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FULL RESEARCH PAPER

Control of late blight in organic potato production: evaluation of copper-free preparations under field, growth chamber and laboratory conditions

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Abstract In order to replace copper fungicides in organic potato production, 53 copper-free preparations (CFPs) based on natural compounds, including plant extracts and microorganisms, and five copper preparations were evaluated for their potential to control *Phytophthora infestans*, the pathogen that causes late blight of potatoes. In in vitro assays, 30% of the CFPs inhibited indirect germination of sporangia, 26% mycelial growth and in growth chamber experiments, 21% efficiently reduced foliar blight of tomato plants. In micro-plot field trials with applications twice a week, the copper preparations were the most effective and reduced foliar blight by 99%. Of the CFPs tested, Oekofluid P, Mycosin and other sulphuric clays, and C-2000 reduced late blight the most, from 63% to 37%. In small-plot trials in 2001, 2002 and 2004, 27 CFPs with different formulations and four copper preparations were examined. In 2004, copper preparations at full and reduced rates and sulphuric clays were applied either weekly or according to the decision support system Bio-PhytoPRE. With Bio-PhytoPRE, copper preparations reduced foliar blight of potatoes by 23–77% and increased tuber yield by 2–28%, depending on the copper rate applied and year. With CFPs, maximal efficacy was 17% and no effect on tuber yield was observed. In vitro and in vivo trials showed that the rainfastness and the persistence of CFPs was low compared with copper preparations. This indicates that the failure of CFPs under field conditions is probably due to a lack of stability under prevailing environmental conditions and not to a lack of efficacy. Until stable formulations for CFPs are developed, an optimised and restricted use of copper fungicides using a decision support system could help to control late blight in organic potato production and to reduce copper input into the environment.

Keywords Copper fungicides · Decision support system · Persistence · *Phytophthora infestans* · Plant extract · Rainfastness

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Introduction

Late blight of potato, caused by *Phytophthora infestans*, is one of the most devastating potato diseases worldwide. At present, only a limited number of moderately susceptible potato varieties meet consumer demands in Switzerland. Direct control of the disease requires regular use of fungicides to prevent infection of the potato foliage. In organic production, the most effective means of direct control are copper fungicides. With the exception of Scandinavian countries and the Netherlands, copper fungicides can still be used in European agriculture. Copper in trace amounts is essential for

metabolic processes in all organisms. However, copper persists in the environment and is therefore ecologically harmful (Flemming and Trevors 1989). Hence, a reduction or replacement of copper compounds in disease control is desirable. Decision support systems for late blight control, such as Bio-PhytoPRE, help to reduce the input of copper fungicides into the environment (Musa-Steenblock and Forrer 2005). For copper replacement, copper-free preparations (CFPs) based on natural compounds including plant extracts (Bowers and Locke 2004; Stephan et al. 2005), algae (Cannell 1993), lichens (Halama and van Haluwin 2004), and microorganisms such as *Trichoderma* spp. (Etebarian et al. 2000), *Bacillus* spp. (Fernando and Linderman 1995), and *Xenorhabdus* spp. (Ng and Webster 1997) proved to be effective against *P. infestans* or other *Phytophthora* species in laboratory and greenhouse experiments. In contrast, under field conditions, no or only minor effects on late blight with CFPs were observed (Blaeser et al. 2002). Furthermore, results from field trials often varied depending on the prevailing experimental conditions.

Since there is a lack of consistent field performance data for commercial and experimental CFPs, we focused on their field evaluation in the presented study. However, in the initial field trials, most of the CFPs performed poorly. Consequently to better appraise the potential of CFPs, in vitro and in vivo assays were integrated in the evaluation. In particular we used simple and rapid laboratory and growth chamber trials to evaluate the efficacy of CFPs to suppress *P. infestans*, and we determined in vitro the rainfastness and in vivo the persistence of CFPs. In the field, the efficacy of a selection of CFPs was examined in micro-plot trials with applications twice a week, in small-plot trials with regular applications once a week, and in small-plot trials with applications of CFPs and copper fungicides in reduced copper dosages according to the decision support system Bio-PhytoPRE.

Materials and methods

Selection of preparations

Preparations were selected based on the literature (Blaeser et al. 2002), previous field trials at Agroscope Reckenholz-Tänikon research station (ART) (Bassin and Forrer 2001; Krebs and Forrer 2001), and company information. Details on the preparations are given in Table 1. Five preparations contained copper, 53 were based on natural compounds, including plant extracts and microorganisms, and five were additives.

Preparation of plant extracts

In 2001, extracts of *Salvia officinalis*, *Potentilla erecta* and *Salix* ssp. were prepared according to Blaeser et al. (2002). One hundred grams of dried and ground plant material (Retsch mill, Retsch GmbH, Haan, Germany) was extracted in ethanol (70%) for 2 h in a water bath at 60°C. The broth was stirred continuously with a magnetic stirrer, filtered through cheesecloth, and stored at 4°C in the dark. The extracts were applied in the field trials in a concentration corresponding to 1% or 2% of dried plant material, and the concentration of ethanol in the spraying broth was 7% or 14% respectively. The extract of fresh *Rheum rhabarbarum* was prepared as follows: 0.5 kg of fresh leaves and stems were cut into pieces and soaked in 1 l of tap water for 4 h at 60°C. The broth was stirred from time to time. After cooling, the extract was filtered through cheesecloth and stored at −20°C. The extract of dry *R. rhabarbarum* was prepared as follows: fresh rhubarb leaves were dried for ten days at 35°C and cut up using a mill (Fuchsmühle, Vienna, Austria). Fifty grams of the ground plant material were extracted for 2 h in a water bath at 60°C in 1 l of tap water. The broth was stirred continuously with a magnetic stirrer, filtered through cheesecloth, and stored at 4°C in the dark. The extracts were prepared fresh the day before spraying.

In 2002 and 2004, modified extracts of *R. rhabarbarum* and *Solidago canadensis* were prepared in the following manner: 50 g of dried and ground leaf material were placed in 1 l of deionised water and stirred for 2 h with a magnetic stirrer at room temperature. The broth was filtered through cheesecloth and deionised water was added to obtain a total volume of 1 l. One gram of the additive methylcellulose was added to improve adhesion. These extracts were applied in a concentration corresponding to 5% of dried plant material. Extracts of *Ocimum basilicum*, *Artemisia annua*, *Sophora flavescens*, and *Malva silvestris* were prepared as follows: 50 g of

Table 1 Copper and copper-free preparations evaluated for efficacy against late blight, *Phytophthora infestans*, in laboratory, growth chamber, and field trials

Preparation group/Preparation name	Ingredients	Manufacturer/Distributor
Copper		
Kocide DF	Copper hydroxide (40% metallic copper)	Burri Agricide, Brügg, Switzerland
SPU-00880-F0-SC[a]	Copper, alginates and phosphonic acid (5% metallic copper)	Spiess-Urania Chemicals GmbH, Hamburg, Germany
Cueva	Copper octanoat (2% metallic copper)	Andermatt Biocontrol AG, Grossdietwil, Switzerland
Peptiram 5	Copper as amino acid complex (5% metallic copper)	Segetis, Vandouevres, Switzerland
Copper protein	Copper as protein complex (0.4% metallic copper)	Proagro GmbH, Abenberg, Germany
Sulphuric clays and minerals		
-Sulphuric clays ($Al_2(SO_4)_3$)		
Mycosin®	Aluminium sulphate, yeast component, horsetail extracts	Dr. Schaette AG, Bad Waldstadt, Germany
VPMS 99	As Mycosin, but other composition/formulation	Dr. Schaette AG, Bad Waldstadt, Germany
VPMS-W 2000	As Mycosin, but other composition/formulation	Dr. Schaette AG, Bad Waldstadt, Germany
VPMS-W 2003	As Mycosin, but other composition/formulation	Dr. Schaette AG, Bad Waldstadt, Germany
VPMSK 2003	As Mycosin, but other composition/formulation	Dr. Schaette AG, Bad Waldstadt, Germany
-Al_2O_3		
Ulmasud B	Aluminium oxide, silicium oxide, sulphur	Andermatt Biocontrol AG, Grossdietwil, Switzerland
-Minerals		
Zeolite	Zeolite	MIFA AG, Frenkendorf, Switzerland
Kaolin	Kaolin	ECC International, St. Austell, Great Britain
Vermiculite	Vermiculite	ETH Zurich, Plant Pathology, Zurich, Switzerland
-Stone meal		
Napfsteinmehl ®	Silica, lime, aluminium oxide	Andermatt Biocontrol AG, Grossdietwil, Switzerland
Microorganisms		
Serenade ®	*Bacillus subtilis* strain QST 713	AgraQuest, Inc., Davis, USA
Sonata ®	*Bacillus pumilus* QST 2808	AgraQuest, Inc., Davis, USA
Trichodex ®	*Trichoderma harzianum* var. T-39	Feinchemie Schwebda GmbH, Köln, Germany
EM 5	Mixture of bacteria, yeasts, actinomycetes and fungi	Bionova Hygiene GmbH, Rotkreuz, Switzerland
Polyversum ®	Pythium oligandrum	Biopreparáty, Horoměřice, Czech Republic
Xenorhabdus bovienii	Culture filtrate of strain 4722	BBA Braunschweig, Germany
Agro-Mos ™	*Lactobacillus plantarum*, liquid fermentation product	Improcrop, Nicholasville, USA
Plant preparations		
Homeopathic		
Biplantol vital NT	Homeopathic plant extracts	Bioplant Naturverfahren GmbH, Konstanz, Germany
Biplantol SOS ®	Homeopathic plant extracts	Bioplant Naturverfahren GmbH, Konstanz, Germany
-Commercialised	Sea algae (*Ascophyllum nodosum*)	Andermatt Biocontrol AG, Grossdietwil, Switzerland

