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Induced resistance in potato to *Phytphthora infestans*—effects of BABA in greenhouse and field tests with different potato varieties

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Abstract We have investigated to what degree induced resistance with β-aminobutyric acid (BABA) can protect potato from late blight infection under Swedish field conditions and if synergistic interactions occur if BABA is applied in combination with a commonly used fungicide, Shirlan. In greenhouse experiments we also investigated the durability of BABA induced resistance, the dose-response relationships in susceptible (Bintje) and partially resistant (Ovatio, Suberb) cultivars and effects of combined applications of BABA and fungicides. We found a clear effect of BABA on *P. infestans* infection of greenhouse grown potato plants. The lesion sizes were reduced by on average 40–50% compared to untreated control. However, this effect lasted for only 4–5 days after BABA treatment and then the efficacy was lower. When BABA was given in combination with the fungicides it appeared to have an additive effect both in greenhouse and field experiments. Higher concentrations of BABA gave a stronger protective effect. The partially resistant cultivars Ovatio and Superb reacted to lower concentrations of BABA where no effect was found in susceptible Bintje. According to our field data, 20–25% reduction of the fungicide dose in combination with BABA gave on average the same result on late blight development as full dose Shirlan alone; while reduced dose of Shirlan alone sometimes resulted in less effective protection. Our results indicate that induced resistance could be used in practice in combinations with fungicides in order to reduce the amount of toxic compounds under north European conditions.

Keywords Solanum tuberosum · β-aminobutyric acid · Late blight · Fungicide · Shirlan

Introduction

Potato late blight caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is today one of the most severe/destructive plant diseases worldwide. *P. infestans* causes foliage death, which lead to significant yield reductions. The ability to infect tubers is also of great importance since attacked tubers become useless for seed, ware or processing.

Today the only efficient management method to control late blight in susceptible and moderately resistant cultivars is frequent use of fungicides, which has a negative impact on the environment as well as on the health of the farmers. Despite this frequent use of fungicides, yield reductions and lowered quality of tubers leads to great economical losses, estimated to be up to several billion dollars annually (CIP 1996; Guenthner et al. 2001). The

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situation became more detrimental in Europe in the 1980s, when the occurrence of the new mating type, assigned A2, made sexual reproduction possible (Hohl and Iselin 1984). Therefore, it is essential to find new methods with minimized use of fungicides that is harmless to both the environment and the farmers that use them. Identification of new resistance and increased efforts in resistance breeding is of great importance.

One method to decrease late blight infections without a negative impact on the environment could be induced resistance, i.e. to apply non-toxic compounds or organisms that stimulate or prime plant defence. Among the most reported effective inducing agents in potato is BABA (DL-β-aminobutyric acid), a non-protein amino acid (Cohen and Gisi 1994; Jakab et al. 2001; Cohen 2002; Kim and Jeun 2007; Altamiranda et al. 2008; Olivieri et al. 2009). Only once has it been reported to have effect also under field conditions and this study was carried out in Israel (Cohen 2002).

BABA has been reported to induce resistance against pathogens in numerous plants including pea, tomato, tobacco, potato, grapevine and *Arabidopsis* (Cohen et al. 1999; Zimmerli et al. 2000; Jakab et al. 2001). Exactly how BABA induces resistance still remains unknown and the course of events seems to vary between different pathosystems. Different mechanisms, involving physical barriers such as callose deposition and lignin accumulation as well as biochemical defence mechanisms with accumulation of PR proteins, phytoalexins and phenols, have been suggested (Cohen et al. 1994; Jakab et al. 2001; Cohen 2002; Si-Ammour et al. 2003; Jeun et al. 2004; Hamiduzzaman et al. 2005). Jeun et al. (2000) reported that cells around penetration sites showed more intense fluorescence after aniline blue staining in BABA treated plants indicating formation of callose and at these sites significant growth inhibition and morphological changes of intercellular hyphae was observed. Macroscopic hypersensitive response (HR) were detected more frequently in induced plants.

Some recent studies have shown that the effect of BABA depends on the developmental stage of potato as well as on the cultivar used, e.g. the level of resistance (Andreu et al. 2006; Altamiranda et al. 2008 and Olivieri et al. 2009). Altamiranda et al. (2008) reported that foliar treatments with BABA 30 days after crop emergence showed a 60% and 20% efficacy against late blight in the moderately resistant cv. Pampeana and the highly susceptible cv. Bintje, respectively. Andreu et al. (2006), found the highest level of BABA protection against late blight at early stages of crop development, i.e. up to 30 days after emergence, and the best effect was found in moderately resistant cultivars.

Olivieri et al. (2009) reported that BABA application also resulted in improved tuber blight resistance and the degree of improvement depended on the basic level of resistance in each cultivar. Furthermore, BABA did not negatively affect tuber yield as can be expected since induced resistance may result in a metabolic cost. Four foliar applications with BABA showed instead increased amounts of tubers per plant and also total dry matter of tubers in both the moderately resistant and the highly susceptible cultivars.

Even if induced resistance alone may not be efficient enough under field conditions it potentially could be used together with fungicides to lower the necessary fungicide dose for efficient control. Baider and Cohen (2003) reported that using a mixture of BABA and the fungicide mancozeb was significantly more effective to control late blight in potato than BABA or mancozeb alone. A mixture containing 83% of BABA was superior to the other mixtures. Thus, the fungicide dose could be lowered extensively and still control late blight as effectively as full dose fungicide. The improved effect was interpreted as synergistic interaction between the two agents.

Despite numerous reports on BABA induced resistance in greenhouse-grown plants, there is only one report involving full-scale field experiments. Cohen (2002) reported significant field control of late blight by BABA. Possible combinatorial effects between induced resistance and fungicides needs to be investigated under field conditions.

