*European Journal of Plant Pathology* **107:** 861–869, 2001.


# Phytogard® and DL-$\beta$-amino butyric acid (BABA) induce resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativa* L)

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Accepted 30 July 2001

*Key words:* induced localized or systemic resistance, pathogenesis related proteins (PR)

## Abstract

Downy mildew of lettuce (*Bremia lactucae*) is a serious disease. An alternative to chemicals is the application of disease resistance inducers. The aim of this study was to test whether DL-$\beta$-amino butyric acid (BABA) and Phytogard® ($K_2HPO_3$) could induce resistance in downy mildew susceptible plants. Aqueous solutions of BABA (0, 10, 20, 30, 50, 80, 100 mM) and Phytogard® (0.0, 5.8, 29.0, 40.6, 58.0 and 87.0 ppm) were sprayed on seven-day-old seedlings 0, 3, 7 and 15 days before or 1–3 days after inoculation with *B. lactucae*. Results obtained showed that Phytogard®- and BABA-induced resistance was dose-dependent. At 40.6 ppm for Phytogard® and 10 mM for BABA, complete protection was obtained. Both compounds had a curative effect and the induced resistance lasted for at least 15 days. It was also shown that both compounds induced systemic resistance in lettuce against downy mildew. Phytogard® at 40.6 ppm completely inhibited spore germination while BABA at 20 mM did not. Pathogenesis related (PR) protein analysis showed that BABA induced weak accumulation of PR-2, but not PR-1, PR-5 and PR-9. Phytogard® induced none of these proteins. The use of these two compounds to protect lettuce from *B. lactucae* is discussed.

## Introduction

Downy mildew of lettuce caused by *Bremia lactucae* is the most serious fungal disease of this crop. The disease can be controlled by chemicals including metalaxyl-based compounds but mutant isolates insensitive to these compounds have been recorded (Crute et al., 1985, 1994; Leroux et al., 1988). Disease resistance (R)-genes are also used to control the disease but recombinant isolates evading recognition conferred by the R-genes can occur.

Since the discovery of the resistance inducer benzothiadiazole (BTH, trade name Bion®) (Friedrich et al., 1996; Reuss et al., 1996; Ryals et al., 1996; Benhamou and Bélanger, 1998; Inbar et al., 1998; Dann et al., 1998; Jensen et al., 1998; Cole, 1999; Ishii et al., 1999; Schweizer et al., 1999; Anfoka, 2000; Colson-Hanks and Deverall, 2000), the use of such compounds has become an attractive alternative for controlling plant pathogens. Resistance inducers are of biotic or abiotic origin (Ryals et al., 1996; Schneider et al., 1996; Kombrink and Somssich, 1997) and render susceptible cultivars resistant to subsequent infections.

Apart from BTH and 2,6-dichloroisonicotinic acid (INA; Metraux et al., 1991; Uknes et al., 1992; Hijwegen and Verhaar, 1993; Kogel et al., 1994), potassium and phosphate salts (for review, Reuveni and Reuveni, 1998), as well as DL-$\beta$-amino butyric acid (BABA; Cohen, 1994a,b, 1996; Cohen and Gisi, 1994; Cohen et al., 1994, 1999; Sunwoo et al., 1996; Siegrist et al., 2000) are well documented as plant protecting agents and are efficient in many pathosystems.

We have shown that BTH (Godard et al., 1999a,b), $K_2HPO_3$ (Bécot et al., 2000), $K_3PO4$ and $H_3PO_3$ (Pajot et al., unpublished) as well as BABA (Silué et al., in press) could protect cauliflower (*Brassica oleracea* var. *botrytis*) from downy mildew of brassicas caused

by *Peronospora parasitica.* The aim of this study was to test whether BABA and $K_2HPO_3$ can protect lettuce (*Lactuca sativa*) from downy mildew (*B. lactucae*).

## Materials and methods

### *Host preparation*

The downy mildew susceptible accession Saladin (Rijk Zwaan, The Netherlands) was used. Seeds were sown in trays filled with peat and grown in the greenhouse (25 ± 0.1 °C days, 16 ± 2 °C nights) for seven days.

### *Chemicals*

DL-$\beta$-amino butyric acid (BABA) was purchased from Sigma (France) and aqueous solutions of 0 (control), 10, 20, 30, 50, 80, 100 mM were prepared. Phytogard® is a liquid solution containing 58% potassium phosphonate ($K_2HPO_3$) and 42% water. It was obtained from CATE (St Pol-de-Léon, France) and is produced by Intrachem Bio (France). Different quantities were mixed in water to obtain final concentrations of 5.8, 29.0, 40.6, 58.0 and 87 ppm.