Table 1 continued

Preparation group/Preparation name	Ingredients	Manufacturer/Distributor
Algifol ™		
Lebermooser	Moss extracts	NIEM Handel, Griesheim, Germany
Elot VIS ®	Plant extract	EDEN Bioscience corp., USA
Kontrapilz	Fennel oil	MioPlant Natura, Zürich, Switzerland
Pandorra ®	Fennel oil	Siegfried-Agro, Zofingen, Switzerland
Envirepel ®	Plant extracts, garlic	Biodomo GmbH, Baden-Baden, Germany
FungEND	Oils of thyme, sesame, and maize	EB Blumenstein, Demirtas, Turkey
Inulex	Extract of *Inula viscosa*	Yigal Cohen, Bar-Ilan University, Ramat-Gan, Israel
Pyralvex ®	Extract of *Rheum palmatum*, roots	Norgine S.A., Rotkreuz, Switzerland
-Prepared at ART		
Rheum rhabarbarum dry	*R. rhabarbarum*, leaf extract in water	J. Huber, Steinmauer, Switzerland
Rheum rhabarbarum fresh	*R. rhabarbarum*, leaf extract in water	J. Huber, Steinmauer, Switzerland
Rheum rhabarbarum	*R. rhabarbarum*, leaf extract in water	BBA Darmstadt, Germany
Potentilla erecta	*P. erecta*, root extract in ethanol	Hänseler AG, Herisau, Switzerland
Salvia officinalis	*S. officinalis*, leaf extract in ethanol	Hänseler AG, Herisau, Switzerland
Salix spp.	*Salix* spp, bark extract in ethanol	Hänseler AG, Herisau, Switzerland
Solidago canadensis	*S. canadensis*, leaf extract in water	BBA Darmstadt, Germany
Malva silvestris	*M. silvestris*, leaf extract in ethanol	Hänseler AG, Herisau, Switzerland
Sophora flavescens	*S. flavescens*, wood extract in ethanol	Bergapotheke, Zürich, Switzerland
Artemisia annua	*A. annua*, leaf extract in water	Agroscope RAC Changins, Nyon, Switzerland
Ocimum basilicum	*O. basilicum*, leaf extract in water	Hänseler AG, Herisau, Switzerland
-Incorporated into soil		
M. silvestris	*M. silvestris*, leaf material	Hänseler AG, Herisau, Switzerland
M. silvestris + *Salvia officinalis*	*M. silvestris* and *S. officinalis*, leaf material	Hänseler AG, Herisau, Switzerland
Phosphonic acid		
Robus	Phosphonic acid	GIP, Jechtingen, Germany
Oekofluid P	Stone meal, plant extracts, sodium silicate, phosphonic acid, lecithin	GIP, Jechtingen, Germany
Disinfectants		
Jet 5	Peracetic acid	Andermatt Biocontrol AG, Grossdietwil, Switzerland
Hydrogen peroxide (H2O2)	Hydrogen peroxide	Sigma-Aldrich, Steinheim, Germany
Resistance inducers		
Bion ®	Acibenzolar-S-Methyl	Novartis Agro, Dielsdorf, Switzerland
Preparations based on natural compounds		
Goemar GA 14	Seaweed cream based on *Ascophylum nodosum*	Stähler Suisse SA, Zofingen, Switzerland
C-2000	Ascorbic, lactic, and citric acid	C. Kempenaar, Wageningen, The Netherlands
Humin Vital 70	Fossil organic material (Leonardit) with 80–92% humic acid	Andermatt Biocontrol AG, Grossdietwil, Switzerland
Whey	Whey	Rüegg dairy, Hinwil, Switzerland

Table 1 continued

Preparation group/Preparation name	Ingredients	Manufacturer/Distributor
Duxon	Fatty acid from vegetable oils saponified with potassium hydroxide	Duxon Aps., Herning, Denmark
Potassium permanganate	Potassium permanganate	Siegfried-Agro, Zofingen, Switzerland
Kendal ®	a) Vegetal extracts, oligosaccarines, glutathione, and phosphonic acidb) As above but without phosphonic acid	Gerlach, Hannover, GermanyGerlach, Hannover, Germany
Enzymatic preparation (e.p.)	Lysozyme	Novaprot GmbH, Stulln, Germany
Wetting agent of e.p.	Wetting agent of enzymatic preparation	Novaprot GmbH, Stulln, Germany
Armicarb 100 ®	Potassium bicarbonate	Church & Dwight Co., Inc. Princeton, USA
Extractants and additives		
Ethanol	Ethanol (96%)	Hänseler AG, Herisau, Switzerland
Tween 20 ®	Polyoxyethylensorbitan monolaurate	Merck, Dietikon, Switzerland
CereNat E30 ®	Wax-rapeseed oil-emulsion	Schümann Sasol Wax GmbH, Hamburg, Germany
Metylan normal	Methylcellulose	Henkel KGaA, Düsseldorf, Germany
TSB-Medium ™	Tryptic soy broth	Difco Laboratories, Detroit, USA

[a] This preparation was only tested in 2001 because the manufacturer was no longer interested in us evaluating it

dried and ground plant material were placed in 0.5 l of ethanol (96%) and stirred with a magnetic stirrer at 60°C for 2 h. The broth was filtered through cheesecloth and ethanol was added to obtain a total volume of 0.5 l. Deionised water was added to obtain a total volume of 1 l of extract. The extracts were applied in the field in a concentration corresponding to 5% dried plant material, and the concentration of ethanol in the spraying broth was 48%. The extracts were freshly prepared the day before spraying.

Incorporation of plant material into the soil

In 2002, at the time of planting of the field trial, in two treatments dried *M. silvestris* (15.6 t ha^{-1}) and a combination of *M. silvestris* (10.4 t ha^{-1}) and *Salvia officinalis* (5.2 t ha^{-1}) were incorporated into the soil using a rotary hoe (10 cm deep).

Laboratory and growth chamber experiments

Inoculum production

Phytophthora infestans was grown and maintained on rye agar (200 g rye seed, 20 g agar agar, 5 g D-glucose, 1000 ml water) (Caten and Jinks 1968) at 18°C in the dark. Isolates of *P. infestans* from the culture collection at ART were screened and suitable isolates for the laboratory and growth chamber experiments were selected.

For the sporangial germination test, potato tuber slices of the variety Bintje were inoculated with a drop of a sporangial suspension. The tuber slices were incubated in covered plastic boxes in the dark at 18°C for 5–7 days. The sporangial suspension was produced by removing the sporangial and mycelial mat from the overgrown tuber slices and suspending it in deionised water. The resulting suspension was strained through cheesecloth and the concentration adjusted to 1×10^4 sporangia ml^{-1}, and directly used without storing.

For the mycelial growth test, mycelial plugs (0.7 mm) were cut from 10 day-old rye agar cultures.

For the inoculation of tomato plants, 10 day-old mycelial and sporangial mats were scraped off the rye agar plates using a microscope slide and suspended in tap water. The suspension was strained through cheesecloth and the concentration adjusted to 1×10^5 sporangia ml^{-1}.

Sporangial germination test

The effect of the preparations on indirect germination of sporangia of *P. infestans* by release of zoospores was assessed on microscope slides. Forty µl of the preparations were placed in the centre of silicon rings (1 cm) on the microscope slide. The preparations were left to desiccate for 24 h to simulate the drying and adherence process on the leaf surface. Subsequently, 40 µl of the sporangial suspension were applied onto the slides, which were then placed on moist filter paper in a plastic box with a lid (22 cm × 35 cm × 2.5 cm). They were incubated in the dark at 4°C to induce indirect germination of sporangia. After 24 h, the number of indirectly germinated sporangia was counted. The experiment included a water control and a reference treatment with the copper fungicide Kocide DF, and was performed with six replications per treatment. The concentration of the preparations to be used was determined in a previous trial as described above in a dilution of two copper fungicides (Kocide DF, Cueva), two synthetic fungicides (Mancozeb, Ridomil Gold), and a copper-free preparation (Mycosin) (results not shown). Consequently, all preparations were examined at the 10-fold dilution of that given in Table 4.

Model test for rainfastness

Preparations showing good efficacies in the sporangial germination test were selected. Rainfastness was assessed in a test similar to the one described by Nienhaus (1969). The preparations were applied to microscope slides dried at room temperature for 12 h and then submerged in tap water for 2 or 4 min. Slides not submerged in water served as a control treatment. After drying the microscope slides for 24 h, the sporangial suspension (1×10^4 sporangia ml^{-1}) was applied and the slides were incubated in the dark at 4°C. After 24 h, the number of indirectly germinated sporangia was counted. The experiments included a water control and a reference treatment with Kocide DF. Each treatment was performed with six replicates.

Mycelial growth test

The effect of the preparations on mycelial growth of *P. infestans* was assessed with an agar incorporation test. Freshly autoclaved rye agar was amended with the preparations when cooled down to approximately 40°C and poured into plastic Petri dishes (9 cm). After 24 h, the agar plates were inoculated with a mycelial plug (7 mm), which was placed upside down in the centre of each plate. The plates were incubated at 18°C in the dark. After eight days, the mycelial growth of *P. infestans* was determined by measuring the colony diameter. The experiment included a non-amended control and a reference treatment with Kocide DF. The concentration of the preparations to be used was determined in a previous mycelial growth test using a dilution series with the same products as described above (results not shown). Consequently, all preparations were examined at the 10-fold dilution of that given in Table 4.

Foliar blight test on tomato plants

The inhibitory effect of the preparations on foliar blight was assessed on tomato plants (*Lycopersicon esculenta*) (cv. Marmande). Tomato seeds were sown in trays and 7 day-old seedlings were transplanted to pots (6 cm × 8 cm) containing a planting substrate (Staudenerde, Obi-Ter, Märwil, Switzerland). They were kept in a growth chamber with a 16 h photoperiod at 24°C day and 20°C night temperatures. Two week-old tomato plants, having two fully developed true leaves, were sprayed with the preparations using a paint sprayer (DeVilbiss MP, Bournemouth, UK) until run-off and put back in the growth chamber with the above mentioned conditions. The tomato plants were treated one day before inoculation to evaluate direct toxic effects of the CFPs, and three days before inoculation to allow the plants to produce resistance-inducing metabolites. Twenty-four hours after the second spraying treatment, the plants were inoculated with a sporangial suspension and placed in a growth chamber with a 16 h photoperiod at 18°C. A plastic hood was placed over the tomato plants to assure high humidity. Seven days after the inoculation, the % diseased leaf area, treated with the CFPs, was assessed. Five plants were used per treatment and the experiment was repeated once. The experiment included an untreated control and a reference treatment with Kocide DF. The concentration of the preparations is given in Table 4.

Persistence of preparations on tomato plants

Preparations showing good inhibitory effects in the foliar blight test on tomato plants were selected. They

were sprayed on tomato plants twice with an interval of 48 h between the two treatments. Three hours or 1, 2, 4 and 8 days after the second treatment, the tomato plants were inoculated with a sporangial suspension of *P. infestans*. Incubation, plant management and disease assessment were conducted as described above. The experiments included an untreated control and a reference treatment with Kocide DF. Six plants were used per treatment and the experiment was repeated once.