The present work investigated to what degree BABA treatment can protect potato from late blight infection under Swedish field conditions and whether synergistic interactions occur if BABA is applied in combination with a commonly used fungicide, Shirlan. In greenhouse experiments we also investigated the durability of BABA induced resistance, the dose-response relationships in susceptible and partially resistant cultivars and effects of combined applications of BABA and fungicides.

Material and methods

Greenhouse experiments

Plant material Good quality seed tubers (S1 according to the Swedish classification system; www.sjv.se) of the potato cultivars Bintje (susceptible), Ovatio (partially resistant) and Superb (partially resistant), obtained from SW Seed AB were used in the experiments. Tubers were planted in 2 l plastic pots containing commercial pot soil (Weibull Horto, Sweden) containing a peat base supplemented with 3 kg m^{-3} dolomite lime, 3 kg m^{-3} limestone, 0.7 kg m^{-3} PG-mix fertilizer (NPK + micronutrients) and 120 kg m^{-3} sand (1–3 mm). The pots were placed in a greenhouse chamber with a temperature of approximately 18°C during day and 15°C during night with a day length of 16 h. Fertilizer Rika S® (Weibull Horto, Sweden) was regularly added with the irrigation water so that no mineral deficiency symptoms appeared.

Inoculation and disease assessment The detached leaf assay according to Eucablight protocols (http://www.eucablight.org), were used for assessment of the infection rate by *P. infestans*. *P. infestans* isolate SE03058, mating type A1, obtained from Björn Andersson, Department of Forest Mycology and Pathology, SLU, Sweden were used in all experiments and it was maintained at rye agar or pea agar media according to standard protocols. For inoculation, Petri dishes with *P. infestans* were flooded with sterile deionised water, the sporangial suspension was filtered through a 100 µm mesh and the concentration of sporangia was adjusted to 36,000 sporangia ml^{-1}. The suspension was incubated at 15°C in darkness for 2–3 h to release zoospores. Leaves were detached from the upper completely developed composite leaves of potato plants at a stage where budding started. Detached leaves were placed on a net in boxes with moistened filter paper at 15°C for 2 h before inoculation. Each leaf was inoculated with a 20 µl drop of the spore suspension along the central vein. The inoculated leaves were kept in a climate chamber at 15°C with the following light regime: 16 h light and 8 h dark. Six to 7 days after inoculation the lesions were scored by measuring the diameter of the lesions. On each leaf we also scored the degree of sporulation as: no sporulation, slight to moderate, or intense sporulation.

Experiment A. Duration of BABA effect Potato plants, cv. Bintje, were grown in the greenhouse as described above. At the stage of first budding the plants were treated with BABA (β-amino-butyric acid; Sigma), either by spraying the canopy with a 1.0 gl^{-1} distilled water solution until run-off or by giving a soil drench with 25 ml 0.2 gl^{-1} BABA per pot. Untreated control plants were given the same amount of tap water without BABA. For each treatment four replicates were used. Each replicate consisted of three plants placed as a group and at each sampling 5 leaves were sampled from this group for inoculation. The replicates were randomly distributed in the greenhouse chamber. The whole experiment was repeated. Detached leaves were inoculated at different times (see Fig. 1) after BABA treatment up to 20 days. The experiment with leaf spray was repeated a third time but this time leaves were inoculated only at day 2 after BABA treatment.

Experiment B. Dose response effects of BABA Potato plants, cv. Bintje and Ovatio, were grown in the greenhouse as described above. At the stage of first budding the plants were treated with BABA (β-amino-butyric acid; Sigma) with the following concentration: 0, 0.05, 0.1, 0.25, 0.5, 1.0 and 2.0 gl^{-1} distilled water by spraying the canopy of the plants until runoff. Two and three days after treatment leaves were sampled from the upper fully developed composite leaves for inoculation as described above. Three replicates were used for each treatment and cultivar. Each replicate consisted of three plants placed as a group and at each sampling 3 or 5 leaves were sampled from this group for inoculation. The replicates were randomly distributed in the greenhouse chamber.

The experiment was repeated with cvs. Bintje, Ovatio and Superb with the following BABA dosages: 0, 0.25, 0.5, 1.0 and 2.0 gl^{-1}.

Experiment C. Combined effects of BABA and fungicides Potato plants, cv. Bintje, were grown in the greenhouse as described above. At the stage of first budding the plants were treated with BABA (β-amino-butyric acid; Sigma) in combination with the fungicides Shirlan (ISK Biosciences Europe S.A., Belgium; Active ingredient: Fluazinam 500 gl^{-1}) or Electis (GOWAN Comércio Internacional e Servicos LDA, Portugal; Active ingredients: Mancozeb 66.7%

w/w and 8.3% Zotamid w/w). On basis of manufacturer's recommendation of full dose in field applications (0.4 lha^{-1} for Shirlan and 1.8 kg ha^{-1} for Electis) assuming a spray volume of 400 lha^{-1} we calculated the corresponding concentration for use in our greenhouse experiments (0.1% v/v for Shirlan and 0.45% w/v for Electis).

Two experiments were carried out. In the first experiment cv. Bintje and Ovatio were used and treated with 0, 20, 50 and 100% of full dose for BABA alone; 0%, 20%, 50% and 100% of full dose for Shirlan alone; Combinations of BABA and fungicides were applied at the following rates: 100+100%, 50+50%, 20+80% and 80+20% of full dose, respectively for Shirlan and BABA. The experiment was repeated one time.

The second experiment was conducted with cvs. Bintje and Ovatio with 0 and 0.05% (w/v) BABA alone; 0 and 0.02% (v/v) Shirlan alone; 0 and 0.09% (w/v) Electis alone; 0.02+0.05%, v/v and w/v, respectively for Shirlan and BABA in combination; 0.09+0.05%, w/v, respectively for Electis and BABA in combination.