### *Fungal isolates*

Six single spore isolates of *B. lactucae* were used. They all originated from Brittany (France) and their characteristics are given in Table 1. The conidia were produced on the susceptible accession Saladin and sporulating cotyledons were gently agitated in sterile water to dislodge the conidia. The inoculum was adjusted to 20,000 conidia/ml. The suspension was sprayed on to the seedlings (250 µl/seedling) and the inoculated seedlings were kept in sealed propagators in the dark for at least eight hours in the growth cabinet (20 ± 0.1 °C days, 16 ± 0.1 °C nights), followed by incubation for seven days with a 12 h photoperiod.

*Table 1.* Characteristics of the six *B. lactucae* isolates tested

| Names | Year of isolation | Location of origin | Status* |
|---|---|---|---|
| BL1A | June 1999 | Brittany (France) | S derived from BL1** |
| BL1B | June 1999 | Brittany (France) | S derived from BL1 |
| BL2A | August 2000 | Brittany (France) | S derived from BL2 |
| BL2B | August 2000 | Brittany (France) | S derived from BL2 |
| BL2C | August 2000 | Brittany (France) | S derived from BL2 |
| BL2D | August 2000 | Brittany (France) | S derived from BL2 |

*S: single spore isolate.
**: BL1 and BL2 were first isolated from two independent samples of diseased lettuce leaves. Single spore isolations were then done.

### *Dose-response analysis*

Seedlings were sprayed with the test solutions (approximately 250 µl/seedling) using compressed air. The treated seedlings were kept in the growth cabinet (20 ± 0.1 °C days, 16 ± 0.1 °C nights, 12 h light) in sealed propagators to avoid exogenous contaminations. The seedlings were inoculated three days after treatment with *B. latucae*. For each concentration, 50–60 seedlings were tested and at least three replicates were carried out in independent experiments.

### *Isolate specificity of the induced resistance*

To test whether the induced resistance was isolate specific, 50–60 treated seedlings were inoculated three days later with each of the six isolates BL1A, BL1B, BL2A, BL2B, BL2C and BL2D. Two replicates were done in independent experiments.

### *Duration of protection*

To assess the duration of protection conferred by Phytogard® or BABA, seedlings were treated and inoculated 0–15 days later. In another experiment the seedlings were first inoculated with downy mildew and then treated 1, 2 and 3 days later with the test solutions. The aim of this experiment was to test whether the compounds had a curative effect. Each experiment was repeated at least three times with 50–60 seedlings per treatment.

### *Systemic mode of action*

To test whether the compounds had a systemic mode of action, one cotyledon per seedling was drop-treated (2 × 20 µl) followed by inoculation of the whole seedlings three days later. Seven days after inoculation, treated and non-treated cotyledons were scored independently for disease expression. 50–60 seedlings were

analysed and a mean disease index (DI) was calculated. Two replicates were carried out.

*Disease rating*

Seven days after inoculation, disease assessment was done by using a six-point (0, 1, 3, 5, 7, 9) scale that corresponds to the class of interaction phenotypes. On this scale, rating 0 corresponds to plants without any visible symptoms. Rating 1 corresponds to plants exhibiting hypersensitive reactions (HR) or HR-like lesions without any conidiophores. When 1–3 conidiophores were produced on the cotyledons with or without HR symptoms, rating 3 was given. Rating 9 corresponds to seedlings showing a heavy sporulation on both sides of the cotyledons. Such seedlings often died. Rating 5 and 7 corresponded to intermediate responses.

A mean DI was calculated for each treatment (50–60 seedlings/treatment) according to the formula:

$$\mathrm{DI} = \frac{\sum_{i=0}^{9}(i \times j)}{n}$$

where $n$ = total number of plants, $i$ = interaction phenotype class and $j$ = number of plants per class. Analysis of variance (ANOVA) was carried out to compare all treatments.

*PR-protein analysis*

To analyse the PR-proteins induced by BABA and Phytogard®, seedlings were treated and harvested 0, 1, 2, 3, 4 and 7 days after spraying. Control seedlings were infected with the virulent isolate BL1A. They were frozen in liquid nitrogen until protein extraction. Each sample consisted of 1.5 g fresh weight of seedlings. Protein extraction and PR-protein analysis, as well as the $\beta$-1,3-glucanase activity, were done according to the method already described (Jung et al., 1995). Four antibodies raised against tobacco acidic PRs were provided by Dr B. Fritig (IBMP, Strasbourg, France): PR-1(C), PR-2(2), PR-5(S), PR-9.

## Results

*Dose-response analysis*

Results obtained with Phytogard® revealed that the level of protection was dose-dependent. At 5.8 ppm the DI was 4.6 while that of the water control was 7.8. The dose of 5.8 ppm led to an efficacy of 41%. At 29.0 ppm, the efficacy was higher than 83% (Figure 1) and higher concentrations provided complete protection (no sporulation).