Field trials

Small-plot field trials

In 2001, 2002, and in 2004, four, two and three small-plot field trials, respectively, were performed at Zürich-Reckenholz, Switzerland. The trials comprised ten treatments with five replications each, using a latin square design. In 2001, each experimental plot was divided into two subplots, each planted with two rows of the moderately susceptible varieties Agria and Ditta and flanked by a row of the low susceptible variety Appell. Two rows, containing the highly susceptible variety Bintje, between the experimental plots served as disease spreader rows to achieve a homogenous distribution of the disease and a severe disease pressure (Fig. 1). When disease severity in the spreader rows reached a level of 5–15%, they were defoliated. Row width was 75 cm with 33 cm spacing between the eight plants in a row. In 2002 and 2004, the trial set-up was similar to that described above, except that ten instead of eight plants were planted in a row, and the variety Ditta was replaced by the variety Nicola. Certified seed potatoes were used. The experimental field was managed according to Swiss organic farming regulations. The agronomic measures and experimental specific treatments for all the trials from 2001, 2002, and 2004 are listed in Table 2. Table 3 describes the treatments in all trials.

Each trial included an untreated control and a reference treatment with the copper-fungicide Kocide DF. Application of Kocide DF at a rate of 0.938 kg ha^{-1} (375 g metallic copper) reliably controlled late blight in our field trials.

In 2001, ten applications were made from May 30 to July 27 with a knapsack sprayer (Birchmeier, Stetten, Switzerland) with a boom (1.5 m long, three nozzles, 50 cm distance, Teejet 110 02 VP, 3.5 bar). The spraying volume was 500 l ha^{-1} in trials 1 and 2 and 1000 l ha^{-1} in trials 3 and 4. The preparations were applied once a week at regular intervals. On June 18, the trials were inoculated during a rainy period by placing *P. infestans*-infected potato leaves collected from naturally infected potato fields into the Bintje spreader rows.

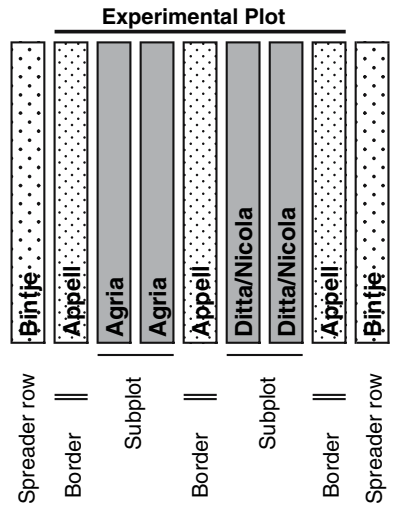


Fig. 1 Layout of an experimental plot in the field trials. Agria and Ditta/Nicola, both moderately susceptible to *Phytophthora infestans*, served for disease and yield assessments. In 2001, the potato variety Ditta was used, and in 2002 and 2004, the potato variety Nicola. Appell, a variety with low susceptibility, was used in the border rows. Bintje, a highly susceptible variety, was planted in the disease spreader row

In 2002, 12 applications were made from May 29 to July 27. In two treatments, the plant material was directly incorporated into the soil during the planting of the field trials. Applications of all other preparations were conducted as described above. The spraying volume was 500 l ha^{-1}. Stone meal was dusted over the respective treatment by hand. On May 30, June 5 and July 5, the Bintje spreader rows were inoculated with *P. infestans*-infected potato leaves as mentioned above.

In 2004, upon the first late blight incidence recorded within a radius of 50 km from the experimental field, on June 8, the decision support system Bio-PhytoPRE recommended a first application for all treatments. Preparations were applied as routine treatments once a week at regular intervals, or according to Bio-PhytoPRE (Musa-Steenblock and Forrer 2005). The spraying volume was 500 l ha^{-1}. On June 11, the Bintje spreader rows were inoculated during a rainy period with a sporangial suspension of *P. infestans* using a mixture of three isolates collected in previous years from the trial region. On June 28,

Table 2 Agronomic measures, soil type and experimental specific treatments for the field trials in 2001, 2002 and 2004 at Zürich-Reckenholz, Switzerland

	Small-plot trials 2001	Small-plot trials 2002	Micro-plot trials 2004	Small-plot trials 2004
Soil type	Loamy Eutric Cambisol	Loamy Gleyic Cambisol	Clayey Eutric Gleysol	Clayey Eutric Gleysol
Agronomic measures				
Fertilisation	Cattle dung (N: 45 kg ha^{-1}; P: 70 kg ha^{-1}; K: 140 kg ha^{-1})	Cattle dung (N: 36 kg ha^{-1}; P: 56 kg ha^{-1}; K: 112 kg ha^{-1})	Cattle dung (N: 45 kg ha^{-1}; P: 70 kg ha^{-1}; K: 140 kg ha^{-1})	Cattle dung (N: 45 kg ha^{-1}; P: 70 kg ha^{-1}; K: 140 kg ha^{-1})
	Autumn 2000	Autumn 2001	Autumn 2003	Autumn 2003
	Liquid manure (N: 48 kg ha^{-1})	Bovine slurry (N: 22.5 kg ha^{-1}; P: 12.3 kg ha^{-1}; K: 33.3 kg ha^{-1})	Biorga (10% N):	Biorga (10% N):
	May 21 and June 13	April 8 and June 3	April 23 (140 kg ha^{-1}), May 18 (300 kg ha^{-1}), and 26 (270 kg ha^{-1}), June 9 (160 kg ha^{-1})	April 23 (140 kg ha^{-1}), May 18 (300 kg ha^{-1}), and 26 (270 kg ha^{-1}), June 9 (160 kg ha^{-1})
Land management	Autumn ploughing	Autumn ploughing	Autumn ploughing	Autumn ploughing
	Harrowing: May 1	Harrowing: April 5	Harrowing: April 20	Harrowing: April 20
	Hoeing: May 21 and 30, June 15	Hoeing: May 10 and18, June 3	Hoeing: May 12 and 18	Hoeing: May 12 and 18
	Ridging up: May 11 and 22	Ridging up: May 2 and 10	Ridging up: May 26, June 9	Ridging up: May 26, June 9
Control of Colorado beetle	Novodor ® (3 l ha^{-1})	Novodor ® (3 l ha^{-1})	Novodor ® (3 l ha^{-1})	Novodor ® (3 l ha^{-1})
	June 27 and July 6	June 17	June 29	June 29
Planting	May 2	April 10	April 15	April 21
Defoliation of Bintje spreader rows	July 5	July 16	June 29	June 29
Defoliation of test varieties	August 3	August 2	July 25	July 25
Harvest	August 15 and 16	August 20	n. a.	August 12 and 13
Yield (weight and calibration)	September 16 to 24	September 13 to 16	n. a.	September 16 and 24

n.a.: not applicable

Table 3 Treatments and application rates for the small-plot field trials in 2001, 2002 and 2004, and the micro-plot field trial in 2004 at Zürich-Reckenholz, Switzerland

	Small-plot trials 2001			Small-plot trials 2002		Micro-plot trials 2004		Small-plot trials 2004		
	Treatment[a]	Application rate (l or kg ha^{-1})[d]	Additives	Treatment[a]	Application rate (l or kg ha^{-1})[d]	Treatment[a]	Application rate (l or kg ha^{-1})[e]	Treatment[a]	Application rate (l or kg ha^{-1})[d]	Spraying interval (days)
Trial 1	Untreated	–	–	Untreated	–	Untreated	–	Untreated	–	–
	Kocide DF (375 g Cu ha^{-1})	0.938 kg	–	Kocide DF (375 g Cu ha^{-1})	0.938 kg	Kocide DF (375 g Cu ha^{-1})	0.938 kg	Kocide DF (375 g Cu ha^{-1})	0.938 kg	7
	Mycosin	5 kg	–	Mycosin	10 kg	Cueva (210 g Cu ha^{-1})	12 l	Kocide DF (200 g Cu ha^{-1})	0.53 kg	7
	Ulmasud B	5 kg	–	VPMS 99	10 kg	Peptiram 5 (150 g Cu ha^{-1})	3 l	Cueva (210 g Cu ha^{-1})	12 l	7
	Humin Vital	5 kg	–	VPMSW 2000	10 kg	Copper protein (40 g Cu ha^{-1})	5 l	Peptiram 5 (150 g Cu ha^{-1})	3 l	7
	Goemar GA 14	2 l	–	C-2000	2 l	Mycosin	10 kg	Kocide DF (375 g Cu ha^{-1})	0.938 kg	DSS[f] + ST[g]
	Mycosin + Goemar GA 14	5 kg + 5 l	–	Trichodex	25 kg	VPMS-W 2003	10 kg	Kocide DF (200 g Cu ha^{-1})	0.53 kg	DSS + ST
	Whey	250 l	–	Serenade	25 kg	VPMS-K 2003	10 kg	Cueva (210 g Cu ha^{-1})	12 l	DSS + ST
	Whey	250 l	CereNat E30	Napfsteinmehl (stone meal)	400 kg	C-2000	2 l	Peptiram 5 (150 g Cu ha^{-1})	3 l	DSS + ST
	SPU-00880-F0-SC (150 g Cu ha^{-1})	3 l	–	Napfsteinmehl[c]	400 kg	Armicarb 100	25 kg	Kocide DF (375 g Cu ha^{-1})	0.938 kg	DSS – ST

