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An integrated concept for early blight control in potatoes

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SUMMARY

In an attempt to develop an integrated early blight management strategy in potatoes, we tested different soil treatments in order to reduce the primary inoculum in the soil. Besides incorporation of calcium cyanamide or biofumigant plant tissue into the furrows, we varied the crop rotation and compared scorings between potato plots with previous crops potatoes and barley, respectively. While we are still working on proving the causal relationship between our treatments and reduced early blight severity, we have already observed significant effects of all of our treatments.

KEYWORDS

Alternaria spp., calcium cyanamide, biofumigation, crop rotation, soil inoculum

INTRODUCTION

Early blight in potatoes is mainly caused by the pathogen *Alternaria solani*. In potato fields we observe a pathogen complex consisting of *A. solani* and *A. alternata*. Yield losses can add up to 25% and in Germany late maturing varieties are affected more heavily than early maturing varieties. So far early blight has been controlled by fungicides, most effectively by the strobilurin azoxystrobin. But only two years after registration of azoxystrobin in the USA, *A. solani* isolates with reduced sensitivity to azoxystrobin have been found (Pasche *et al.*, 2004). The altered sensitivity is caused by the F129L mutation of the cytochrome b gene. Until now there have been no proven reports of a loss of sensitivity among European isolates in the field, but the mutation is also spreading among isolates in Europe (Leiminger *et al.*, 2013). So we have to pose the question: do we have alternatives or complements for early blight control?

In the last decades early blight control has been achieved by inhibiting or reducing the secondary spread of *A. solani* spores between plants. Yet, the primary source of inoculum is fungal material which is overseasoning on plant debris within the soil. Therefore we thought of ways to affect this primary inoculum and we conducted several field trials with different soil treatments. The factors we varied were the previous crop, the nitrogen fertilization, the soil application of azoxystrobin, and biofumigation. Biofumigation means the suppression of soil-borne pathogens, pests, and weeds by isothiocyanates (ITCs), which derive from hydrolisation of glucosinolates by myrosinase in disrupted plant cells (Angus *et al.*, 1994, Kirkegaard *et al.*, 1993). Shredded plants of the brassicaceous plant family can be incorporated into infested soil to release their ITCs and in this way to inhibit pathogens. We expected that the early blight pressure in potatoes with previous crop potato should be higher than after the previous crop barley. Furthermore we believed that biofumigation and even soil application of azoxystrobin after planting would reduce early blight severity. As fourth element we varied the type of nitrogen fertilizer and expected calcium cyanamide to have a fungicidal side effect on Alternaria spores and mycelium.

MATERIALS & METHODS

Field trials

The field trials were conducted in two locations near to Munich, Bavaria. As early blight susceptible potato variety we chose the late maturing "Maxilla". Plant density was 40,000 plants per hectare. Trials were designed as a randomized complete block consisting of four replications. Field plots were comprised of six rows (0.66 m between rows) and were 7.5 m long (29.7 m²). For suppression of late blight infections we sprayed Ranman (active ingredient: cyazofamid) weekly in all plots of our trials. In our biofumigation trial in 2012 we incorporated leaf material of different crops into the furrows of the potato plots (table 1) for simulating different crop rotations.

Table 1. *Crop species, variety, and amount of green manure amendments of the biofumigation field trial and the reported characteristic isothiocyanate (ITC)*

Treatment	Green manure amendment	Released ITC	Amount per plot (kg fresh weight)
1	Dried infested leaves of S. tuberosum "Kuras"	___________	0.5
2	none (control)	___________	___
3	Solanum tuberosum "Maxilla"	___________	7.5
4	Phacelia tanacetifolia"Lila"	___________	7.5
5	Sinapis alba "Albatros"	4-hydroxybenzyl-ITC	7.5
6	Raphanus sativus "Defender"	4-(Methylsulfinyl)-3-butenyl-ITC	7.5

In a second trial (2010-2012) we compared calcium cyanamide ($CaCN_2$) fertilization to a calcium ammonium nitrate ($CaNH_4NO_3$) fertilizer and to Ortiva (active ingredient: azoxystrobin) soil application. In the years 2011 and 2012, this trial was planted in two fields with comparable soil parameters except for the crop rotation. So we had eight replications for each treatment, four with potatoes and four with barley as previous crop. Details can be seen in table 2.