Three replicates were used for each treatment and cultivar in these experiments. Each replicate consisted of three plants placed as a group and at each sampling 3 leaves were sampled from this group for inoculation. The replicates were randomly distributed in the greenhouse chamber and the canopy was sprayed with the different solutions until runoff. Two and three days after treatment leaves were sampled from the upper fully developed composite leaves for detached leaf assay as described above. In the combined treatments the synergy factor (SF) was calculated according to the Abbott method:

$$SF = \%Cobs/\%Cexp$$

where %Cobs is the observed percent disease protection and %Cexp is the expected percent disease protection. %Cexp was calculated in the following way:

$$\%Cexp = A + B - (A^{*}B)/100$$

where A is the percent protection from compound A only and B is the percent protection by compound B only.

Field experiments

Field experiments were carried out during 2007 and 2008. During 2007 two susceptible cultivars, i.e. Bintje and King Edward were used and in 2008 Bintje and the partially resistant Superb were used. The field trials were carried out at two different places, Borgeby and Mosslunda, in southern Sweden (Scania) by the Swedish Rural Economy and Agricultural Societies.

The field trials were performed according to Good Experimental Practice (GEP) consistent with EU directive 93/71, KIFS 2004:4, STAFS 2001:1 and Standard Operative Procedures, SLU 2004.

Experimental design At each place the trials were performed with a randomized block design with four blocks. Within each block the different treatments were randomly distributed. Each plot was 5 rows of 10 m length, from which the middle 3 rows were harvested. Between block 1 and 2 and between block 3 and 4 three rows of untreated plants were grown, serving as infector plants. No inoculation was carried out.

Field assessment of late blight Late blight (percent infection) was scored according to an assessment key by Syrén and Wiik (1993), a modification of the EPPO-scale (OEPP/EPPO 2004) comprising more exact assessments in the beginning of the epidemics. Visual inspections were done at the day of the first sign of infection until the maturation of the crop with intervals of 5–7 days. The relative area under the disease progress curve (rAUDPC) and the date of 10% infection was calculated for each plot. We define rAUDPC as the area under disease progress curve divided by the total area during the assessment period.

Yield and tuber blight Staff at the Swedish Rural Economy and Agricultural Societies determined the yield per plot. Sub-samples of 6 kg per plot were investigated for the presence of tuber blight.

Experiment in 2007 BABA and the fungicide Shirlan was applied in the following combinations at field trials at two different places, Borgeby (cv. King Edward) and Mosslunda (cv. Bintje): Untreated control; Shirlan 0.4 L ha^{-1}/kg ha^{-1}; BABA 0.12 and 0.6 kg ha^{-1}; Combinations of Shirlan/BABA 0.32/0.024, 0.2/0.06, 0.08/0.096, 0.16/0.048, 0.12/0.036

and 0.08/0.024 L ha^{-1}/kg ha^{-1}, respectively. The treatments were applied once a week until crop maturation, in total 13 times. Occasionally, a single day deviation from this scheme happened due to unfavourable whether conditions for spraying.

Experiment in 2008 Two cultivars were used, Bintje (susceptible) and Superb (partially resistant). The following treatments were applied for each cultivar: Untreated control; Shirlan 0.4, 0,3 and 0,2 l ha^{-1}; BABA 0.2 kg ha^{-1}; Combinations of Shirlan/BABA 0.3/0.1, 0.2/0.2 and 0.2/0.1 L ha^{-1}/kg ha^{-1}, respectively. The field trial was carried in two places, Borgeby and Mosslunda, in a similar manner. The treatments were applied once a week until crop maturation, in total 13 times. Occasionally, a single day deviation from this scheme happened due to unfavourable whether conditions for spraying.

Statistical analysis

The effect of treatments in the greenhouse experiments were investigated with analysis of variance (GLM) using the SAS software on relative values of lesion sizes (durability experiment) and log-transformed values of lesion sizes (dose-response experiment and combination experiments). Differences between treatments were tested with Ryan-Einot-Gabriel-Welsh multiple range test.

The effect of different treatments in the field experiments was investigated with Ryan-Einot-Gabriel-Welsh multiple range test. The effect of BABA in field was investigated with analysis of variance (GLM) using SAS on log-transformed values. A multiple regression analysis on the effects of Shirlan and BABA was done with the Minitab software.

Results

Greenhouse experiments

Durability of BABA-effect The effect of BABA-treatment on the lesion development in cv. Bintje up to 20 days after treatment was investigated (Fig. 1). There was a clear significant effect of leaf spray with BABA on lesion size development (GLM over all three experiments: $p<0.0001$). However, the largest effect was found up to 4 days after treatment where the lesion diameter was about 50% lower compared to untreated control (a significant interaction between lesion size and days after treatment was found; $p=0.0002$). When the data for 8–20 days after treatment was analyzed separately no significant effect of BABA was found. The effect of BABA given as a soil drench was less evident since lesion sizes were reduced with only 10–20%; still the overall effect was statistically significant (GLM, $p<0.003$).

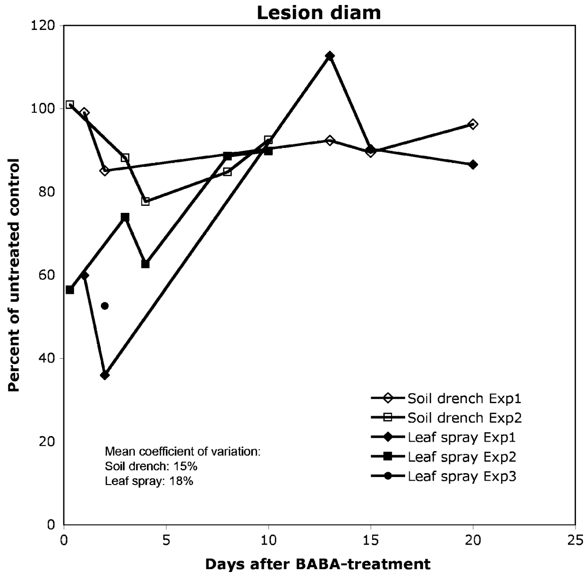

Fig. 1 Effect of BABA on *Phythophthora infestans* infection of potato, cv. Bintje. BABA was applied either as a soil drench or by spraying the leaf canopy. Different days after treatment detached leaves were sampled and inoculated with *P. infestans* and the diameter of the developed lesions were measured. In the figure values are expressed relative to untreated control that was set to 100