Table 3 continued

	Small-plot trials 2001			Small-plot trials 2002		Micro-plot trials 2004		Small-plot trials 2004		
	Treatment[a]	Application rate (l or kg ha^{-1})[d]	Additives	Treatment[a]	Application rate (l or kg ha^{-1})[d]	Treatment[a]	Application rate (l or kg ha^{-1})[e]	Treatment[a]	Application rate (l or kg ha^{-1})[d]	Spraying interval (days)
Trial 2	Untreated	–	–	Untreated	–	Untreated	–	Untreated	–	–
	Kocide DF (375 g Cu ha^{-1})	0.938 kg	–	Kocide DF (375 g Cu ha^{-1})	0.938 kg	Kocide DF (375 g Cu ha^{-1})	0.938 kg	Kocide DF (375 g Cu ha^{-1})	0.938 kg	7
	R. rhabarbarum dry	25 kg	–	*R. rhabarbarum*	25 kg	Trichodex	25 kg	Mycosin	10 kg	7
	R. rhabarbarum fresh	250 kg	–	*S. canadensis*	25 kg	Serenade	25 kg	VPMS-W 2003	10 kg	7
	Salix ssp.	10 kg	Tween 20	*O. basilicum*	25 kg	Sonata	5 l	VPMS-K 2003	10 kg	7
	Salix ssp.	10 kg	CereNat E30	*A. annua*	25 kg	*X. bovienii*	500 l	C-2000	2 l	7
	C-2000	1 l	–	*S. flavescens*	25 kg	Oekofluid P	7.5 l	Kocide DF (375 g Cu ha^{-1})	0.938 kg	DSS + ST
	C-2000	2 l	–	*M. silvestris*	25 kg	FungEND	0.25 l	Mycosin	10 kg	DSS + ST
	EM 5 leaf	1 l	–	*M. silvestris* (soil)	15.6×10^3 kg	Enzymatic preparation (e.p.)	50 l	VPMS-K 2003	10 kg	DSS + ST
	EM 5 soil[b] and leaf	1 l	–	*M. silvestris* + *S. officinalis* (soil)	10.4×10^3 kg + 5.2×10^3 kg	Wetting agent (from e.p.)	50 l	C-2000	2 l	DSS + ST
Trial 3/4[h]	Untreated	–	–					Untreated	–	–
	Kocide (375 g Cu ha^{-1})	0.938 kg	–					Kocide DF (375 g Cu ha^{-1})	0.938 kg	7
	S. officinalis	10 kg	Tween 20/ CerNatE 30					Trichodex	25 kg	7
	S. officinalis	20 kg	Tween 20/ CerNatE 30					Serenade	25 kg	7
	P. erecta	10 kg	Tween 20/ CerNatE 30					Sonata	5 l	7
	P. erecta	20 kg	Tween 20/ CerNatE 30					*R. rhabarbarum*	25 kg	7
	P. erecta + *S. officinalis*	5 kg + 5 kg	Tween 20/ CerNatE 30					*S. canadensis*	25 kg	7

Table 3 continued

Small-plot trials 2001			Small-plot trials 2002		Micro-plot trials 2004		Small-plot trials 2004		
Treatment[a]	Application rate (l or kg ha^{-1})[d]	Additives	Treatment[a]	Application rate (l or kg ha^{-1})[d]	Treatment[a]	Application rate (l or kg ha^{-1})[e]	Treatment[a]	Application rate (l or kg ha^{-1})[d]	Spraying interval (days)
P. erecta + *S. officinalis*	10 kg + 10 kg	Tween 20/ CerNatE 30					*X. bovienii*	500 l	7
Ethanol (7%)	70 l	Tween 20/ CerNatE 30					Enzymatic preparation	50 l	7
Ethanol (14%)	140 l	Tween 20/ CerNatE 30					Armicarb 100	25 kg	7

Spraying volume was 500 l ha^{-1}, with the exception of trial 3 and 4 in 2001 with 1000 l ha^{-1}

[a] For full names, ingredients, and descriptions see Table 1

[b] EM 5 was sprayed onto the soil on May 2, 14 and 31

[c] Stone meal was dusted onto the potato plants on May 29, June 11 and 29, and July 16

[d] Spraying interval was 7 days

[e] Spraying interval was 3–4 days

[f] DSS: Decision support system Bio-PhytoPRE for organic potato production

[g] ST: Stop treatment with two applications within three days

[h] In 2001 the CFPs in trial 3 were sprayed with the additive Tween 20 and in trial 4 with the additive CereNat E 30

the first late blight symptoms appeared in the experimental plots. Then, Bio-PhytoPRE recommended a disease stop treatment with two successive spraying treatments within three days (Table 3). With Bio-PhytoPRE, minimal spraying intervals varied depending on the copper content of the preparation. The efficacy of the CFPs was considered to be equal to copper fungicides with a dosage of 200 g Cu ha^{-1} (Musa-Steenblock and Forrer 2005). At the end of the trials, the potato tubers from each treatment were harvested and total yield determined.

Micro-plot field trials

In 2004, two micro-plot field trials were performed at Zürich-Reckenholz, Switzerland with the goal to achieve an approximately permanent coverage of the potato foliage with the CFPs. The trials comprised ten treatments, each with four replications. A randomised complete block design was used. Each experimental plot was planted with one row of the variety Agria. Row width was 75 cm with 33 cm spacing between the five plants per row. The rows were separated on each side by one row of the low susceptible variety Appell. At the end of each plot, a Bintje plant served as the disease spreader. Application of the preparations was initiated on June 10. On June 11, the Bintje spreader plants were inoculated with a sporangial suspension of *P. infestans*. From June 10 to July 25, twelve applications were performed using a hand-held sprayer (Spray-Matic 5S, Teejet XR 11003, Birchmeier, Stetten, Switzerland) adapted for use with compressed air (4 bar). The preparations were applied twice a week at regular intervals until run-off. Each trial included a reference treatment with Kocide DF (375 g Cu ha^{-1}) and an untreated control. The plants were not harvested because the plot size was too small to determine the yield. The application rate of the preparations is given in Table 3.

Meteorological data

Weather data were measured in the experimental field with an OPUS II weather station equipped with air temperature and air humidity sensors, as well as a rain gauge (LUFFT, Feldbach, Germany). For the decision support system (DSS) Bio-PhytoPRE, current weather data and a 48 h weather forecast (MeteoSwiss) were recorded to calculate and predict main infection and sporulation periods (MISP) (Cao et al. 1997). Hence, Bio-PhytoPRE application recommendations depended on various factors, including the prediction of MISP periods, susceptibility of the variety, the preparation's level of protection, rainfall since the last application, and the current growth stage of the potatoes (Musa-Steenblock and Forrer 2005).

Efficacy, disease and yield assessment, and statistical analysis

For the laboratory and growth chamber experiments, the relative efficacy of the preparations to inhibit indirect germination of sporangia, mycelial growth, and the relative efficacy on foliar blight on tomato plants was calculated.

In the micro-plot and small-plot field trials, foliar blight was assessed by estimating % diseased leaf area for each plot every three to four days. In 2001 and 2004, the disease assessment was continued until complete defoliation of the potatoes in the untreated plots and in 2002, it was continued until the start of senescence of the potato foliage. For each plot, the area under the disease progress curve (AUDPC) was calculated. The effect of treatment and variety on foliar blight was determined by an analysis of variance (ANOVA) on the AUDPC at $P < 0.05$ (PROC MIXED, Statistical Analysis Software System, release 6.12 for Windows, SAS Institute, Cary, NC, USA). A Tukey HSD-test for pairwise comparison was applied to determine significant differences of the treatments at $P < 0.05$. The effect of treatment and variety on total tuber yield was determined by an ANOVA as described above. Blighted tubers were counted in all three field trial years at harvest and six weeks thereafter. Since only very low tuber blight incidences were observed no data are given. All residual values of the ANOVAs were examined for normality by the Shapiro-Wilk test and the homogeneity of variances, and all data met these assumptions.

Results

Laboratory and growth chamber experiments

Sporangial germination test

Sixteen (30%) of the 53 CFPs inhibited indirect germination of sporangia completely, namely the sulphuric

clays Mycosin, VPMS 99, VPMS-W 2000, VPMS-W 2003, and VPMS-K 2003, extracts of *P. erecta*, *S. officinalis*, *Salix* spp., *M. silvestris*, *S. flavescens*, *A. annua*, and *O. basilicum*, and the preparations based on natural compounds Robus, Oekofluid P, potassium permanganate, Armicarb 100, as well as three of the four copper preparations, namely the reference Kocide DF, Cueva, and Peptiram 5 (Table 4). The germination rate in the untreated control was 85%.

Rainfastness

Most of the preparations applied on microscope slides lost inhibitory properties when the microscope slides were submerged in water for 2 or 4 min. The inhibition of indirect germination of sporangia decreased with increasing time of immersion, with the exception of the reference treatment with Kocide DF, and the copper preparations Cueva and Peptiram 5 (Table 5A). The germination rate in the untreated control was 82%.

Mycelial growth test

Fourteen (26%) of the CFPs inhibited mycelial growth completely, namely the sulphuric clays Mycosin, VPMS 99, VPMS-W 2000, VPMS-W 2003, VPMS-K 2003, the preparations based on microorganisms Serenade, Sonata, Trichodex, and Polyversum, and the preparations based on natural compounds, including Robus, Jet 5, hydrogen peroxide, Armicarb 100, as well as three of the four copper preparations, namely the reference Kocide DF, Cueva and Peptiram 5 (Table 4). The microorganisms from the microbial preparations all grew on the rye agar medium.

Foliar blight test on tomato plants

Eleven (21%) of the CFPs, namely the sulphuric clays Mycosin, VPMS-W 2003, VPMS-K 2003, the extract of *M. silvestris*, *P. erecta*, *S. flavescens* and *O. basilicum*, the culture broth of *X. bovienii*, the preparations based on natural compounds C-2000, Kendal with phosphonic acid, and Armicarb 100 as well as the copper preparations, namely the reference Kocide DF, Cueva and Peptiram 5, inhibited foliar blight on tomato plants with an efficacy higher than 80% (Table 4). Since extracts of *A. annua* and the preparation of potassium permanganate were phytotoxic to tomato plants (data not shown), the efficacy of these preparations could not be determined. Foliar blight in the untreated control varied between 98% and 100%.

Persistence of preparations on tomato plants

The efficacy of the preparations on foliar blight of tomato plants decreased within eight days from initially 86–100% down to 0–73%, depending on the preparation. The reference treatment with Kocide DF was the most efficient preparation over all time periods; however, the persistence of the CFPs Mycosin, VPMS-K 2003, and VPMS-W2003 was nearly as good as that of the copper preparations Kocide DF, Cueva and Peptiram 5 (Table 5B). Foliar blight in the untreated control varied between 95% and 100%.

Field trials

Weather conditions, epidemics and spraying schedules

In 2001, the first disease occurrence in Switzerland was recorded on May 11. Applications of the preparations were initiated on May 30 for all treatments. On June 29, an additional treatment was applied because of heavy rainfall. The weather conditions were conducive to infection during the whole trial period (Fig. 2A). Late blight developed rapidly, and foliar blight in the untreated controls reached 100% 28 days after first appearance of late blight symptoms in the experimental plots on June 27 (Fig. 3A).