Table 2. *Nitrogen fertilization, fungicide application, and previous crops of our multiannual second trial*

Treatment	Fertilizer	Fungicide	Previous crop	Years
1	0 kg N	___	barley	2010-2012
2	CaNH4NO3 (160 kg N)	___	barley	2010-2012
3	CaCN2 (160 kg N)	___	barley	2010-2012
4	0 kg N	Ortiva (3 l/ha)	barley	2010-2012
5	CaNH4NO3 (160 kg N)	Ortiva (3 l/ha)	barley	2010-2012
6	0 kg N	___	potato	2011-2012
7	CaNH4NO3 (160 kg N)	___	potato	2011-2012
8	CaCN2 (160 kg N)	___	potato	2011-2012
9	0 kg N	Ortiva (3 l/ha)	potato	2011-2012
10	CaNH4NO3 (160 kg N)	Ortiva (3 l/ha)	potato	2011-2012

For revealing the effects of the treatments of interest on early blight development we compared the area under the disease progress curve (AUDPC) of potato plants according to the formula of Das *et al.* (1992). Scores were taken as percent value of necrotic leaf area every ten days. Of each plot we scored ten plants and thereof three leaf levels (top, middle, and bottom).

Lab experiment

In a lab trial we observed the effect of biofumigant ITCs at direct contact with spores of *A. solani*. A dilution series of different ITCs was added to a standard culture medium and poured into petri dishes. Final concentrations of ITCs ranged between 1 µM and 10 mM. After solidification, a spore solution was streaked on the medium resulting in approximately 800 spores per petri dish and dishes were sealed with parafilm afterwards for inhibiting evaporation of volatiles. The spore solution was derived from 14 day old single spore isolates growing on SNA medium (Nirenberg, 1981). Each ITC was tested repeatedly with four different single spore isolates. The ITCs of interest were allyl-, ethyl-, methyl-, benzyl-, and phenylethyl-ITC (Sigma Aldrich Corp.,MO-St. Louis). Spore germination was evaluated after an incubation period of four days by counting 100 spores per petri dish and differentiating between germinated and non-germinated spores.

Scorings and AUDPC values were tested for significant differences by SPSS version 21.0.0.0 (IBM Corp., NY-Armonk).

RESULTS

The biofumigation trial in 2012 revealed a differentiated early blight development. Amendments of white mustard and leaf radish resulted in significantly reduced leaf symptoms compared to the amendments with potato leaves, whereas Phacelia ranged in between.

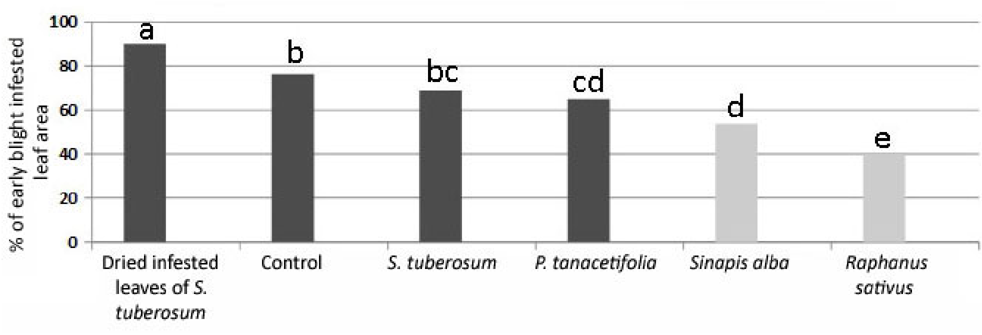

Figure 1. *Effect of green manure amendments on early blight symptoms of top leaves in the late season of 2012. Different letters above columns mean significant differences (Tukey, α=0,05)*

Lab experiments showed a clear inhibition of spore germination by ITCs. Yet, the effective concentration varied between ITCs and allyl-ITC has been the most potent one with 100 µM being enough to inhibit germination.

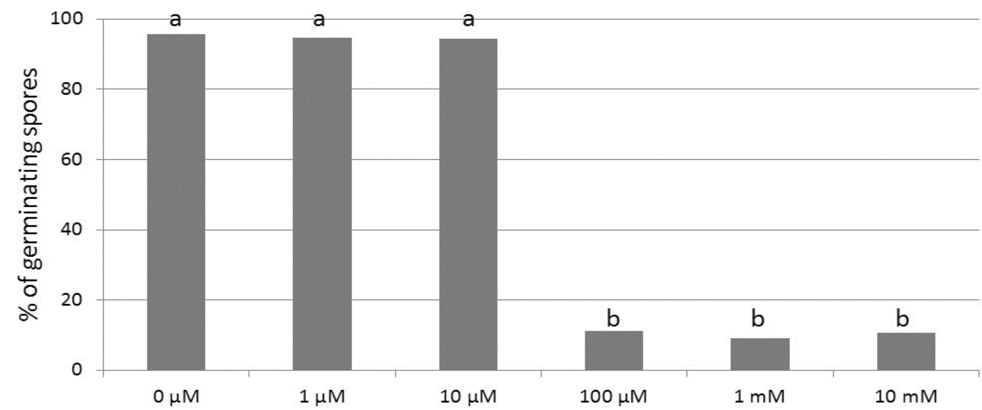

Figure 2. *Spore germination of A. solani on media with increasing allyl-ITC concentrations. Different letters above columns mean significant differences (Tukey, α=0,05)*

In our second trial be observed that early blight epidemics are delayed by approximately 15 to 20 days after wheat as previous crop compared to plots with potatoes as previous crop. The mean AUDPC of the plots with previous crop potato was significantly higher than the mean AUDPC of plots with previous crop barley (α=0.05).

