Evaluation of innovative products to reduce copper applications to control potato late blight in organic production systems

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SUMMARY

The main objective of this project, VETAB project, is to determine alternatives to massive copper utilization to control potato late blight (*Phytophtora infestans* (Mont.) de Bary) in organic systems.
To reach such a target, we first performed a screening of candidate products and additives based on their efficiency in the laboratory, under controlled conditions. We evaluated a wide range of products: formulations with a low level of copper, antagonists suspensions, amino-acid extracts, plants extracts, potassium salts, sulphur formulation, organically stabilised peroxide and rhamnolipids. The product's suspensions were applied by vaporization on potato plants. Two different protocols of application were elaborated. To test the fungicide protection action, the product was applied four days before inoculation of the pathogen. To evaluate the defence stimulating effect, the product was applied several times during the plant growth before inoculation of the pathogen. The last vaporization was performed 4 days before inoculation. We also evaluated the resistance of the product to washing risk. Pathogen suspension was applied as droplets of $5x10^4$ spo/ml on detached leaves. The leaves were then incubated (18°C, RH > 90%, 6 days) in order to record symptoms development.
The best results were obtained with formulations integrating reduced doses of copper and with potassium salts.
In conclusion, a wide range of products and additives are proposed on the market but very few of those have a real efficiency. The performance of the most efficient products has to be confirmed in field trials.

INTRODUCTION

Recently, the European Union, in the regulation 2091/92, imposed to reduce the amount of copper application to control fungal diseases in organic production. It imposes to reduce the amount of copper metal to 6 kg per year and per hectare since January 2006. The point is that, today, there are no known effective alternative to copper to control potato late blight in organic systems. So, the VETAB project, co-financed by the European Union in the INTERREG III Wallonie-France-Flandre program, aimed to explore the new control opportunities. To do so, all the products proposed as potential alternatives were identified, collected and tested in the laboratory.

MATERIALS AND METHODS

Products tested: First of all, a range of products (table 1), as wide as possible, was identified and collected from different sources (internet, organic products distributors, growers advisers, researchers,…).
Thereafter, 2 protocols were used in link to the mode of action of these products, indicated by their distributor. The first protocol aimed to test the efficacy of the products distributed as fungicide while the second one aimed to test the efficacy of the products presented as helicitors. The choice of the product concentration to be used was also made according to distributor advices.

Table 1: List of the products tested in March and November 2006 for their fungicide and/or helicitor action(s)

Product name	**Dosage**	**Fungicide***	**Helicitor***
Bordeaux mixture	3kg/ha	-	-
Distilled water	-	-	-
Microsulfo	1,5kg/ha	1	
PK2	2L/ha	1 and 2	2
Solucuivre	2L/ha	1	1
PK2 + Solucuivre	2L/ha and 2L/ha	2	2
Zonix	0,5L/ha	1	
Ecoclearprox	3L/ha	1	
Allicine	75ml/ha and 150ml/ha	2	
Ilsamin	4L/ha		1
Milsana	1,5L/ha		1
Optiplant	1,5L/ha		1
Splinter + Bordeaux mixture	0,65L/ha and 3kg/ha	1	
Kendal with PK2	3L/ha and 2L/ha		1
Siliforce with PK2	0,3L/ha and 2L/ha		1

* 1: products tested in March 2006
2: products tested in November 2006

Products description.
- Bordeaux mixture (3 kg/ha), widely used by the growers to control fungal diseases in organic farming (Tomlin, 2000), was used as positive reference. Its fungicidal activity on the spore is based on the accumulation of free copper ions in the cell till a toxic concentration and the formation of complexes, with sulfhydryl, carboxylic and hydroxyl groups, resulting in a non-specific denaturation of enzymes of the respiration chain (Schwinn et al., 1991). It was tested at the dose of 3 kg/ha to correspond to the new EU prerogatives.
- Distilled water was used as negative control.
- Microsulfo is a sulphur based product commercialised to control a wide range of fungal diseases in organic farming (Tomlin, 2000).
- PK2 is a potassium phosphate. The efficacy of phosphite-based compounds on oomycete has been reported in the literature. Cohen and Coffey (1986) report the studies of Thizy *et al*. (1978) showing that various salts of phosphorous acid display activity against various oomycetes. The studies of Erwin and Ribeiro (1996) reported by Miller *et al*. (2006) confirmed that phosphites could be used to control 19 species of *Phytophtora*.
- Solucuivre is a copper tallate including an amount of 5% of copper.
- Zonix is a product containing rhamnolipids considered as biosurfactant that could explode the zoospores membranes.