In 2002, the first disease occurrence in Switzerland was recorded on May 22. For all treatments, applications of the preparations were initiated on May 29. The weather conditions were conducive for infection at the beginning of the epidemic but were only moderate throughout July (Fig. 2B). First disease symptoms were detected in the experimental plots on June 3. Late blight developed moderately, since foliar blight in the untreated controls only reached 76–83% when the test varieties in the trials were defoliated (Fig. 3B).

In 2004, the first disease occurrence in Switzerland was recorded on June 8. As the weather conditions were conducive to late blight infections the following days, Bio-PhytoPRE recommended a first application on June 8 for all treatments in the small-plot trials. Subsequently, applications were made at weekly intervals for the routine treatment or according to

Table 4 Efficacy of copper and copper-free preparations on indirect germination of sporangia and mycelial growth of *Phytophthora infestans* in in vitro trials, and on foliar blight of tomato plants in growth chamber trials

Preparation group [1]/ Preparation name	Concentration (%)	% Inhibition of sporangia germination	% Inhibition of mycelial growth	% Efficacy on tomato plants
Copper				
Kocide DF	0.8	100.0	100.0	100.0
Cueva	2	100.0	100.0	100.0
Peptiram 5	0.6	100.0	100.0	100.0
Copper protein	1	96.1	54.5	53.3
Sulphuric clays and minerals				
Mycosin	2	100.0	100.0	96.0
VPMS 99	2	100.0	100.0	75.0
VPMS-W 2000	2	100.0	100.0	70.0
VPMS-W 2003	2	100.0	100.0	90.3
VPMS-K 2003	2	100.0	100.0	97.5
Ulmasud B	0.5	22.7	44.1	39.0
Napfsteinmehl	1	0.0	0.0	0.0
Zeolite	1	11.8	61.8	0.0
Kaolin	1	8.2	0.0	0.0
Vermiculite	1	6.8	0.0	4.0
Microorgamisms				
Serenade	1	1.6	100.0	2.5
Sonata	1	7.7	100.0	28.0
Trichodex	1	35.7	100.0	1.2
EM 5	0.2	17.3	11.5	0.0
Polyversum	0.4	12.1	100.0	0.0
X. bovienii	1	98.1	69.0	85.0
Agro-Mos	0.3	0.0	0.0	2.0
Plant preparations				
Biplantol vital NT	2	9.2	0.0	4.0
Biplantol SOS	2	0.0	0.0	0.0
Algifol	0.1	13.4	0.0	3.3
Lebermooser	0.5	9.7	0.0	16.0
Elot VIS	20	4.3	100.0	2.0
Kontrapilz	0.4	8.9	66.9	0.0
Pandorra	0.4	23.7	32.5	3.3
Pyralvex	1	11.8	0.7	63.0
Envirepel	0.5	8.9	0.0	0.0
Inulex	1	16.4	98.9	74.5
FungEND	0.05	0.0	52.6	19.2
R. rhabarbarum	5	62.1	0.0	38.3
S. canadensis	5	2.4	0.4	2.0
P. erecta	5	100.0	43.2	81.1
S. officinalis	5	100.0	42.2	57.6
Salix ssp.	5	100.0	40.3	40.8
M. silvestris	5	100.0	46.4	100.0
S. flavescens	5	100.0	73.5	99.2
A. annua	5	100.0	54.5	Not tested [3]
O. basilicum	5	100.0	40.2	99.5
Phosphonic acid				
Robus	1	100.0	100.0	74.3
Oekofluid P	1.5	100.0	19.8	14.0
Disinfectants				
Jet 5	0.8	14.7	100.0	0.0
Hydrogen peroxide	1	6.9	100.0	0.0
Inducers				
Bion	0.012	6.5	5.5	49.5
Preparations based on natural compounds				
Duxon	0.002	0.0	0.5	54.5
Goemar GA 14	0.8	7.9	0.0	5.0
Humin Vital 70	1	8.3	6.4	10.2
Whey	50	1.3	0.0	0.0
C-2000 [2]	2	92.3	59.6	99.5
Potassium permanganate	1.25	100.0	90.7	Not tested [3]
Kendal with phosphonic acid	0.5	8.5	67.3	80.5
Kendal without phosphonic acid	0.5	20.9	12.1	0.0
Enzymatic preparation (e.p.)	10	98.7	91.3	48.0
Wetting agent of e.p.	10	9.1	86.2	62.5
Armicarb 100	1	100.0	100.0	100.0
Additives [2]				
Metylan Normal	0.1	10.4	0.0	2.5
Ethanol	1	35.0	13.3	0.0
Tween 20	0.0125	22.2	15.4	0.0
CereNat E30	0.8	21.0	82.5	0.0
TSB-Medium	1	24.6	3.9	0.0

Table 4 continued

Results are given as relative efficacies (%) compared with the untreated control

[a] For full names, ingredients and descriptions see Table 1

[b] Neither considered in the evaluation nor counted as separate preparation

[c] Not tested: Preparation was phytotoxic to tomato plants

■ = 100% inhibition; ■ = 80–100% inhibition; □ = 50–80% inhibition; No shading <50% inhibition

Bio-PhytoPRE with Kocide DF at high (375 g Cu ha^{-1}) and Cueva, Peptiram 5, Mycosin, VPMS-W 2003, VPMS-K 2003, and C-2000 at low (200 g Cu ha^{-1}) application rates. In the micro-plot field trials, the first spraying treatment was initiated on June 10. Weather conditions for late blight infections were conducive during most of the trial season (Fig. 2C). Late blight developed rapidly, since foliar blight in the untreated controls reached 100% 26 days after first appearance of late blight symptoms in the experimental plots on June 28 (Fig. 3C).

Small-plot field trial set-up

With disease spreader rows that were inoculated artificially in all three years, disease developed homogenously in the experimental plots providing good conditions for a reliable screening of CFPs. In the three experimental years, the epidemic of *P. infestans* developed similarly in all three years and both varieties (Fig. 3).

Small-plot field trials 2001—Foliar blight and yield

In all four trials, disease severity was higher for the variety Ditta than for the variety Agria (trial 1: d.f. = 1, $F = 238.8$, $P < 0.05$; trial 2: d.f. = 1, $F = 120.4$, $P < 0.05$; trial 3: d.f. = 1, $F = 292.6$, $P < 0.05$; trial 4: d.f. = 1, $F = 41.7$, $P < 0.05$). The reference treatment with Kocide DF was the most efficient in all four trials and reduced foliar blight by between 90% and 95%. The copper-containing preparation SPU-0080-F-SC was as effective as Kocide DF. The efficacy of CFPs was 6% at most and none of them reduced foliar blight significantly. Neither the formulation with Tween 20 nor CereNat E30 nor the spraying volume influenced the efficacy of the preparations (data not shown).

In all four trials, total tuber yield of the variety Agria was higher than that of the variety Ditta, irrespective of treatment (trial 1: d.f. = 1, $F = 6.1$, $P < 0.05$; trial 2: d.f. = 9, $F = 1.9$, $P < 0.05$; trial 3: d.f. = 9, $F = 2.5$, $P < 0.05$; trial 4: d.f. = 9, $F = 2.6$, $P < 0.05$). The highest yield in all four trials was achieved with the reference Kocide DF. It was significantly higher than that of any copper-free preparation. Compared with the untreated control, the yield increase for the mean of the two varieties with the reference treatment Kocide DF varied between 35% and 53%. Total tuber yield of the copper preparation SPU-0080-F-SC was similar to the reference treatment with Kocide DF. In all four trials, none of the CFPs had an effect on tuber yield (data not shown).

Small-plot field trials 2002—Foliar blight and yield

In both trials, disease severity was higher for the variety Nicola than for the variety Agria. As in 2001, the reference treatment with Kocide DF was the most efficient in reducing foliar blight, varying between 84% and 88% (Fig. 4). In trial 1, all treatments except the stone meal treatment with weekly applications reduced foliar blight significantly compared with the untreated control. The highest efficacies of CFPs were observed with sulphuric clays, such as Mycosin (35%), VPMS 99 (34%), and VPMS-W 2000 (35%), followed by the organic acid C-2000 (28%) and the preparations containing microorganisms. In trial 2, the three plant extracts *O. basilicum*, *A. annua*, and *M. silvestris* reduced foliar blight significantly; however, disease reduction was low (8% at most). For the extracts of *R. rhabarbarum*, *S. canadensis*, and *S. flavescens* as well as for the two treatments with plant material incorporated into the soil, foliar blight was similar to the untreated control.

In both trials, the tuber yield of the variety Agria was higher than that of Nicola, irrespective of the treatment (Fig. 4). Compared with the untreated control, the yield increase for the mean of both

Table 5 (A) Indirect germination of sporangia of *Phytophthora infestans* on microscope slides coated with preparations after immersion for 2 or 4 min in water. (B) Effect of time interval between application of preparations and the inoculation of tomato plants with *P. infestans* on foliar blight. A sporangial suspension was applied 0, 1, 2, 4 or 8 days after the spraying treatment

A

Preparations [1)]	% inhibition of sporangia germination Time of immersion in water (min) 0	2	4
Untreated	0.0	0.0	0.0
Kocide DF	100.0	100.0	100.0
Cueva	100.0	100.0	100.0
Peptiram 5	100.0	98.9	100.0
Copper protein	100.0	18.2	12.8
Mycosin	100.0	0.0	0.0
VPMS-K 2003	100.0	10.8	1.2
VPMS-W 2003	100.0	9.7	4.3
Armicarb 100	100.0	28.0	18.9
C-2000	100.0	44.5	30.2
Enzymatic preparation	94.1	11.9	0.6
Xenorhabdus bovienii	78.2	0.0	0.0
Oekofluid P	96.6	49.4	7.3

B

Preparations [1)]	% efficacy against foliar blight Days after treatment 0	1	2	4	8
Untreated	0.0	0.0	0.0	0.0	0.0
Kocide DF	100.0	100.0	96.7	97.0	72.7
Cueva	100.0	100.0	95.8	91.3	26.3
Peptiram 5	100.0	100.0	97.5	91.3	55.7
Copper protein	86.0	85.0	68.0	42.5	21.0
Mycosin	100.0	100.0	81.3	66.1	63.0
VPMS-K 2003	99.8	99.0	90.5	78.8	74.0
VPMS-W 2003	97.5	92.5	85.0	81.3	50.5
Armicarb 100	100.0	100.0	57.5	40.0	0.0
C-2000	95.0	59.7	30.0	43.8	0.0
Enzymatic preparation	98.3	62.5	27.5	56.5	20.0
Xenorhabdus bovienii	100.0	77.5	42.5	9.7	41.7