Dose-response effect of BABA A clear dose-response effect of the BABA treatment of greenhouse grown potato plants were found (GLM, $p<0.0001$; Fig. 2). The lesion diameter was significantly lower on the partially resistant cultivars Ovatio and Superb (GLM, $p<0.0001$). It also appeared that the partially resistant cultivars reacted more strongly on the BABA treatment than Bintje, indicated by a significant interaction between cultivar and BABA dose (GLM, $p=0.022$). At lower doses, e.g. 0.5 gl^{-1}, a clear effect of BABA on lesion size was found on Ovatio but not on Bintje. We also observed differences in macroscopic hyper-

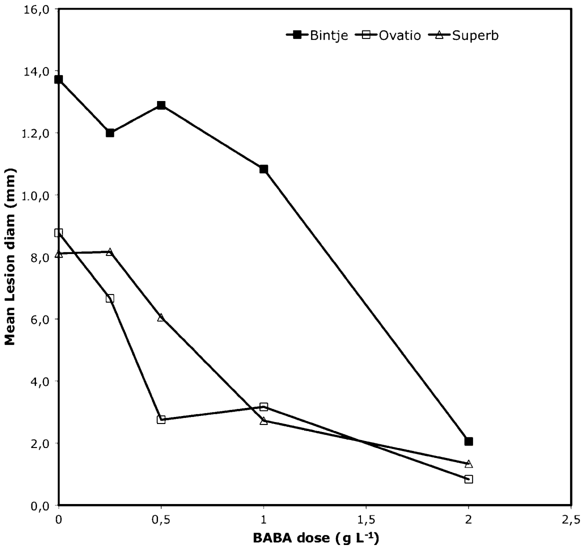

Fig. 2 Dose response effects of BABA on *Phythophthora infestans* infection of three potato cultivars, Bintje, Superb and Ovatio. 2 days after BABA treatment detached leaves were sampled and inoculated with *P. infestans* and the diameter of the developed lesions were measured

sensitivity reactions between Bintje and Ovatio. On Bintje the BABA treatment did not cause any visible symptoms on the leaves. However, after inoculation with *P. infestans*, brown necrotic spots were observed on BABA-treated leaves outside the infection site while these spontaneous lesions did not appear on non-treated control leaves. In contrast, leaves of Ovatio treated with BABA showed these lesions also without *P. infestans* infection. In a repeated experiment similar results were found.

Effect of combined treatment with BABA and fungicides Two experiments were carried out. In the first one different concentration and combinations of the fungicide Shirlan and the inducing compound BABA were used on cvs. Bintje and Ovatio (Fig. 3). BABA alone had a better effect on Ovatio and a clear dose-response effect was found. The combinations between BABA and the fungicide had a better effect than fungicide alone. The variation in lesion sizes was rather large. Analysis of variance (GLM) showed significant effects of Shirlan ($p<0.0001$), BABA ($p<0.0001$) and cultivar ($p=0.0004$). There was also a significant interaction between Shirlan and BABA treatment ($p=0.04$).

Also in the second experiment Ovatio was more resistant indicated by smaller lesions after infection with *P. infestans* (Fig. 4). Overall there was a significant interaction between treatment and cultivar (ANOVA, $p<0.0001$). As in the case of the above dose response experiment BABA had a better effect on Ovatio compared to Bintje. The effect on combinations seemed more to be an additive effect than synergistic, although when the effect of the two fungicides in combination with BABA was analysed separately there appeared to be a significant interaction between the fungicides and BABA for Ovatio ($p=0.016$ and $p<0.0001$, for Shirlan and Electis, respectively) but not for Bintje. The calculated synergy factors (SF) were quite low, at the highest 1.2, also indicating a more additive than synergistic effect. However, the SF values were somewhat higher in combinatons with relatively lower dose Shirlan and higher BABA compared to the treatment with high dose Shirlan and low BABA (Fig. 3).

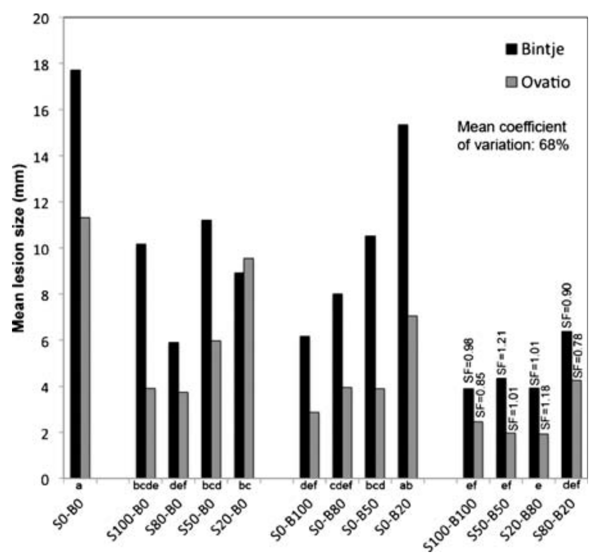

Fig. 3 Effects of BABA and the fungicide Shirlan, alone and in different combinations, on *Phythophthora infestans* infection of two potato cultivars, Bintje and Ovatio. 2 days after treatment detached leaves were sampled and inoculated with *P. infestans* and the diameter of the developed lesions were measured. The applied concentrations are given as percentage of full dose, e.g. S50–B50 means 50% of full dose Shirlan and 50% of full dose BABA (see Material and methods). Different letters under the bars indicate significant differences between treatments according to Ryan-Einot-Gabriel-Welsh multiple range test. SF = synergy factor calculated according to the Abbott method