BABA at 10 mM stopped the disease. At this concentration the mean DI was lower than 0.2 (Figure 2) indicating that the seedlings had no visible symptoms or that HR-like necrosis were sometimes observed. Higher concentrations (20–100 mM) also provided

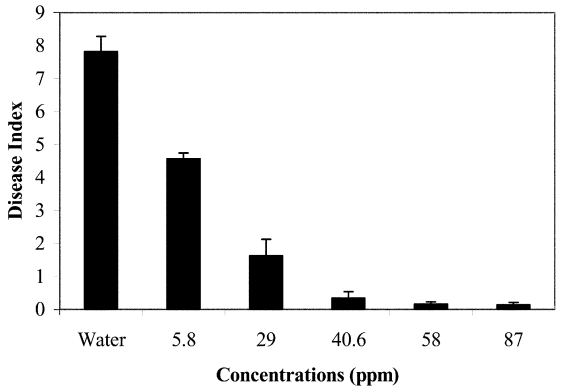


*Figure 1.* Dose-response analysis in Phytogard®-treated seedlings. 50–60 seven-days-old seedlings were treated with various (5.8–87 ppm) Phytogard® solutions and challenged three days later with the downy mildew isolate BL1A. Control seedlings were treated with water. Disease assessment was then done seven days later and a mean disease index was calculated for each treatment. Bars represent standard deviations.

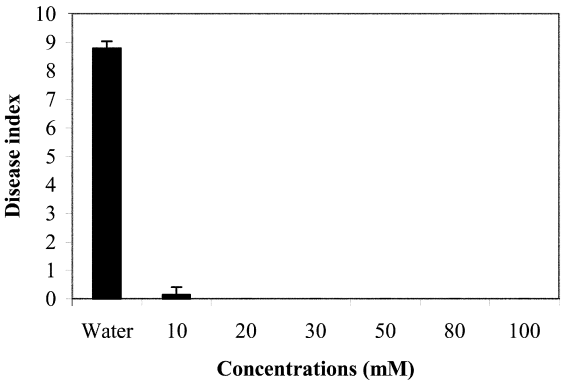


*Figure 2.* Dose-response analysis in BABA-treated seedlings. 50–60 seven-days-old seedlings were treated with various (0–100 mM) BABA solutions and challenged with the downy mildew isolate BL1A three days later. Disease assessment was then done seven days later and a mean disease index was calculated for each treatment. Bars represent standard deviations.

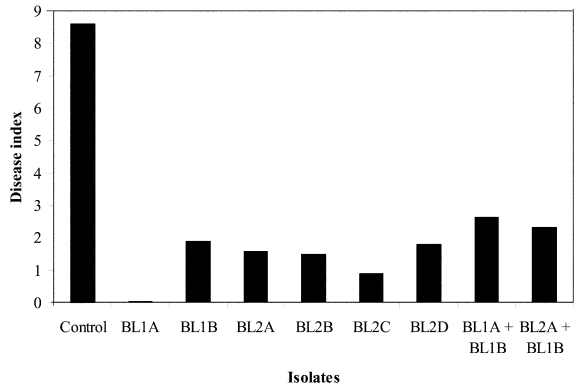


*Figure 3.* Efficacy of Phytogard® to control downy mildew of lettuce. 50–60 seedlings were treated with a 40.6 ppm solution and challenged with each of the six isolates or their mixture. Disease assessment was done seven days after inoculation. Data are means of two independent replicates. The disease index of the water control is the mean of all water controls when testing each isolate independently.

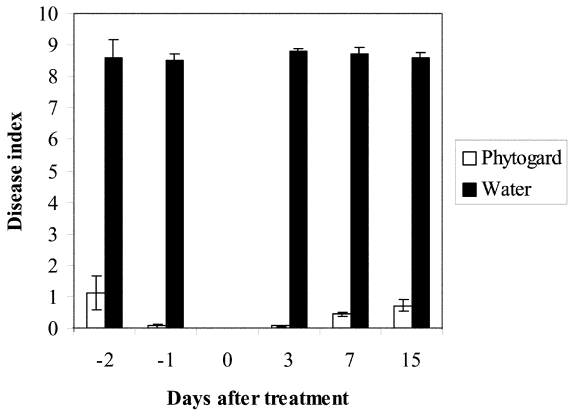


*Figure 4.* Assessment of the length of protection conferred by Phytogard® (40.6 ppm) on lettuce. Seven days-old seedlings were treated with Phytogard® and inoculated with the downy mildew isolate BL1A −2, −1, 0, 3, 7 and 15 days later. Control plants were treated with water and also inoculated with isolate BL1A. Bars represent standard deviations.

complete protection of the seedlings and were not phytotoxic.

*Isolate specificity of the induced resistance*

When the six *B. lactucae* isolates were tested individually on seedlings treated with a 40.6 ppm Phytogard® solution, none of them caused severe disease and DIs obtained were comparable, that is, lower than 3.0 (Figure 3). When the treated seedlings were inoculated with a mixture of spores derived from different isolates, similar results were obtained (Figure 3).