Results are given as relative efficacies (%) compared with the untreated control

[a] For full names, ingredients, and descriptions, see Table 1

– Not tested in this bioassay

■ = 100% inhibition; ■ = 80–100% inhibition; □ = 50–80% inhibition; No shading < 50% inhibition

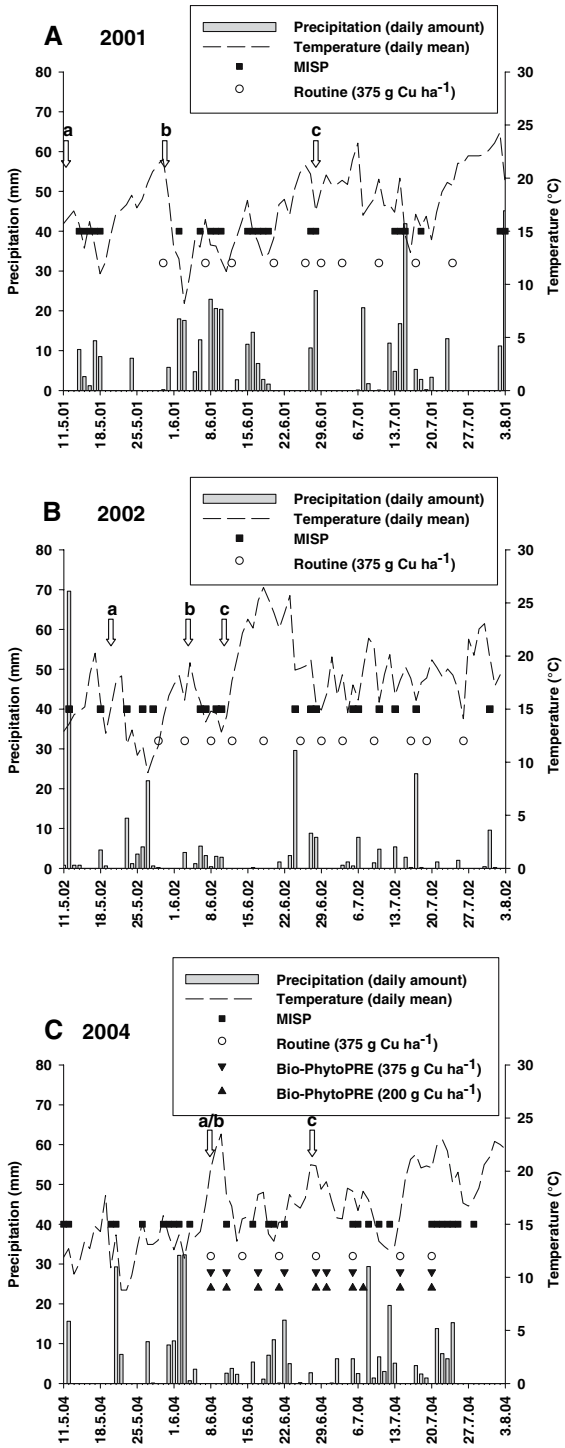
varieties with the reference treatment Kocide DF was about 8%. In trial 1, none of the CFPs had an effect on tuber yield (Fig. 4). In trial 2, plant material incorporated into the soil increased total yield to the same level as the reference with Kocide DF (Fig. 4).

Micro-plot field trial 2004—Foliar blight

In trial 1, all treatments significantly reduced foliar blight (Fig. 5). The efficacy of the copper preparations was higher than that of the CFPs. The reference treatment with Kocide DF, and Cueva were the most efficient and reduced foliar blight by 99%. Efficacy decreased with the decreasing copper content of the preparations, down to 57% for Copper protein. Mycosin was the most efficient copper-free preparation in trial 1. The efficacy of VPMS-W 2003, VPMS-K 2003, and C-2000 did not differ substantially, between 44% and 37%, and Armicarb 100 was the least efficient preparation (23%). Similarly, in trial 2, treatments differed significantly (Fig. 5). The reference treatment with Kocide DF was the most efficient preparation to inhibit foliar blight (99%), followed by Oekofluid P (63%). The preparations containing microorganisms and the enzymatic preparation inhibited foliar blight by between 9% and 29%.

Small-plot field trials 2004—Foliar blight and yield

In all three trials, disease severity was higher for the variety Nicola than for the variety Agria. In trial 1, all copper treatments significantly reduced foliar blight (Fig. 6). Applications according to Bio-PhytoPRE increased the efficacy for all the copper preparations used. Efficacy of Peptiram 5 containing 150 g Cu ha^{-1} was lowest, followed by treatments containing 200 g Cu ha^{-1} (Cueva and Kocide DF) and the treatments with 375 g Cu ha^{-1} (Kocide DF). The stop treatment had no significant effect on the efficacy (Fig. 6). In the two routine treatments, seven sprays were applied, whereas in the treatments according to Bio-PhytoPRE, nine or ten applications were made with the high or low copper dosage, respectively, when a total of 2.8 kg Cu ha^{-1} or 1.4 kg Cu ha^{-1} was applied in the routine treatment, and



◀**Fig. 2** Weather conditions and main infection and sporulation periods MISP (■) based on Bio-PhytoPRE at the field trial site at Zürich-Reckenholz in the year 2001 (**A**) and 2002 (**B**) and 2004 (**C**). Treatments were applied in a regular spraying interval (◯) or according to the decision support system Bio-PhytoPRE (▼;▲). Arrows: a: First disease incidence in Switzerland; b: First spraying treatment in experimental plots; c: First disease incidence in experimental plots

3.4 kg Cu ha^{-1} or 2.0 kg Cu ha^{-1} with Bio-PhytoPRE. In trial 2, all treatments reduced foliar blight compared with the untreated control. There was no significant difference of foliar blight between the routine treatments and the treatments according to Bio-PhytoPRE (Fig. 6). The reference treatment with Kocide DF was the most efficient. The efficacies of Mycosin, VPMS-W 2003, VPMS-K 2003, and C-2000 were substantially lower and varied between 9% and 17%. In trial 3, six treatments reduced foliar blight significantly compared with the untreated control. The culture filtrate of *X. bovienii* and the enzymatic preparation had no effect on foliar blight, and with Armicarb 100 foliar blight was even significantly higher than in the untreated control. Again, the reference treatment with Kocide DF was the most efficient preparation. Of all CFPs, Trichodex showed the best performance (Fig. 6).

In all three trials and throughout all treatments, the tuber yield of the variety Agria was higher than that of the variety Nicola. The yield increase of the treatments with the reference Kocide DF was between 15% and 25% compared with the untreated control. In trial 1 we observed a close relationship between the copper dosage and the total tuber yield, irrespective of whether routine or Bio-PhytoPRE treatments were carried out (Fig. 6). The stop treatment had no effect on total tuber yield. In trial 2, none of the CFPs had an effect on tuber yield (Fig. 6). Similarly, in trial 3, none of the CFPs had a significant effect on tuber yield, with the exception of Armicarb 100, which even reduced tuber yield (Fig. 6).

Discussion

Our aim was to evaluate CFPs in the field with reported activity against late blight of potatoes. Field trials showed that none of them reduced the disease compared with the untreated control and none reached the efficacy of the reference with a copper fungicide. Thus, we were uncertain of whether the CFPs were per se inactive or whether adverse environmental conditions masked their control

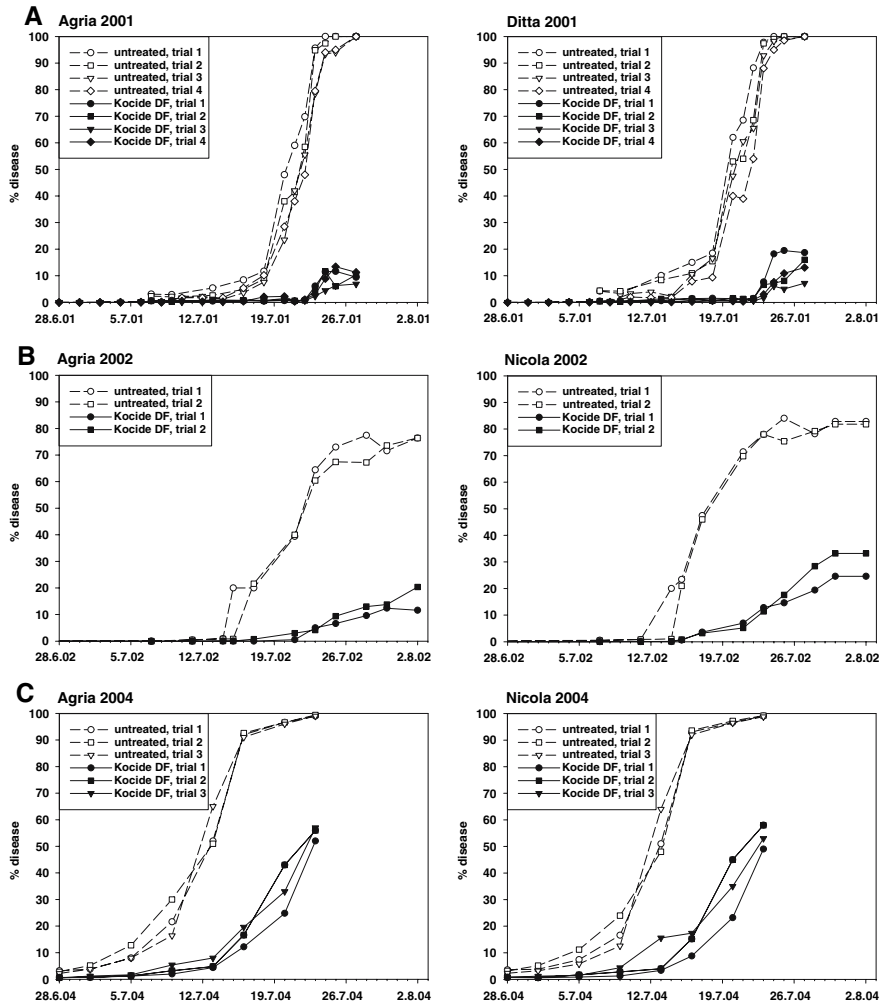


Fig. 3 Late blight epidemics in the untreated plots and the reference treatment with the copper fungicide Kocide DF of all small-plot trials in 2001, 2002 and 2004 at Zürich-Reckenholz

potential. Subsequently, CFPs were evaluated in in vitro and in vivo experiments. This approach allows for the detection and estimation of disease controlling effects and for the comparison of novel preparations with known products. Consequently, the inherent potential of CFPs can be characterized. In vitro and in vivo inhibition of *P. infestans* by CFPs and copper compounds was determined with a sporangial germination test, a mycelial growth test, and a growth chamber test using tomato plants. The in vitro tests allow for the assessment of direct antifungal effects, whereas the in vivo test, in addition, allows for an evaluation of effects caused by the interaction between plant, pathogen and preparation. In our trials, all preparations, which were effective on tomato plants or under field conditions, showed activity against *P. infestans* in the in vitro experiments. Hence, our results suggest that the preparations examined in this study act directly and had a toxic effect on *P. infestans*. Therefore, we doubt that any of the CFPs in our study act through induction of resistance. In our trials, only about 25% of the CFPs that claimed to be active against *P. infestans* performed well in our in vitro and 20% in the in vivo assays. Overall, our results confirm that laboratory and growth chamber trials are a useful means to select the best candidate preparations for field trials.

