed out that only a limited number of products that have been developed within the research project, has also been tested in the open field. The likelihood exists that at least some of the tested new products did not provide adequate disease control in the open field due to the fact that formulations were not advanced enough by the time of testing. For other products, companies were not able to up-scale production in time, and thus to produce the amounts required for practically relevant field trials(i.e. applications with spray equipment, very similar to that commonly used by growers). In some trials, CO-FREE agents showed promising and comparable results to copper (CoFree A2, CoFree 24-2 and CoFree19/19), but further development appears necessary for final use in practice. Finally, The sector Organic Farming of the Research Centre Laimburg and ITAB/GRAB are still willing and interested in evaluating new plant protection products for the control of fungal diseases, and in developing new methods and tools (including cropping systems and cultivar susceptibilities), which allow for a reduction in the use of copper compounds.

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