- Ecoclearprox is an hydrogen peroxyde stabilised with organic molecule.
- Allicine is a product containing garlic extracts. Garlic is known as insect repellent (Copping, 2001) but could also have an antiseptic effect.
- Ilsamin is a mixture of amino-acids with different size length.
- Milsana is a mixture of different plant extracts.
- Optiplant is a mixture of plants extracts containing deactivated late blight strains.
- Splinter is a mixture of short amino-acids chain aiming to enhance rainfastness. We will test its rain protection effect with Bordeaux mixture.
- Kendal is a mixture of N and K2O, this product is presented by a South African company as a good additive to potassium phosphite to control late blight on potato. Knowing this information we decided to test it with PK2.
- Siliforce is a product supposed to give a mechanical protection by silicic acid (Bowen, et al., 1992). We will test this product with PK2 as proposed by the Siliforce belgian distributor.

Vegetal material. The trials were performed on detached leaves of the cultivar Bintje produced *in vitro*. We choose Bintje because this cultivar is very susceptible to lowly resistant to foliage late blight (www.europotato.org). After 3 weeks growing, the plants were transplanted in 1,5 litres flowerpots filled with vermiculite. The plants were then grown up, under hydroponics, in a climatic chamber at 16°C and 16/8 of photoperiod. The first application of candidates helicitors was performed after 2 weeks growing in climatic chamber in assay 1 (March 2006) and one week in assay 2 (November 2006).

Pathogen inoculum isolate. The isolate that has been used in the trials is referenced with the number: 00-091/2. This late blight isolate, characterized as being aggressive, has been collected in 2000, in the Walloon region (Belgium).

Application of the product with the aerograph. 10 ml of the tested product were pulverized, at the chosen concentration, on the potato plant. The pulverization was performed on all the parts of the plant from a distance of 50 cm.
To test heliciting effect, the product was applied several times (3 times in essay 1 and 5 times in essay 2) during the plant growth. To test fungicides effect, the product was applied at the same date than the last application of helicitors (figure 1).

Rainfastness test. Fungicides rainfastness sensitivity was tested 2 days after treatment through an application of an artificial rain of 25 mm (controlled by pluviometer).

Inoculation. Four days after the last treatment, we took 3 leaves per plant, one at the top, one in the middle and one at the lower part of the plant. Those 3 leaves, each with 5 leaflets, were placed in a transparent incubation plastic box (24 cm x 18 cm x 10 cm). Those boxes contained papers humidified with 20 ml of distilled water, at the bottom. The leafstalks were covered by a humid peace of cotton to keep the leaves turgescent. Each of the 15 leaflets were inoculated by 4 droplets (2 droplets per half leaflet) of 10 µl of late blight suspension with $5x10^4$ spo/ml before to be incubated in a lighted chamber under 18°C and 16/8 of photoperiod.

Observations and data analysis. Three observations were performed, respectively after 3, 4 and 6 days of incubation (figure 1). The observations were made following a 5 grades scale. The grade 0 referred to the absence of symptoms. The grade 1 referred to little diameters black spots. The grade 2 referred to large brownish spots without white fructifications. Grade 3 referred to large brownish spots with presence of white mycelium. Finally, grade 4 referred

to leaves fully invades by mycelium and fructifications. The observations were made per half leaflet.
One plastic box, with 15 potato leaflets, was one repetition. In other words, the one day result of one repetition was given by the mean of all the leaflets notations value. Then, we calculated the relative stAUDPC (standard Area Under Disease Progression Curve divided by trial duration since leaflets inoculation). There were 4 replications per object and 6 repetitions for the controls (Bordeaux mixture and water), the four replications were dispatched in different climatic chambers in a 4 fully randomized blocks device.
A two ways ANOVA, including the block (random factor, 4 levels) and the product with or without rainfastness test (fix factor, 20 levels for March 2006 trials and 21 levels for November 2006 trials) was performed on the data of each essay. Thereafter a multiple mean comparison objects was made using the Student-Newman-Keuls method (Dagnelie, 1975) to segregate the different objects.