As a first step, the micro-plot field trials proved to be very useful to evaluate the efficacy of CFPs in the field. The prevailing detrimental environmental conditions were partly counteracted by frequent spraying, creating a nearly permanent protection of the potato foliage. The low number of five plants per plot

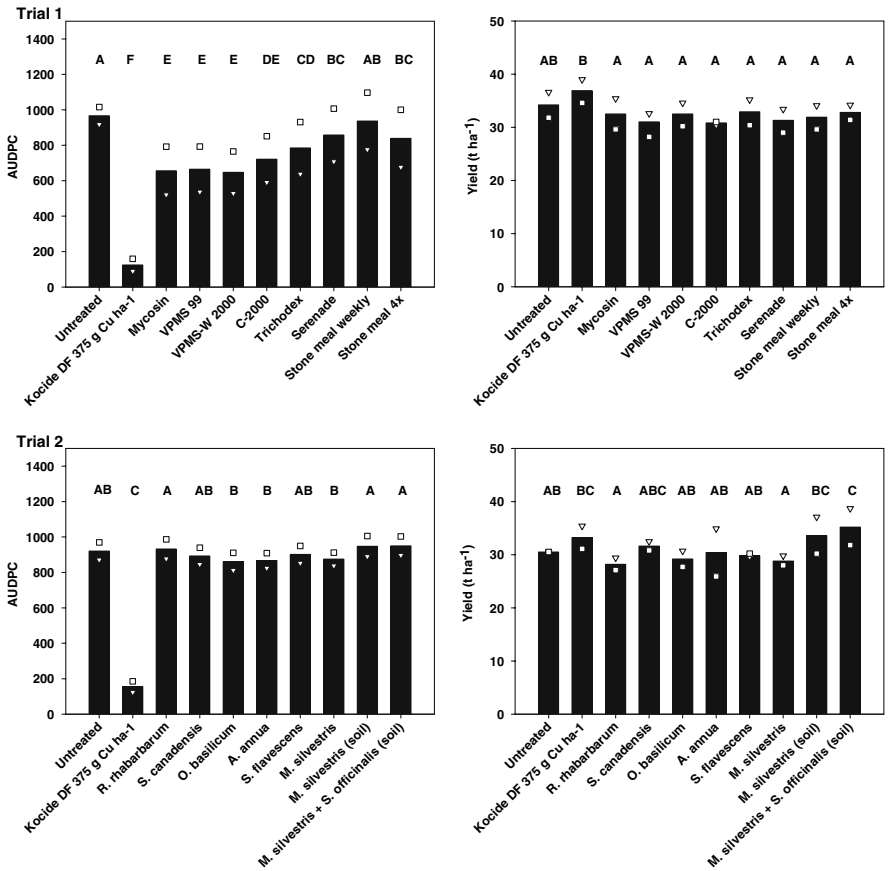


Fig. 4 Small-plot field trials in 2002 at Zürich-Reckenholz, Switzerland: Efficacy of copper and copper-free preparations on foliar blight (AUDPC) and total tuber yield (t ha^{-1}) of the potato varieties Agria (▽) and Nicola (□) separately and as mean values of the two varieties (bars). Means of data followed by different letters are significantly different (PROC MIXED, followed by Tukey HSD-test at $P < 0.05$). Trial 1: copper-free preparations; trial 2: plant extracts and plant material incorporated into the soil. AUDPC: Area under the disease progress curve

used was well suited to estimate the potential of CFPs using a small amount of space, time and material under field conditions.

The well-balanced trial design of the small-plot trials conducted under practice-like conditions served to detect even low levels of efficacies of CFPs. The disease spreader rows planted with a susceptible potato variety between the experimental plots ensured a homogenous distribution of disease in the experimental plots. The disease progress curves in the untreated controls and in the reference treatments with the copper fungicide Kocide DF provide good evidence for the homogenous disease pressure, since they were similar within a year and variety. Hence, we conclude that the set-up of the small-plot trial was suitable for the evaluation of copper-free and copper preparations.

For the commercial use of a plant protection agent, predictable and consistent disease control is required (Jacobson and Backman 1993; Froyd 1997). Most of the CFPs evaluated in our trials lack this crucial prerequisite, since they were not active under complex field conditions with variable environmental conditions. Concrete explanations for why the results obtained under controlled conditions may not be reproduced under field conditions are not available; however, detrimental environmental effects are thought to be the major constraints (Rodgers 1993). Possible reasons are photolability (Lange et al. 1993), degradation on the phyllosphere by microorganisms (Spurr 1990) or the general sensitivity of preparations to environmental conditions (Jacobson and Backman 1993; Hofstein and Chapple 1999). In addition, loss of biological activity and stability of natural compounds was found to be higher in the field than under controlled conditions (Benner 1993). Rainfall was the most important factor affecting longevity of fungicides (Forbes 2001) or the efficacy of copper fungicides in the field (Somers and Thomas 1956). All these processes remove the protective layer from the foliage, which leaves it vulnerable to infection. The model bioassay for rainfastness using a

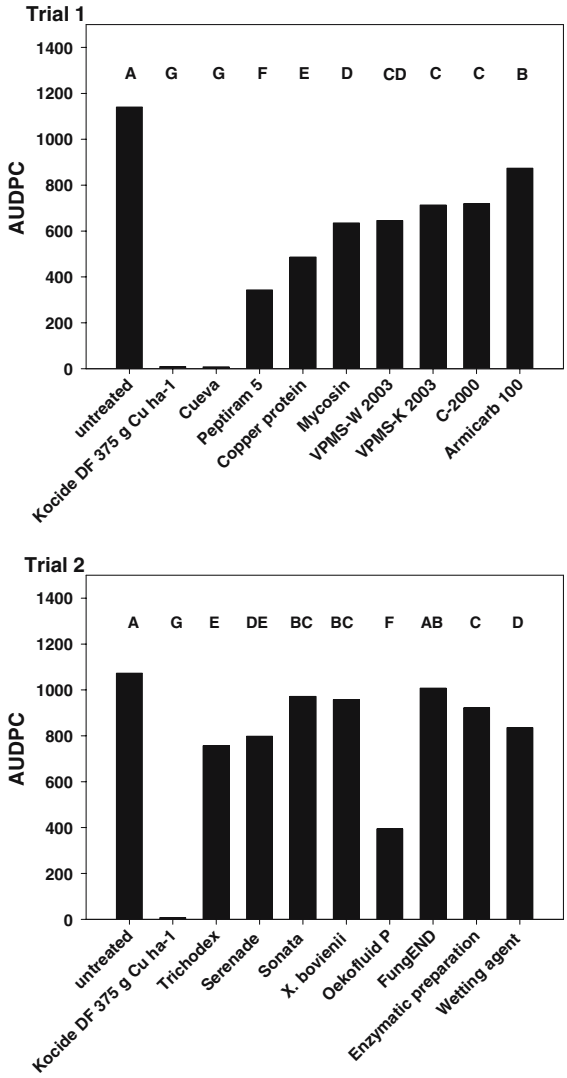


Fig. 5 Micro-plot field trials in 2004 at Zürich-Reckenholz: Efficacy of copper and copper-free preparations on foliar blight of potato, caused by *Phytophthora infestans* (AUDPC). Results are given as mean values. Untreated and Kocide DF treated plots served as control treatments. Means of data followed by different letters are significantly different (PROC MIXED, followed by Tukey HSD-test at $P < 0.05$). AUDPC: Area under the disease progress curve

microscope slide technique revealed that a short time of immersion in water drastically reduced the efficacy of CFPs. This effect is probably due to wash-off or dilution and was not observed with the copper preparations. In addition, the persistence of CFPs on tomato plants was shorter than that of the copper preparations. Similar effects were observed by Stephan et al. (2005) who showed that the efficacy of CFPs decreased with the prolongation of the time interval between treatment and inoculation of the potato plants. Hence, for improved field efficacy of CFPs, additional applications may be required, particularly during rainy weather and in periods with dew in order to counteract the lack of rainfastness.

Many plants possess antifungal properties. In the in vitro tests, seven out of nine plant extracts tested completely inhibited indirect germination of sporangia and reduced mycelial growth. When tested on tomato plants, only three reached an efficacy >80%, including extracts of *P. erecta* and *S. officinalis*. According to Blaeser et al. (2002), extracts of *P. erecta* modified the cell walls of *P. infestans* while extracts of *S. officinalis* inhibited the germination and motility of zoospores. In two field trials conducted by Blaeser et al. (2002), extracts of *S. officinalis* and *P. erecta* reduced foliar blight and increased the yield up to 12%–17%. In a field trial conducted in Germany, extracts of *S. canadensis* and *R. rhabarbarum* delayed disease development significantly and increased the tuber yield (Meinck and Schmitt 1998). Moreover, extracts of *R. rhabarbarum* reduced foliar blight at the beginning of the epidemic. In contrast, in our study, none of the four extracts was effective in any of the field trials, and they showed only minor efficacies in the laboratory and growth chamber trials. Additionally, none of the other plant-derived preparations sufficiently controlled foliar blight or increased the yield of potatoes. The antimicrobial potential of plant extracts is certainly influenced by the age, stage, and origin of the plant material, the extraction method, and the extractant (Eloff 1998). Often, the composition and the active principle of plant extracts are unknown, rendering it difficult to achieve consistent results even though the same methods were used.