*Systemic mode of action*

When one cotyledon per seedling was treated with a 40.6 ppm Phytogard® solution and whole seedlings challenged three days later with *B. lactucae,* treated and non-treated cotyledons were protected from the disease. The corresponding DI was 0.1 and 1.4 for treated and non-treated cotyledons respectively, while that of the water control was 8.7. This indicate that Phytogard® has a systemic mode of action.

When one cotyledon per seedling was treated with a 20 mM solution of BABA followed by challenged of whole seedlings with *B. lactucae* three days later, treated and non-treated cotyledons had a mean DI of 0.0 and 0.8, respectively. The mean DI of water controls was 8.3. These results indicate that BABA acts in a systemic manner.

*Duration of the protection*

Figure 4 shows that when Phytogard®-treated seedlings were inoculated 3, 7 and 15 days after the treatment, they were completely protected from the disease. In fact, the DIs were always lower than 1.0 indicating the absence of visible symptoms or, on a few seedlings only, the presence of small HR-like necrotic lesions. Similar results were obtained when the treatment was done one day after the inoculation (Figure 4). Phytogard® has therefore a curative effect. When plants were treated two days after inoculation, a few conidiophores were produced on some cotyledons. However, the mean DI remained lower than 3.0 indicating that a good level of protection was still observed.

BABA-treated plants inoculated 0, 3, 7 and 15 days later were completely protected from the disease (Figure 5). In fact, the mean DIs obtained were lower than 1.0 (no sporulation). When the treatment was done one and three days after inoculation, complete protection (DIs lower than 0.5) was still observed. It can therefore be concluded that BABA has a curative mode of action in controlling lettuce downy mildew.

*Action on conidia germination*

When suspended in a 40.6 ppm Phytogard® solution, none of the conidia germinated. The mean germination rate of the water control was 77%. Therefore, Phytogard® has a direct fongitoxic effect. On the other

hand, BABA at 20 mM did not inhibit spore germination, indicating an indirect mode of action.

*PR-proteins*

BABA slightly induced accumulation of the acidic PR-2 (Figure 6A) 3–7 days after treatment. This result was confirmed by the $\beta$-1,3-glucanase activity test, showing that enzyme activity increased with time in BABA-treated plants (data not shown). In fact, $\beta$-1,3-glucanase activity expressed in $10^{-9}$ moles/g fresh weight (FW) of plant material did not exceed $4 \times 10^{-9}$ moles/g FW in the water control seven days after treatment. In BABA-treated seedlings, the enzyme activity increased with time and reached $16 \times 10^{-9}$ moles/g FW seven days after. PR-2-like proteins were also induced in seedlings infected with the virulent isolate BL1A but the induction was late (7–9 days post inoculation) and weak (Figure 6B). PR-1, PR-5 and PR-9 were not detected in plants treated with BABA (results not shown), though for the analysis of these PRs, we did not have positive controls. Phytogard® induced none of the four PR-proteins (results not shown).

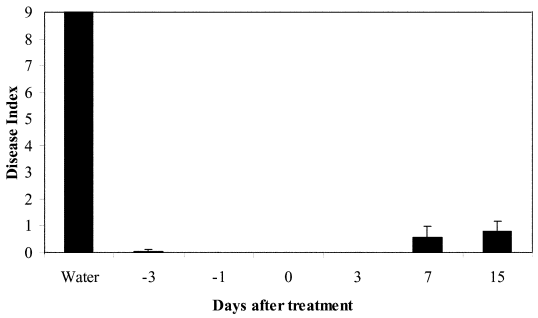


*Figure 5.* Assessment of the length of protection conferred by BABA treatment (20 mM) on lettuce. Seven-days-old seedlings were treated with BABA and inoculated with the downy mildew isolate BL1A −3, −1, 0, 3, 7 and 15 days later. Control plants were treated with water and inoculated with isolate BL1A. Bars represent standard deviations.

## Discussion

This study has shown that Phytogard® ($K_2HPO_3$) protects lettuce against downy mildew caused by *B. lactucae*. When applied at 40.6 ppm before inoculation, the seedlings were completely protected from the disease and showed no visible symptoms. When applied after inoculation, results obtained also showed that $K_2HPO_3$ prevented disease development. This suggests that $K_2HPO_3$ has a direct mode of action against pathogen development in lettuce tissues. As *B. lactucae* is an obligate pathogen, we monitored whether the compound inhibited conidia germination *in vitro*. The results showed that Phytogard® inhibited spore germination. To date, the mode of action of $K_2HPO_3$ for the control of downy mildew on lettuce is