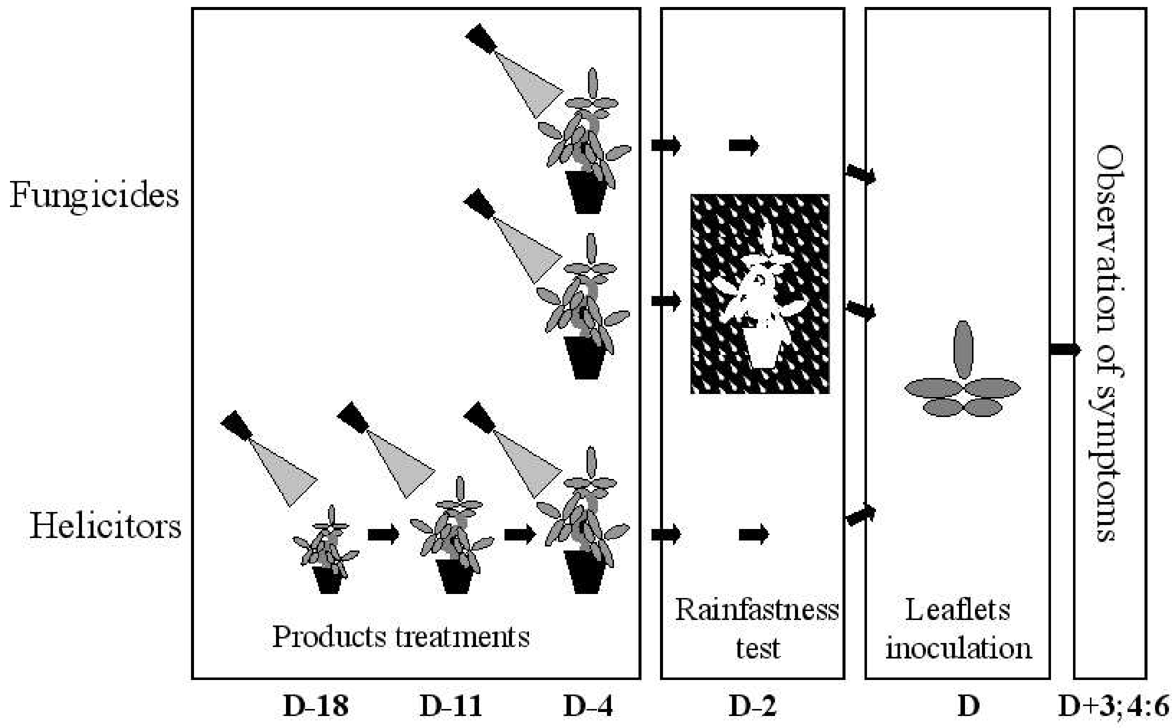

Figure 1: Presentation of the different manipulations and of their planning.

RESULTS

The two ways ANOVA allowed to identify significant differences among the products tested in March 2006 ($F(19,38) = 12.63$; $p < 0.001$) as in November 2006 ($F(20,40) = 58.40$; $p < 0.001$). Using a multiple mean comparison, all the products were compared to the positive and negative controls: Bordeaux mixture and distilled water.
Only two products tested as fungicides, PK2 ($p > 0.50$) and Solucuivre ($p > 0.50$), gave a protection similar to Bordeaux mixture. The association of those two products gave also a protection similar to the one of Bordeaux mixture ($p > 0.50$). The others candidates didn’t reach a protection level as good as Bordeaux mixture. Those products are Ecoclearprox ($p < 0.001$), Microsulfo ($p < 0.001$), Zonix ($p < 0.001$), Allicin at 75 ml/ha ($p < 0.001$) and Allicin at 150 ml/ha ($p < 0.001$). We also noted that those products couldn’t be differentiate from the distilled water negative control (p always higher than 0.50) (table 2).

Table 2: Products performances against late blight infection development quantified through the RstAUDPC index.