In outdoor trials conducted at ART with potted potato plants, soil-incorporated dried plant material of *S. officinalis*, *M. silvestris* and *O. basilicum* reduced foliar blight and in addition, *M. silvestris* and *O. basilicum* increased tuber yield (Krebs and Forrer 2001). However, in the field trials the disease suppressing effect could not be reproduced with soil-incorporated *S. officinalis* and *M. silvestris*. However, tuber yield was significantly higher. We assume that the nitrogen input from the incorporated plants was the cause for the yield increase, because the N-min

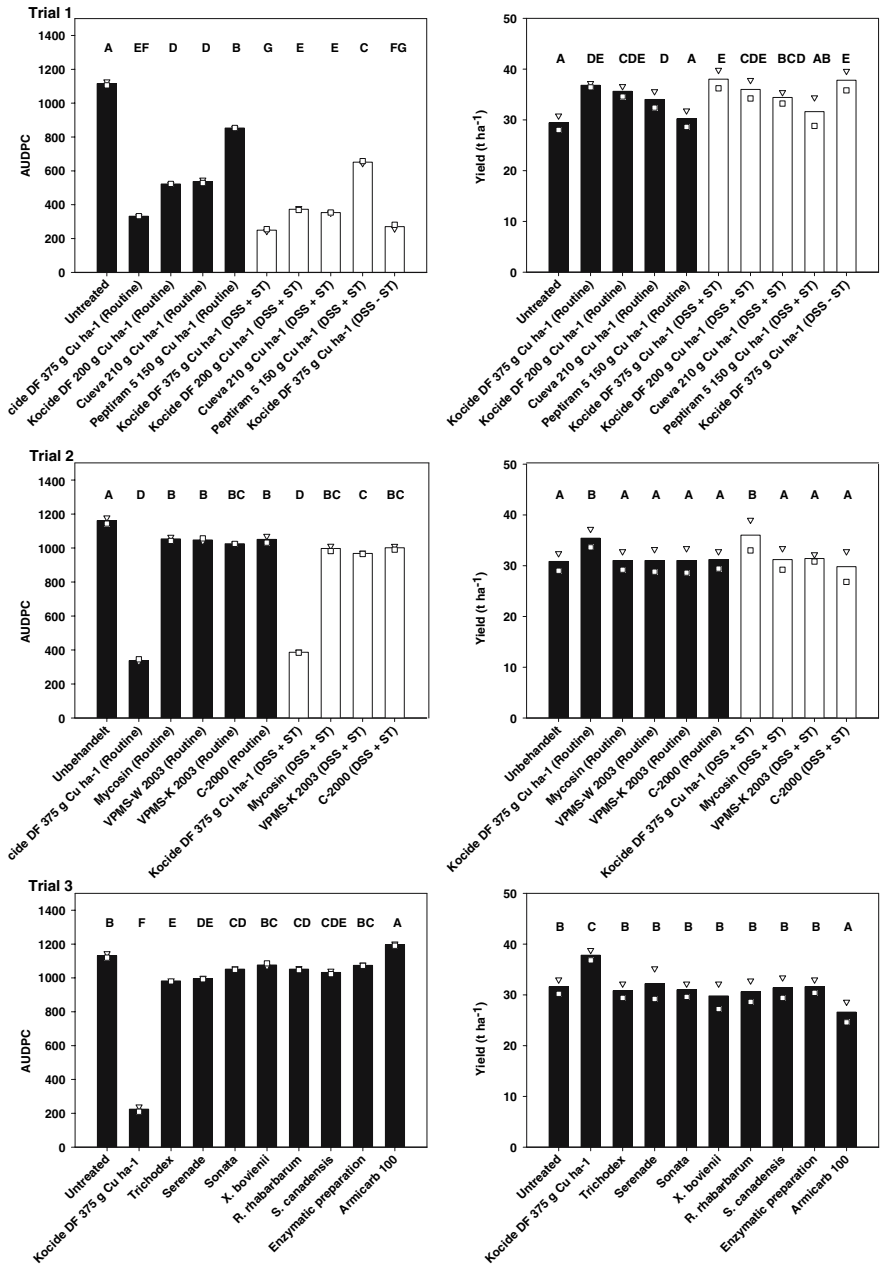


Fig. 6 Small-plot field trials in 2004 at ART, Switzerland: Efficacy of copper and copper-free preparations on foliar blight (AUDPC) and total tuber yield (t ha^{-1}) of the varieties Agria (▽) and Nicola (□)) separately and as mean values of the two varieties (bars). Means of data followed by different letters are significantly different (PROC MIXED, followed by Tukey HSD-test at $P < 0.05$). Trial 1: copper treatments applied as routine (black bars) or according to the decision support system Bio-PhytoPRE (white bars); trial 2: sulphuric clays, C-2000 applied as routine (black bars) or according to the decision support system Bio-PhytoPRE (white bars); trial 3: other copper-free preparations applied as routine. AUDPC: Area under the disease progress curve; DSS: decision support system; ST: stop treatment with two consecutive applications

values were two to six times higher than in untreated plots.

Biological control agents are often selected based on antagonistic effects observed in vitro (Hemming 1990; Lynch 1990). Many bacteria (Daayf et al. 2003) and fungi (Lange et al. 1993; Chambers and Scott 1995) show strong antifungal activity in vitro against pathogens such as *Phytophthora* species. We therefore presume that the good efficacies of the preparations containing microorganisms in the mycelial growth test might be the result of optimal culture conditions for the production of antibiotic metabolites. However, *Phytophthora* species are often very susceptible to the presence of other microorganisms or their metabolites and therefore are easily suppressed on agar plates (Malajczuk 1983). Consequently, the potential of biological control agents to inhibit *P. infestans* may be strongly overestimated. Our

in vivo results supported this hypothesis. Four out of seven bio-control agents completely inhibited the mycelial growth of *P. infestans* but only *X. bovienii* had a sufficient effect in vivo on foliar blight of tomato plants. The *B. subtilis* strain QST 713, the bio-control agent of Serenade, produces metabolites that inhibit the growth of *P. infestans* (Stephan et al. 2005). Effects against other *Phytophthora* species are reported for *X. bovienii* (Ng and Webster 1997) and *Trichoderma* spp. (Etebarian et al. 2000). Therefore, we hypothesize that metabolites in the culture filtrate of *X. bovienii* are the main reason for its high efficacy in our bioassay for sporangial germination and the in vivo test on tomato plants. But again, the field efficacy of the culture filtrate of *X. bovienii*, as well as the preparations containing *T. harzianum* and *B. subtilis* was low. This may be attributed to the detrimental environmental field conditions. As a result, micro-organisms may not multiply (Folman et al. 2004), decline rapidly after application (Rodgers 1993), or may not produce antifungal metabolites. In addition, Lukezic et al. (1990) observed that bacterial control agents did not colonize the entire leaf surface of plants, leaving sites vulnerable for infection.

The preparations containing sulphuric clays inhibited indirect germination of sporangia, mycelial growth, and reduced foliar blight on tomato plants. We hypothesize that the aluminium-ions in these sulphuric clays may be responsible for their high effectiveness. Aluminium-ions were shown to inhibit sporangial production, sporangial germination, and mycelial growth of *P. infestans* in laboratory trials (Andrivon 1995). In addition, it was observed that aluminium mediated the suppression of soil-borne *Phytophthora* species (Fichtner et al. 2001). In 2002, they showed the best efficacies of all CFPs tested against foliar blight in the small-plot field trials. Once again, however, these results were inconsistent. Even though the sulphuric clays Mycosin and VPMS-K were applied in small-plot trials in 2004 according to Bio-PhytoPRE shortly before or after an infection period, no acceptable disease protection was achieved.

Armicarb 100 and the enzymatic preparation showed good efficacy in the laboratory and growth chamber trials. Furthermore, in the micro-plot trials with frequent applications, they reduced foliar blight. However, in the small-plot field trials, they were not effective. In the micro-plot and small-plot field trials, Armicarb 100 was phytotoxic to the potato plants. Similarly, geraniums treated with Armicarb 100 resulted in even higher disease rating values (Palmer et al. 1995).

Oekofluid P, containing phosphonic acid, was the most efficient copper-free preparation in the in vitro and the micro-plot field trials. This confirms the results from a previous field screening of CFPs at ART, where only a preparation containing phosphonic acid significantly reduced foliar blight of potatoes (Bassin and Forrer 2001). Phosphonic acid prevented or reduced infection by pathogens that belong to the Oomycota (Johnson et al. 2004; Wicks et al. 1991). However, phosphonic acid appears to remain stable in plants, since applications against downy mildew of grapes and late blight of potato resulted in residues in wine or potato tubers (Speiser et al. 2000). Residues in organic products are not tolerated and consequently, phosphonic acid is a subject of controversy in organic agriculture despite high efficacy and low environmental impact (Nowack 2003; HERA 2004).

The efficacy of stone meal, frequently used in biodynamic agriculture, was similar to the untreated control in our laboratory and the growth chamber trials, and its effect on reducing foliar blight in the field was minor and no effect on tuber yield was observed. Similarly, the efficacy of other minerals, other plant preparations, disinfectants, resistance inducers, and other preparations based on natural compounds in the laboratory and growth chamber trials was not different from the untreated control, and hence further testing was discontinued.

Copper fungicides are a controversial element in organic agriculture, but in our laboratory, growth chamber and field trials they proved to be the most effective and reliable means for late blight control. Only copper reduced foliar blight to very low levels and sustained or increased tuber yield. Agriculture is one of the major sources of environmentally harmful copper. As long as effective copper-free substitutes are not available, reduced application rates or less frequent applications of copper fungicides could help to decrease copper input into the environment. As shown in our field trials, reduced rates of copper fungicides applied according to Bio-PhytoPRE or with a first application according to Bio-PhytoPRE followed by applications at regular intervals are a promising means for satisfactory disease control

without yield losses (Musa-Steenblock and Forrer 2005).

The results of our study demonstrate that certain CFPs have the potential to control *P. infestans* under controlled environmental conditions. For a successful introduction of a copper-free preparation, proof of field efficacy under variable environmental conditions is of primary importance in the final evaluation process. Hence, for a comprehensive evaluation of CFPs, a combination of standardized laboratory and growth chamber trials as well as field trials are needed. Further research should be focused primarily on the development of effective formulations. In the meantime, the control of potato late blight with an optimised and restricted use of copper fungicides seems to be a viable way to minimise copper input into the environment.

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