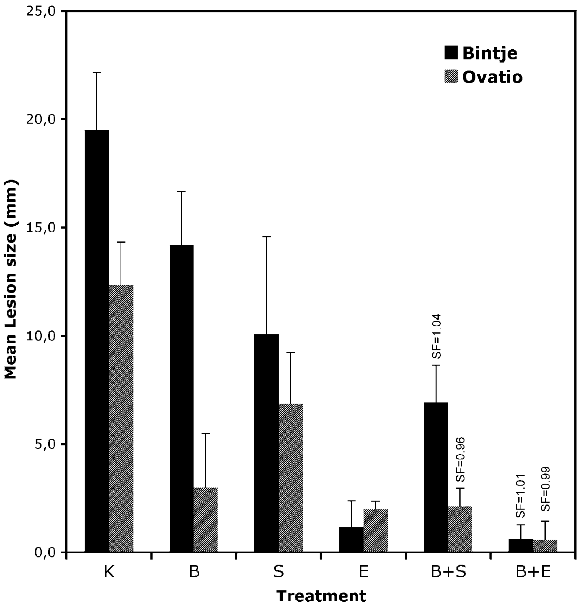

Fig. 4 Effects of BABA and the fungicides Shirlan and Electis, alone and in combinations, on *Phythophthora infestans* infection of two potato cultivars, Bintje and Ovatio. 2 days after treatment, detached leaves were sampled and inoculated with *P. infestans* and the diameter of the developed lesions were measured. Bars represent standard deviation. SF = synergy factor calculated according to the Abbott method

Field experiments

Experiment 2007 The first visible symptoms of late blight appeared already at the end of June. In Mosslunda, flooding caused by heavy rains affected both late blight infections and, to a higher degree, the yield. When infections started to appear the progress of the disease was relatively quick in plots not treated with a high dose of fungicide. There was an overall significant effect of treatment with BABA alone (0.6 kg/ha) on relative area under the disease progress curve (AUDPC, see Material and methods) during the first half of the disease progression (ANOVA, ProcGLM, $p<0.05$), but the effect within each experiment was not significant (Table 1). When plots damaged by flooding were excluded from the analysis the effect was significant on AUDPC over the whole period ($p=0.02$). However, the effect of BABA was weak, occurred mainly in the beginning of the infection period, and it only delayed the time up to 10% infection by on average one day. Full dose Shirlan did not stop the late blight attacks either, but delayed the infection with 12–16 days.

Reducing the amount of Shirlan by 20% and combining it with a low dose of BABA gave mean values of AUDPC that did not differ from full dose Shirlan. Half dose fungicide combined with BABA gave a significant higher AUDPC compared to full dose fungicide over the whole period but the difference during the first period was not statistically significant (Table 1). Lower doses of fungicide combined with BABA resulted in higher levels of late blight infections compared to standard doses.

The yield in plots treated with BABA alone did not significantly differ from untreated control. In Mosslunda half of the plots were excluded from the analysis due to damage by flooding. Full dose Shirlan resulted in significant higher yield compared to control, but half or 80% of full dose Shirlan combined with BABA did not result in significant lower yield compared to full dose Shirlan (Table 1).

In 2007 the average rate of tuber blight varied between 0 and 6% (w/w) but no relations with the treatments were found and the variation among replicate plots was large (data not shown).

Experiment 2008 In 2008 the late blight attacks started much later than in 2007 and the first symptoms appeared in beginning of August. However, the rate of disease development was extremely fast, especially in Mosslunda (Fig. 5). None of the treatments resulted in sufficient control of late blight. Also this year, treatment with BABA alone had a small but significant effect (Fig. 4, Table 2). ANOVA (GLM Proc) showed that the effect of BABA on AUDPC was significant during the first period ($p=0.0033$) and also for the entire period ($p=0.0086$). No significant interactions between BABA treatment and cultivar or field trial location were found. However, when the effect of BABA treatment was analysed separately for the two cultivars, the effect was significant on cv. Superb during the first period ($p=0.03$) but not significant in Bintje.

Lower doses, i.e 75% or 50% of that recommended for Shirlan in combination with BABA, never had a statistically significant higher AUDPC than full dose Shirlan (Table 2). However, 50 % Shirlan alone had a significantly higher AUDPC than full dose Shirlan in cv. Bintje, while in the partially resistant cv. Superb that difference was not significant.

The treatments 0.2 and 0.3 kg Shirlan alone or in combination with BABA were analysed and the BABA

Table 1 Relative area under disease progression curve (whole period and first half of infection period) and yield from field trials in 2007 with different application rates of BABA and Shirlan. Different letters indicate significant differences according to Ryan-Einot-Gabriel-Welsh multiple range test. α=0.05. For vertical comparisons within each sub-table only

Treatment	BABA kg ha^{-1}	Shirlan L ha^{-1}	AUDPCr	AUDPCr, first period	Yield ton ha^{-1}
Borgeby, Cv. King Edward					
A	0	0	0.72a	0.204a	27.6e
B	0	0.4	0.25d	0.007d	61.4ab
C	0.12	0	0.69a	0.167b	28.3e
D	0.6	0	0.68a	0.149b	26.9e
E	0.024	0.32	0.26d	0.006d	65.9a
F	0.06	0.2	0.37c	0.018d	56.2bc
G	0.096	0.08	0.57b	0.076c	37.9d
H	0.048	0.16	0.42c	0.021d	52.0c
I	0.036	0.12	0.54b	0.066c	43.1d
J	0.024	0.08	0.60b	0.087c	37.2d
Mosslunda Cv. Bintje					
A	0	0	0.81a	0.524a	40.8[a]
B	0	0.4	0.23cd	0.014d	91.6
C	0.12	0	0.79a	0.486ab	43.1
D	0.6	0	0.75ab	0.417abc	46.7
E	0.024	0.32	0.21e	0.011d	84.0
F	0.06	0.2	0.33cd	0.049d	87.5
G	0.096	0.08	0.57bc	0.174cd	66.5
H	0.048	0.16	0.44cd	0.065d	76.6
I	0.036	0.12	0.56bc	0.189cd	75.2
J	0.024	0.08	0.62abc	0.234bcd	70.0
Mean Shirlan 0.4			0.242	0.0105	76.5
Mean Shirlan 0.32+BABA			0.236	0.0084	75.0
Mean Shirlan 0.2+BABA			0.352	0.0336	71.8

[a] No multiple range test performed due to only two replicates, the other two damaged by flooding

treatment in addition to Shirlan resulted in significantly lower AUDPC (p=<0.001). When the cultivars were analysed separately we found a significant effect of BABA in Superb (p=0.0001) but not in Bintje.