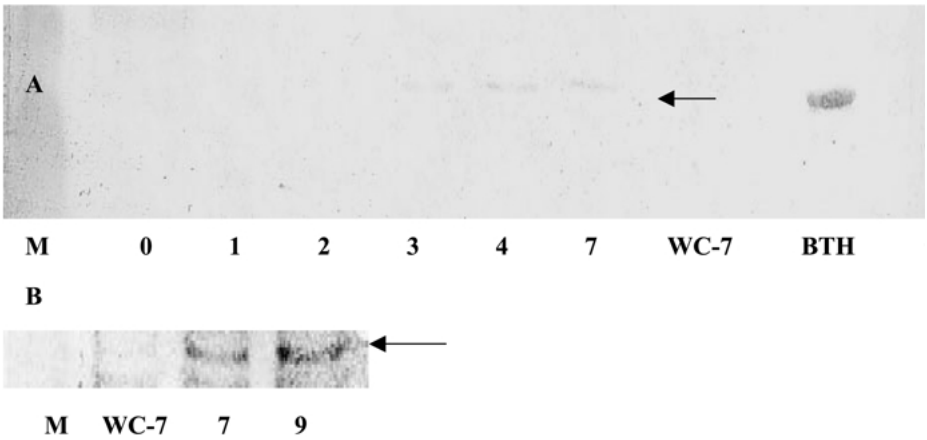


*Figure 6.* Accumulation of PR-2 in seedlings of lettuce treated with BABA (Panel A) or inoculated with the *B. lactucae* isolate BL1A virulent on the lettuce accession Saladin (Panel B). Seedlings were harvested 0–7 days after treatment. Control seedlings (WC-7) were treated with water and harvested seven days later. A positive control consisted of cauliflower seedlings treated with BTH and harvested seven days later. The antiserum used was raised against tobacco PR2-2. Arrows show the PR-2 band. Numbers indicate days after treatment (Panel A) or inoculation (Panel B). M is the protein size marker.

still not understood but other studies on Aliette, a similar compound, have suggested that, after degradation in soil and plant, phosphonate ions ($HPO_3$) were released. These ions are known for their antimicrobial activity (Cohen and Coffey, 1986; Ouimette and Coffey, 1989; Fenn and Coffey, 1989). Although challenge inoculation of Phytogard®-treated seedlings was delayed by up 15 days, the fungitoxic effect observed in *in vitro* assays may also be effective on seedlings.

Systemic-mode-of-action assays showed that non-treated cotyledons were protected from the disease, indicating that induced systemic resistance (systemic acquired resistance) was induced by Phytogard®. It can therefore be concluded that protection of lettuce from downy mildew by this compound is based on both an indirect and probably a direct fungitoxic effect.

This study has shown that $K_2HPO_3$ was efficient against six isolates tested and lasted for at least 15 days. In another study, $K_2HPO_3$ caused complete, but localized, protection in cauliflower against downy mildew of brassicas caused by *P. parasitica* (Bécot et al., 2000). Similar compounds, for example, $KH_2PO_4$, $K_2HPO_4$, $K_2SO_4$, $NH_4H_2PO_4$ were shown to be systemic resistance inducers in many pathosystems (for review, Reuveni et al., 1998) including maize/*Puccinia sorghii* (Reuveni et al., 1994, 1996), maize/*Exserohilum turcicum* (Reuveni et al., 1996), cucumber/*Sphaerotheca fuligenea* (Reuveni et al., 1993, 1995a,b, 1997) and pepper/*Leveillula taurica* (Reuveni et al., 1998). Taken together, the results indicate that the systemic mode of action of these compounds probably depends on their mobility in the plant tissue or on an induced systemic signal(s). It is therefore possible that $K_2HPO_3$ or its induced signals(s) migrate poorly in the cauliflower tissues whereas in lettuce, its translocation is better. This hypothesis awaits testing in cauliflower and lettuce by using radiolabelled compounds.

In order to test if $K_2HPO_3$ induced resistance by activating the synthesis of molecules involved in plant defence, the accumulation of PR-proteins was monitored. Although many PRs have no proven function in disease, they have been shown to be good indicators of the induced-resistant state (Van Loon, 1997). $K_2HPO_3$-treated plants did not accumulate the acidic PR-1, PR-2, PR-5 and PR-9. In their recent review, Pieterse and Van Loon (1999) provided several examples of different types of induced resistance that was not associated with the accumulation of PRs, for example, resistance induced by exogenous application of salicylic acid. However, it is not clear whether $K_2HPO_3$-treated lettuce plants that became resistant to *B. lactucae* do not accumulate PRs at all, or whether they accumulate PRs that are different from the downy mildew-induced ones. Another possibility is that Phytogard® only potentiates the induction of PRs that are only detectable after challenge inoculation. This has already been found in *Arabidopsis thaliana*/*Pseudomonas syringae* pv *tomato* (Zimmerli et al., 2000) and *B. oleracea* var. *botrytis*/*P. parasitica* (Silué et al., in press) in which BABA was used as the resistance inducer.