Product	Mean of RstAUDPC	Standard deviation	Product	Mean of RstAUDPC	Standard deviation
Controls March 2006					
Bordeaux Mixture	0.328	0.113	Distilled water	0.899	0.197
Fungicides March 2006					
PK2	0.299	0.232	Microsulfo	1.006	0.182
Solucuivre	0.505	0.124	Zonix	0.974	0.210
Ecoclearprox	0.934	0.176			
Helicitors March 2006					
Ilsamin	0.779	0.306	Milsana	0.766	0.135
Ecoclearprox	1.027	0.275	Optiplant	0.924	0.222
Additives March 2006					
PK2 + Siliforce	0.394	0.127	Bordeaux Mixture +Splinter	0.356	0.041
PK2 + Kendal	0.350	0.102			
Controls November 2006					
Bordeaux mixture	0.521	0.296	Distilled water	1.527	0.186
Fungicides November 2006					
PK2	1.043	0.049	Allicin (75 ml/ha)	1.510	0.223
PK2 + Solucuivre	0.653	0.106	Allicin (150 ml/ha)	1.449	0.158
Helicitors November 2006					
PK2	0.581	0.051	PK2 + Solucuivre	0.468	0.0316

Knowing the efficiency of Bordeaux mixture, PK2 and Solucuivre, rainfastness was tested by comparison of modalities with and without rain application. No significant impact of rain application on those products efficiency was highlighted (Bordeaux Mixture: $p > 0.50$; PK2: $p > 0.10$ and Solucuivre: $p > 0.50$) (table 3).

Table 3: Rainfastness of the three products identified as efficient against potato late blight. RstAUDPC index was used to compare the performances of these products with and without rain simulation.

Rain modality	**No artificial rain**		**Artificial rain**	
Product	**Mean of RstAUDPC**	**Standard deviation**	**Mean of RstAUDPC**	**Standard deviation**
March 2006				
Bordeaux mixture	0.328	0.113	0.408	0.193
Bordeaux mixture + Splinter	0.356	0.041	0.574	0.333
Solucuivre	0.505	0.124	0.587	0.247
November 2006				
Bordeaux mixture	0.521	0.296	0.712	0.161
PK2	1.043	0.049	0.788	0.190

The case of the tested helicitors, Ecoclearprox, Ilsamin, Milsana and Optiplant, was clearer. None of them could offer a protection level similar to the one reached by Bordeaux mixture (respectively: $p < 0.001$; $p = 0.022$; 0.032 and 0.001). These products, can't be distinguished from the negative control ($p > 0.50$). As the literature announces that phosphite could induce a plant-defence response (Zainuri *et al.* 1997 cited by Miller *et al*. 2006), we tested the efficiency of PK2 and of the association of PK2 and Solucuivre as helicitors. The results we get could confirm this hypothesis concerning PK2 ($p < 0,001$) but not for PK2 + Solucuivre ($p = 0.853$) as shown in table 2. Indeed, we get a better efficiency with 5 applications of PK2 instead of 1.

The effect of additives was evaluated in combination with another product as shown in table 1. The addition of Siliforce ($p > 0.50$) and Kendal didn't improve the efficacy of PK2 ($p > 0.50$). In the same way, we tested the efficiency of Splinter additive to enhance rainfastness of Bordeaux mixture. No significant effect was highlighted following the application of 25 mm of artificial rain ($p > 0.50$) (table 3). Moreover, the addition of Splinter didn't improve the efficacy of Bordeaux mixture by promoting a better penetration in the potato leaves (table 3).

DISCUSSION AND CONCLUSION

It is important to note that all the results we get are linked to our laboratory conditions. Under such conditions, Microsulfo, Zonix, Ecoclearprox, Allicin, Ilsamin, Milsana and Optiplant weren't effective to control late blight infestation while PK2 and Solucuivre were as effective as the positive control, the Bordeaux mixture.

The effect of Solucuivre is, of course, linked to the effect of copper, even if the concentration of this element is very low: 5 % in comparison to 20 % for Bordeaux mixture. So, under our laboratory conditions, it was possible to reduce 6 times the copper application while reaching the same level of efficacy.

PK2 is a product with a very simple structure, mainly composed by potassium phosphite, and with a good efficiency, in the laboratory, to control late blight. Indeed PK2 was as affective as Bordeaux mixture in all the tests performed as fungicide or as helicitor. PK2 presented a better efficiency if used as helicitor. The question is: “Could this product, with a structure as simple as the one of Bordeaux mixture, be accepted for organic farming?” The most interesting is that this product degrades itself in potassium phosphate, an un-reactive salt acting as plant nutrient. So, this product is far to be as dangerous as copper for the environment.

Concerning the additives, Kendal and Siliforce couldn't enhance the effect of PK2. On the other hand, to evaluate the efficacy of Splinter to enhance rainfastness of copper based products, the treated plant have to be exposed to a more important level of rain that the 25 mm tested in this trial. In our laboratory conditions, this rain fall wasn't enough to wash out the Bordeaux mixture.

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