In 2008, the untreated control had in most cases a significant lower yield than treatments with fungicides or combinations but the differences were smaller than in 2007 (Table 2). However, the different levels of fungicides and combinations did not result in significant differences in yield.

As in 2007, no relation between treatment and the rate of tuber blight was found. This year the average rate varied between 0 and 18% and the highest values were found in Mosslunda.

Overall analysis

A regression analysis of AUDPC-values (relative to untreated control) was carried out with combined data from both years. The regression analysis resulted in the following:

$$\begin{aligned}&\text{AUDPC (relative to untreated control)}\\&\quad = 1 - 2.44 \times \text{BABA} - 4.57 \times \text{Shirlan} + 5.53 \times \text{BABA}\\&\qquad \times \text{Shirlan} + 4.51 \times (\text{BABA})^2 + 6.00 \times (\text{Shirlan})^2\\&R^2 = 0.82\end{aligned}$$

In this equation, AUDPC is described as a function of Shirlan and BABA dose. This is a second degree equation, including an interaction term, with positive parameters for the second degree terms and therefore has a minimum value. The minimum value of this function was 0.052 kg ha^{-1} BABA combined with 0.36 l ha^{-1} Shirlan. Thus, according to this model a somewhat lower dose of Shirlan combined with a low dose BABA perform better than full dose Shirlan.

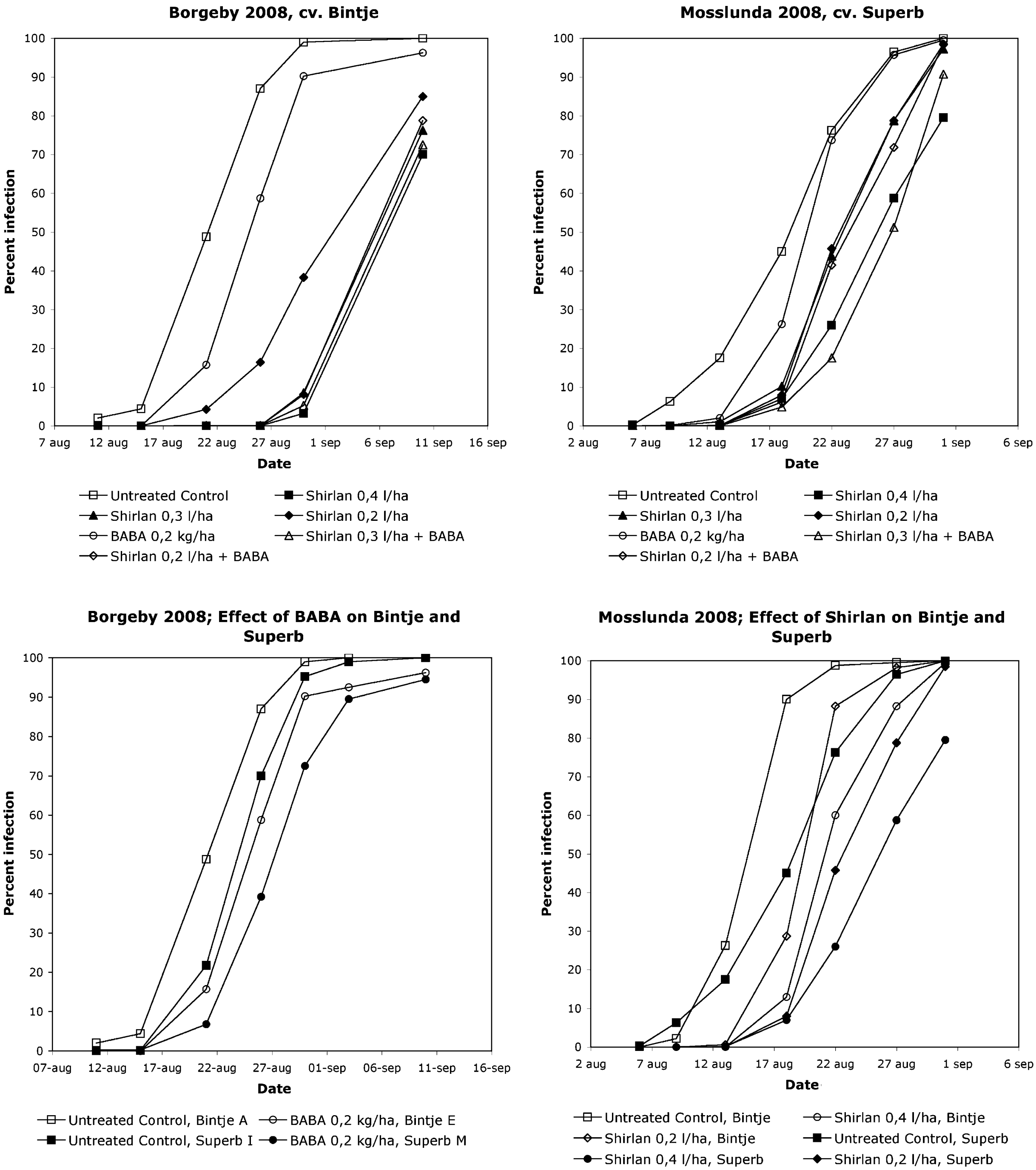

Fig. 5 Development of *Phythophthora infestans* infections in field experiments with potato cvs. Bintje and Superb treated with different doses of Shirlan and BABA once per week