This study has also shown that low amounts of BABA (10 mM) protect lettuce from *B. lactucae*. The protection was long lasting (at least 15 days) and systemic. Furthermore, the compound had no effect on spore germination *in vitro*, suggesting that resistance was induced. Cohen et al. (1999) have shown that BABA-induced resistance in grapevine against *Plamopara viticola* was associated with the translocation of 3.5–29.9% of the total amount of the compound from treated to non-treated leaves. This great variation depended on the age of the treated leaf, and young treated leaves translocated BABA better than did older leaves. It appears therefore that the systemic mode of action of BABA found in lettuce was associated with efficient translocation of the compound.

BABA had a curative effect on *B. lactucae* development. The compound was also curative on downy mildew of brassicas (Silué et al., in press). These results contrast with those published by Zimmerli et al. (2000) who stated that BABA had no curative effect on *P. parasitica* on *A. thaliana*. The experiment carried out by these authors could not allow this conclusion to be drawn since the seedlings were treated with BABA six days after inoculation with *P. parasitica*, one day before sporulation. It is known that even systemic fungicides (e.g. metalaxyl) do not prevent disease development in these conditions. Concentrations higher than 10 mM (20–100 mM) were also protective but still not phytotoxic. In cauliflower, BABA concentrations higher than 20 mM provided complete protection against downy mildew (*P. parasitica*) but they were phytotoxic (Silué et al., in press). Root treatment with a 20 mM solution was also phytotoxic in cauliflower. Therefore, phytotoxicity of BABA seems to be plant species-dependent.

The mode of action by which BABA prevents downy mildew of lettuce is still not known. However, we could show that this compound weakly induced the acidic PR-2. Accumulation of PR-2 protein was stronger in lettuce seedlings inoculated with a virulent

isolate of *B. lactucae*. In cauliflower, BABA-induced plants showed no PR-protein accumulation. However, when the BABA-treated plants were inoculated with *P. parasitica*, a massive accumulation of PR-2 was found (Silué et al., in press). Zimmerli et al. (2000) obtained a similar result in the pathosystem *A. thaliana*/ *P. syringae* pv *tomato*. Therefore, it would be interesting to test whether potentiation of PR-protein accumulation also occurs in lettuce. Another possibility is that in cauliflower and lettuce, BABA induced other compounds involved in resistance mechanisms such as phytoalexins or nitric oxide.

This study has shown that $K_2HPO_3$ and BABA protected lettuce against the most serious fungal pathogen, *B. lactucae*, probably by inducing resistance in the plant. Our results have shown that seedlings treated with these compounds and challenged with different single-spore isolates or isolate mixtures were protected to a similar extent. It is therefore likely that resistance induced by these compounds is not isolate-specific. Field tests will help to solve this issue. Most of the disease resistance inducers identified to date have no curative effect. Therefore, they must be applied on plants before any disease occurs. These conditions can be easily monitored in the laboratory, but not so in the fields and this may therefore explain why field results do not always fit those obtained in the laboratory.

Testing of BABA and Phytogard® in the field will reveal their usefulness as crop protection agents (alone or in mixture with other disease resistance inducers) against *B. lactucae*. Even if their efficacies in the field were lower than in the laboratory, these compounds could help to reduce the input of other chemicals used against *B. lactucae.* To date, effects of these two compounds on the environment are not known since they are still not used in fields. However, BABA seems to have no direct effect on microorganisms. Phytogard® at low amounts inhibits spore germination. It should be checked whether both compounds have any negative effect on the environment. Results obtained will condition their effective use in the field either alone, or with low classical fungicides.

## Acknowledgements

Special thanks to the CERAFEL (Morlaix), ONIFHLOR (Paris), The Conseil Régional de Bretagne, The Conseil Général du Finistère (Quimper) and the Ministère de l'Agriculture (Paris) for financial support. Professor Y. Cohen (Bar-Ilan University, Israel) is acknowledged for reviewing the manuscript.

## References

Anfoka GH (2000) Benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester induces systemic resistance in tomato (*Lycopersicon esculentum*. Mill cv. Volledung) to cucumber mosaic virus. Crop Protection 19: 401–405

Bécot S, Pajot E, Le Corre D, Monot C and Silué D (2000) Phytogard® ($K_2HPO_3$) induces localized resistance in cauliflower to downy mildew of crucifers. Crop Protection 19: 417–425

Benhamou N and Bélanger R (1998) Induction of resistance to Pythium damping-off in cucumber plants by Benzothiadiazole: ultrastructure and cytochemistry of the host response. The Plant Journal 14: 13–21

Cohen Y and Coffrey MD (1986) Systemic fungicides and the control of oomycetes. Annual Review of Phytopathology 24: 311–338

Cohen Y (1994a) Local and systemic control of *Phytophthora infestans* in tomato plants by DL-$\beta$-amino-n-butanoic acids. Phytopathology 84: 55–59

Cohen Y (1994b) $\beta$-Aminobutyric acid induces systemic resistance in tobacco against *Peronospore tabacina*. Physiological and Molecular Plant Pathology 44: 273–288