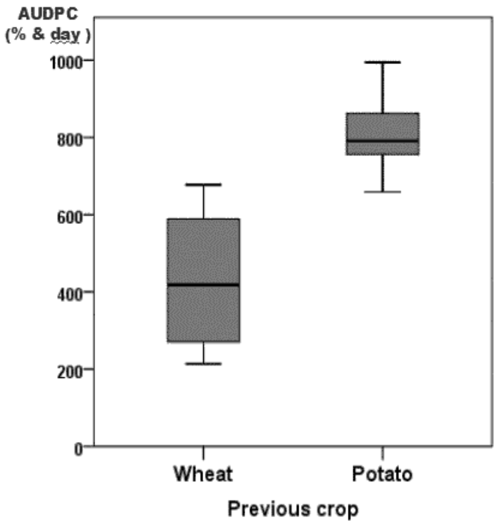

Figure 3. *Effect of previous crops wheat and potatoes on the early blight AUDPC of potatoes in 2011*

With regard to nitrogen fertilization, calcium cyanamide-fertilized plants had significantly less early blight symptoms than plants treated with calcium ammonium nitrate (α=0.05). The AUDPC of calcium cyanamide-treated plants was comparable to plants sprayed with Ortiva.

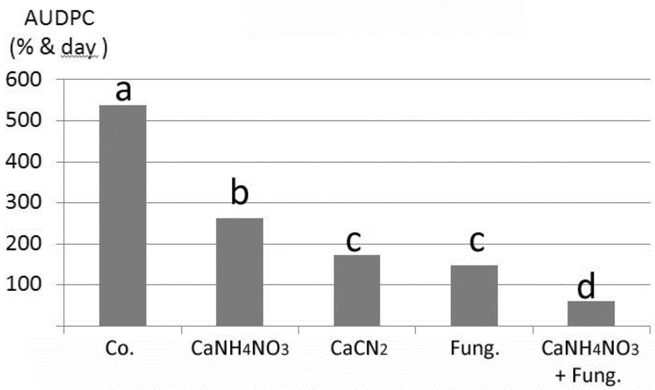

Figure 4. *Comparison of calcium cyanamide, calcium ammonium nitrate, and the fungicide Ortiva with regard to their effect on the early blight AUDPC in 2010. Different letters above columns mean significant differences (Tukey, α=0,05)*

DISCUSSION

Our experiments give us reason to believe that there is more than an explicit fungicide treatment to control early blight in potatoes. Integrated disease management strategies include preventive aspects like growing disease resistant varieties and choosing an appropriate location for the respective crop. The aim to maintain healthy crops can be achieved by meeting their nutritional demands. Other factors are disease monitoring as well as biological and mechanical control methods. For some of these aspects we have already found promising approaches in early blight control. Now after having observed significant effects, we are trying to elucidate the causal relationship behind these effects.

The fungicidal side effect of degradation products of calcium cyanamide which has been reported by Finck and Börner (1985) can until now only assumed to be the reason for the delayed early blight development in cyanamide-treated plots. In lab assays, we have no evidence for any lethal effects of the degradation products of calcium cyanamide on spores or mycelium of Alternaria spp. so far. But assays are still under progress. As an alternative explanation to the fungicidal potential we consider the slower degradation of calcium cyanamide and thus the more adequate availability of nitrogen during early blight epidemics to be a reason for obviously healthier plants. Nevertheless, effects are significant and constant over years.

A very promising method for early blight control seems to be biofumigation. There are many factors which affect the efficiency of the procedure like fertilization and growth stage of biofumigant plants, the time span between maceration of biofumigant plant tissue and incorporation into soil, and soil temperature and humidity during and after incorporation, and some more. Therefore further research is necessary to support farmers for a better practicability of this method. But if conditions are optimal, a significant reduction of early blight symptoms is possible.

First scorings of the field season 2013 seem to confirm the tendency of last year: after wheat as previous crop early blight epidemics are delayed by approximately 15 to 20 days compared to plots with potatoes as previous crop. The most probable reason for accelerated and enhanced early blight development in the plots with previous crop potatoes is a higher concentration of soil inoculum. We have already developed a DNA extraction method for soil samples and aim to provide evidence for the presence and for the amount of soil inoculum by PCR and qPCR, respectively. This will help us to compare the extent of leaf infestation with the concentration of *A. solani* and *A. alternata* inoculum in soil and to reveal a suspected correlation.

Encouraged by our findings about the effects of crop rotation, biofumigation, and calcium cyanamide on early blight development on potatoes, we hope to find even more modules for a comprehensive strategy to control this disease.

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