Discussion

We found a clear effect of BABA on *P. infestans* infection of greenhouse grown potato plants. The lesion sizes were reduced by 40–50% compared to the untreated control, which is in accordance with many earlier studies of BABA induced resistance in potato (Jeun et al. 2000; Jakab et al. 2001; Cohen 2002; Si-Ammour et al. 2003; Altamiranda et al. 2008). Also in field trials we found an effect of BABA alone,

Table 2 Relative area under disease progression curve (whole period and first half of infection period) and yield from field trials in 2008 with different application rates of BABA and Shirlan. Different letters indicate significant differences according to Ryan-Einot-Gabriel-Welsh multiple range test. α=0.05. For vertical comparisons within each sub-table only

Treatment	BABA	Shirlan	rAUDPC		rAUDPC first period		Yield	
	kg ha^{-1}	L ha^{-1}					ton ha^{-1}	
Cultivar:			Bintje	Superb	Bintje	Superb	Bintje	Superb
Borgeby								
A	0	0	0.66a	0.57a	0.4033a	0.2561a	55.3b	66.5c
B	0	0.4	0.11c	0.02c	0.0002c	0.0002c	68.7a	83.1a
C	0	0.3	0.15bc	0.04c	0.0004c	0.0002c	70.5a	79.7ab
D	0	0.2	0.30b	0.09c	0.0574c	0.0003c	62.9a	73.5abc
E	0.2	0	0.52a	0.44b	0.2066b	0.1269b	64.5a	67.9bc
F	0.1	0.3	0.14bc	0.03c	0.0002c	0.0002c	67.2a	82.1a
G	0.2	0.2	0.15bc	0.08c	0.0003c	0.0002c	68.0a	78.6ab
H	0.1	0.2	0.14bc	0.05c	0.0002c	0.0003c	66.2a	78.1ab
Mosslunda								
A	0	0	0.65a	0.51a	0.3792a	0.2169a	77.0bc	76.2b
B	0	0.4	0.37b	0.23c	0.0419b	0.0230b	92.0a	88.0ab
C	0	0.3	0.41b	0.32bc	0.0677b	0.0364b	85.0ab	87.2ab
D	0	0.2	0.47b	0.32bc	0.0944b	0.0259b	80.4abc	94.2a
E	0.2	0	0.68a	0.44ab	0.4404a	0.0913ab	71.4c	80.9ab
F	0.1	0.3	0.46b	0.21c	0.0814b	0.0158b	82.8abc	88.9ab
G	0.2	0.2	0.47b	0.31bc	0.1167b	0.0238b	83.5abc	82.5ab
H	0.1	0.2	0.43b	0.28bc	0.0669b	0.0169b	85.6ab	86.1ab
Average								
A	0	0	0.65a	0.54a	0.3912a	0.2365a	66.2c	71.3b
B	0	0.4	0.24c	0.12b	0.0210b	0.0116c	80.4a	85.5a
C	0	0.3	0.28bc	0.18b	0.0340b	0.0183c	77.7a	83.4a
D	0	0.2	0.38b	0.20b	0.0759b	0.0131c	71.6abc	83.8a
E	0.2	0	0.60a	0.44a	0.3235a	0.1091b	67.9bc	74.4ab
F	0.1	0.3	0.30bc	0.12b	0.0408b	0.0080c	75.0ab	85.5a
G	0.2	0.2	0.31bc	0.19b	0.0585b	0.0120c	75.8ab	80.6ab
H	0.1	0.2	0.29bc	0.17b	0.0336b	0.0086c	75.9ab	82.1ab
Mean Shirlan 0.3 and 0.2			0.33	0.19	0.055	0.016	74.7	83.6
Mean Shirlan 0.3 and 0.2 with BABA			0.30	0.16	0.044	0.010	75.6	82.7

although the effect was small and delayed the infection process by only 1–3 days. When BABA was given in combination with the fungicides it appeared to have an additive effect both in greenhouse and field experiments. BABA combined with a reduced dose of fungicide (20–25%) had as good effect as full dose fungicide alone.

There are few reports on duration of the improved resistance due to BABA treatment but it appear to depend on the pathosystem (see review by Cohen 2002). A single dose of BABA on tomato was reported to be effective for 12 days against *P. infestans* (Cohen 1994) while persistence up to 30 days was reported for pearl millet against *Sclerospora graminicola* (Shailasree et al. 2001). In our greenhouse experiments a clear effect was found up to 4–5 days after treatment, but declined after that. Andreu et al. (2006) studied the duration of the effect of BABA and fosetyl-Al on *P. infestans* in different greenhouse grown potato varieties. In some varieties at least 50% protection was evident up to 20 days after treatment, which is much longer than in this study. However, the effect was not as good at later developmental stages and in some varieties the duration was much shorter. They used about four times higher concentration of BABA compared to us in their experiments, which may be one reason behind the longer persistence. In our field experiments BABA and fungicides were applied once a week and sometimes the interval became 8–9 days due to unfavourable weather conditions for spraying. It is possible that a better effect of BABA would have been obtained if the treatments had been applied at shorter intervals. In many countries it is now necessary to apply fungicides at short intervals, i.e. twice a week, due to the aggressive late blight attacks.

Jeun et al. (2000) reported soil drenches to be more effective than foliar application, in contrast to our results, although they applied higher concentrations (40 mM ≈ 4 gl^{-1}) on leaves and about the same amount per pot as for the soil drench.

Our experiments on the dose-response relationship showed that a higher concentration of BABA gave a stronger protective effect. At the highest dose (2 gl^{-1}) only very small lesions developed after inoculation, in particular in the more resistant cultivars. The cultivars with a higher level of resistance (Ovatio and Superb) showed induced resistance at lower concentrations of BABA when no effect was found in cv. Bintje. Cultivar differences in sensitivity to BABA were also evident by macroscopic brown spot development after BABA treatment with higher doses on cvs. Ovatio and Superb, but not on cv. Bintje, in greenhouse experiments (data not shown). In Bintje, spots developed first after *P. infestans* infection, but only on plants pre-treated with BABA. More knowledge about the mechanism behind priming might in the future facilitate selection of cultivars with stronger defence reactions in response to BABA.