Cohen Y and Gisi U (1994) Systemic translocation of 14C-DL-$\beta$-aminobutyric acid in tomato plants in relation to induced resistance against *Phytophthora infestans*. Physiological and Molecular Plant Pathology 45: 441–456

Cohen Y, Niderman T, Mosinger E, Fluhr R (1994) $\beta$-aminobutyric acid induces pathogenesis-related proteins in tomato (*Lycopersicon esculentum* Mill.) and resistance against *Phytophthora infestans*. Plant Physiology 104: 59–66

Cohen Y (1996) Induced resistance against fungal diseases by aminobutyric acid. In: Lyr H, Russel PE and Sisler HD (eds) Modern Fungicides and Antifungal Compounds (pp 461–466) Intercept, Andover

Cohen Y, Reuveni M and Baider A (1999) Local and systemic activity of BABA (DL-3-aminobutyric acid) against Plasmopara viticola in grapevines. European Journal of Plant Pathology 105: 351–361

Cole DL (1999) The efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance, against bacterial and fungal diseases of tobacco. Crop Protection 18: 267–273

Colson-Hanks ES and Deverall BJ (2000) Effect of 2,6-dichloroisonicotinic acid, its formulation materials and benzothiadiazole on systemic resistance to Alternaria leaf spots in cotton. Plant Pathology 49: 171–178

Crute IR, Norwood JM and Gordon DL (1985) Resistance to phenylamide fungicides lettuce and Brassica downy mildew. Proceedings of the Mixture Centenary Meeting, Bordeaux, British Crop Protection Council Monograph No 31, pp 311–314

Crute IR, Gordon PI and Moss NA (1994) Variation for responses to phenylamides in UK populations of *Bremia lactucae* (lettuce downy mildew) and *Peronospora parasitica* (Brassica downy mildew). In: Heaney S, Slawson D, Hollomon DW, Smith M,

Russel PE and Parry DW (eds) British Crop Protection Council Monograph No 60, pp 155–162

Dann E, Diers B, Byrum J and Hammerschmidt R (1998) Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotium* in fields and greenhouse studies. European Journal of Plant Pathology 104: 271–278

Fenn ME and Coffey MD (1989) Quantification of phosphonate and ethyl phosphate in tobacco and tomato tissues and significance for the mode of action of two phosphonate fungicides. Phytopathology 79: 76–82

Friedrich L, Lawton K, Dincher S, Winter A, Staub T, Uknes S, Kessmann H and Ryals J (1996) Benzothiadiazole induces systemic acquired resistance in tobacco. The Plant Journal 10: 61–70

Godard JF., Ziadi S, Monot C, Le Corre D and Silué D (1999a) CGA 245704, a benzothiadiazole-derived compound induces resistance in cauliflower (*Brassica oleracera* var *botrytis*) to downy mildew of crucifers caused by *Peronospora parasitica*. In: Lyr H, Russell PE, Dehne HW, Sisler HD (eds) Modern Fungicides and Antifungal Compounds (pp 367–372) Intercept, Andover

Godard JF, Ziadi S, Monot C, Le Corre D and Silué D (1999b) CGA 245704, Benzothiadiazole (BTH) induces resistance in cauliflower (*Brassica oleracea* var *Botrytis*) to downy mildew of crucifers caused by *Peronospora parasitica*. Crop Protection 18: 397–405

Hijwegen T and Verhaar MA (1993) Induced resistance to *Peronospora parasitica* in red cabbage. Netherlands Journal of Plant Pathology 99(Suppl 3): 103–107

Inbar M, Doostdar H, Sonoda RM, Leibee GL and Mayer RT (1998) Elicitors of plant defensive systems reduce insect densities and disease incidence. Journal of Chemical Ecology 24: 135–149

Ishii H, Tomita Y, Horio T, Narusaka Y, Nakazawa Y, Nishimura K and Iwamoto S (1999) Induced resistance of acibenzolar-S-methyl (CGA 245704) to cucumber and Japanese pear diseases. European Journal of Plant Pathology 105: 77–85

Jensen BD, Latunde-Dada AO, Hudson D and Lucas JA (1998) Protection of Brassica seedlings against downy mildew and damping-off by seed treatment with CGA 245704, an activator of systemic acquired resistance. Pesticide Science 52: 63–69

Jung JL, Maurel S, Fritig B and Hahne G (1995) Different pathogenesis-related proteins are expressed in sunflower (*Heliantus annus L.*) in response to physical, chemical and Stress factors. Journal of Plant Physiology 145: 153–160

Kogel KH, Beckhove U, Dreschers J, Munch S and Romme Y (1994) Acquired resistance in barley. The resistance mechanism induced by 2,6-dichloroisonicotinic acid is a phenocopy of a genetically based mechanism governing race-specific powdery mildew resistance. Plant Physiology 106: 1269–1277

Kombrink E and Somssich JE (1997) Pathogenesis-related proteins and plant defence. In: Caroll G and Tudzynski P (eds) The Mycota V Part A Plant Relationships (pp 107–128) Springer Verlag, Berlin, Heidelberg

Leroux P, Maisonneuve B and Bellec Y (1988) Détection en France de souches de Bremia lactucae, agent du mildiou de la laitue, résistantes au métalaxyl et à l'oxadixyl. PHM-Revue Horticole 292: 37–40

Métraux JP, Ahl Goy P, Staub T, Speich J, Steinmann A, Ryals J and Ward E (1991) Induced systemic resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens. In: Hennecke H and Verma DPS (eds) Advances in Molecular Genetics of Plant-Microbe Interactions Vol 1 (pp 432–439), Kluwer Academic Publishers, Dordrecht, The Netherlands

Ouimette DG and Coffey MD (1989) Comparative antifungal activity of four phosphonate compounds against isolates of nine Phytophthora species. Phytopathology 79: 761–767

Pieterse CMJ and Van Loon LC (1999). Salicylic acid-independent plant defence pathways. Trends in Plant Science 4: 52–58

Reuss W, Mueller K, Knauf-Beiter G, Kunz W and Staub T (1996) Plant activator CGA 245704: an innovative approach for disease control in cereals and tobacco. In: Proccedings of The Brighton Crop Conference, Pest & Disease, pp 53–60

Reuveni M, Agapov V and Reuveni M (1993) Induction of systemic resistance to powdery mildew and growth increase in cucumber by phosphonates. Biological Agriculture and Horticulture 9: 305–315

Reuveni R, Agapov V and Reuveni M (1994) Foliar spray of phosphates induces growth increase and systemic resistance to *Puccinia sorghi* in maize. Plant Pathology 43: 245–250

Reuveni M, Agapov V and Reuveni R (1995a) Suppression of cucumber powdery mildew (*Sphaerotheca fulginea*) by foliar spray of phosphate and potassium salts. Plant Pathology 44: 31–39

Reuveni R, Reuveni M and Agapov V (1995b) Induced systemic protection to powdery mildew in cucumber plants by phosphonate and potassium fertilizers: effect of inoculum concentration and post-inoculation treatment. Canadian Journal of Plant Pathology 17: 247–251

Reuveni R, Reuveni M and Agapov V (1996) Foliar spray of NPK fertilizers induce systemic protection against *Puccinia sorghi* and *Exserohilum turcicum* and growth response in maize. European Journal of Plant Pathology 102: 339–348

Reuveni M, Agapov V and Reuveni M (1997) A foliar spray of micronutrient solutions induces local and systemic protection against powdery mildew (*Sphaerotheca fulginea*) in cucumber plants. European Journal of Plant Pathology 103: 581–588

Reuveni R, Dor G and Reuveni M (1998) Local and systemic control of powdery mildew (*Leveillula taurica*) on pepper plants by foliar spray of mono-potassium phosphate. Crop Protection 17: 703–709

Reuveni R and Reuveni M (1998) Foliar-fertilizer therapy. A concept in integrated pest management. Crop Protection 17: 111–118

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner H-Y and Hunt MD (1996) Systemic acquired resistance. The Plant Cell 8: 1809–1819

Schneider M, Schweizer P, Meuwly P and Métraux J-P (1996) Systemic acquired resistance in plants. International Review of Cytology 168: 303–340

Schweizer P, Schlangenhauf E, Schaffrath U and Dudler R (1999) Different patterns of host genes are induced by *Pseudomonas syringae*, a biological inducer of resistance, and the chemical

inducer benzothiadiazole (BTH). European Journal of Plant Pathology 105: 659–665

Siegrist J, Orober M and Buchenauer H (2000) $\beta$-amino butyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. Physiological and Molecular Plant Pathology 56: 95–106

Silué D, Pajot E and Cohen J. DL-$\beta$-amino-*n*-butanoic acid (BABA) induces resistance in cauliflower against downy mildew (*Peronospora parasitica*). Plant Pathology (in press)

Sunwoo JY, Lee YK and Hwang BK (1996) Induced resistance against *Phytophthora capsici* in pepper plants in response to DL-$\beta$-amino-n-butyric acid. European Journal of Plant Pathology 102: 663–670

Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D, Slusarenko A, Ward E and Ryals J (1992) Acquired resistance in Arabidopsis. The Plant Cell 4: 645–656

Van Loon LC (1997) Induced resistance in plants and role of pathogenesis-related proteins. European Journal of Plant Pathology 103: 753–765

Zimmerli L, Jakab G, Métraux JP and Mauch-Mani B (2000) Potentiation of pathogenesis-specific defense mechanisms in Arabidopsis by $\beta$-aminobutyric acid. Proceedings of the National Academy of Sciences USA 7: 12920–12925