On average the effect of BABA seemed to be greater on the partial resistant cv. Superb compared to cv. Bintje also in the field trials, although that was not as evident as in the greenhouse experiments. However, BABA alone delayed the time to 10% infection with on average 1.5 days in Bintje and 3 days in Superb. Furthermore, there appeared to be an interaction between BABA and cultivar when BABA was given on top of a reduced fungicide dose. As a comparison, full dose of the fungicide Shirlan delayed the infection by 11 days in Bintje and 14 days in Ovatio. Altamiranda et al. (2008) found in greenhouse trials no significant protective effect on the highly susceptible cv. Bintje, while in the more resistant cv. Pampeana up to 60% protection was obtained. Olivieri et al. (2009) reported that varieties with higher level of resistance responded more strongly to BABA, also for tuber blight resistance. We also analysed the level of tuber blight in the field experiments but were not able to establish any statistical relationship with the treatments.

That cultivars with higher level of basal resistance respond stronger to BABA suggests that BABA potentiates basal resistance mechanisms against *P. infestans*. The mechanisms behind BABA activation of defence is not well known, although correlations with callose deposition, HR frequencies, accumulation of phenolics, phytoalexins and hydrolytic enzymes etc has been documented after infection (Jeun et al. 2000; Jakab et al. 2001; Cohen 2002; Andreau et al. 2006; Olivieri et al. 2009). Still, there is no reliable marker for BABA induced resistance identified. BABA induced resistance may depend on relatively broad signalling pathways such as those involving abscisic acid (ABA, Goellner and Conrath 2008). Recent research on priming induced by salicylic acid derivates indicate that the induced state (primed state) is associated with accumulation of non-activated mitogen-activated protein kinases (MPKs; Beckers et al. 2009). More knowledge about the mechanism behind full priming might in the future facilitate selection of cultivars with stronger defence reactions in response to BABA. Breeding through improved potentiation of basal resistance might be expected to be a more durable strategy than introgression of single R genes from wild potato varieties, since *P. infestans* has a great ability to overcome many of these.

One important question is whether application of BABA, priming plant defence, has a metabolic cost that would affect the yield level. However, we did not find any signs of yield change after 13 times application of BABA in the field. Olivieri et al. (2009) analysed the effect of repeated BABA-treatments on tuber yield in greenhouse experiments and found no negative effect. Instead, the BABA treatment improved the yield of harvested tubers in cvs. Pampaena and Bintje. The cost and benefits of priming for defence in *Arabidopsis* has been investigated in greenhouse experiments using mutants insensitive for the inducing agent, constitutive priming mutant and constitutively activated defence mutant (Van Hulten et al. 2006). It was shown that priming involved less fitness cost than induced direct defence activation and the benefit of priming-mediated resistance was higher than the cost due to disease. Walters et al. (2009) applied saccharin as priming agent in barley against the fungus *Rhynchsporium secalis*. In field experiments they found no measurable effect of saccharin on plant fitness. However, the treatment provided significant benefits under high disease pressure.

We did find an effect of BABA in full-scale field trials although the effect of the compound alone was relatively small. It also have to be kept in mind that the field trials were made in fields with very high infection pressure due to concentrations of trials with infector plants and untreated control plots. We speculate that BABA treatment in a common agricultural field would have delayed the infection for a longer period of time. In our greenhouse experiments we scored the sporulation rate of *P. infestans* on the infected detached leaves as no sporulation, weak sporulation or strong sporulation (data not shown). In general untreated controls had strong sporulation while BABA-treated leaves showed weak sporulation. However, in this type of field trial the effect of BABA on the sporulation rate cannot be expected to affect the epidemic since heavy amounts of spores were always available in the rows with untreated infector plants besides each plot. It would be worthwhile to measure the effect of BABA on the sporulation rate more carefully.

We did not find any clear evidence for a synergistic effect with Shirlan in field trials, as reported before in greenhouse trials for other fungicides given in combination with BABA. In the greenhouse experiments there may have been a weak synergistic effect since we found a statistical interaction between BABA and the fungicide. However, the calculated Abbot synergy factor was at highest 1.2 indicating a more additive that synergistic effect. Baider and Cohen (2003) showed a clear synergistic effect between BABA and the fungicide mancozeb against *P. infestans* both in tomato and potato. A mixture of 1/6 Mancozeb and 5/6 BABA gave in most cases the best effect in greenhouse trials, better than full dose mancozeb. Synergy effects with other fungicides have also been described in patents (Cohen et al. 2002) and the degree of synergy with BABA appeared to vary with different fungicides.

BABA has been considered as a non-toxic compound that has no direct effect on pathogens in most studies (e.g. Cohen 2002). Still, no careful toxicologial investigation has been reported. In a recent publication Fischer et al. (2009) found direct effects on fungi, since BABA inhibited the mycelial growth of *Botrytis cinerea* and affected *Saccharomyces cerevisiae* growth in a concentration dependent manner. However, Baider and Cohen (2003) did not find any effect of BABA on sporangial germination and mycelial growth of *P. infestans*.

According to our field data 20–25% reduction of the fungicide dose in combination with BABA gave on average the same result on late blight development as full dose Shirlan alone, while reduced dose of Shirlan alone sometimes resulted in less effective protection. However, more field experiments are necessary to confirm that a reduced dose in practice can be used without risk, if combined with BABA. Further studies to find the most effective combination are also required. Since different cultivars appear to react differently future experiments could include tests of other varieties with different type of resistance and the frequency of treatments in the field since the durability of BABA in our experiments appeared to be less than a week.

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