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Induced resistance of acibenzolar-S-methyl (CGA 245704) to cucumber and Japanese pear diseases

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Abstract

Antifungal activity of the novel compound acibenzolar-S-methyl (CGA245704: benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester) was examined *in vitro*. No remarkable activity was observed on mycelial growth and conidial germination of almost all fungi tested. Only melon isolates of *Didymella bryoniae* were sensitive to this compound. On potted plants, acibenzolar-S-methyl showed control efficacy on anthracnose and scab of cucumber and rust of Japanese pear but not on *Fusarium* wilt of cucumber. In field trials, the occurrence of both rust and scab on Japanese pear was suppressed with this compound. Based on these experiments, it was suggested that acibenzolar-S-methyl induced resistance to some but not all diseases on cucumber and Japanese pear. Induction of disease resistance in cucumber was rapidly triggered after treatment with acibenzolar-S-methyl.

Introduction

Most chemical agents used for crop disease control are categorized as either fungicides or bactericides, which possess a direct mode of action on the pathogens. Currently, resistance development by plant pathogens to fungicides is a factor limiting quality food production worldwide due to the decrease in efficacy of fungicides (Brent, 1995). Furthermore, in the development and use of chemical agents for crop disease control, considerable attention must be given to the preservation of the global environment. Modern fungicides used at present may influence non-target organisms such as saprophytic fungi resulting in the disturbance of fungal populations on plant surfaces.

However, only a few non-fungitoxic chemicals are now available for the control of fungal diseases. Probenazole, which was the first commercialized disease-resistance inducer is an example of such a compound and is widely used for the control of rice blast in Japan. Interestingly, with this chemical agent no reports have yet been made on the failure of disease control due to the occurrence of resistant fungal strains.

Besides probenazole, other chemical inducers of disease resistance in plants have been described including salicylic acid (SA), 2,6-dichloroisonicotinic acid (INA) and 3-aminobutyric acid (BABA) (Kessmann et al., 1994; Cohen, 1994; Sticher et al., 1997; Willits and Ryals, 1998). Furthermore, one of the benzothiadiazole compounds (BTHs), acibenzolar-S-methyl (CGA245704: benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester) was developed by Novartis Crop Protection AG and was introduced in 1996 as a 'plant activator' for the control of wheat powdery mildew in Germany and Switzerland (Ruess et al., 1996). It has already been briefly reported that acibenzolar-S-methyl does not exhibit significant direct activity against phytopathogenic fungi and bacteria

(Kessmann et al., 1995). Instead, the compound, induces systemic acquired resistance (SAR) to diseases and protects wheat, tobacco, *Arabidopsis* and others from various pathogens (Görlach et al., 1996; Friedrich et al., 1996; Lawton et al., 1996; Benhamou and Bélanger, 1998; Jensen et al., 1998; Morris et al., 1998). As reviewed recently (Lyon and Newton, 1997), there is the desire to reduce toxic or harmful pesticide inputs in agriculture. In such circumstances, the use of 'plant activators' may offer new opportunities for disease control within an environmentally-friendly integrated crop protection system.

In the present study, the authors tested control efficacy of acibenzolar-S-methyl on fungal diseases on cucumber (*Cucumis sativus* L.), Japanese pear (*Pyrus pyrifolia* Nakai var. *culta* Nakai) and others under greenhouse and field conditions. The timing of triggering resistance induction was also examined. Brief results of our experiments have been reported previously (Ishii et al., 1998a,b).

Materials and methods

Fungal strains and compounds. The fungal strains used in this study are listed on Table 1. The compound acibenzolar-S-methyl (50% water dispersible granule [50WG] and technical grade) and its direct metabolite benzo(1,2,3)thiadiazole-7-carboxylic acid (technical grade; Kunz et al., 1997) were generously provided by Novartis Crop Protection AG. The dithiocarbamate fungicide polycarbamate (dizinc bis[dimethyldithiocarbamate]ethylenebis [dithiocarbamate], 75% wettable powder [75WP]) was purchased.

Mycelial growth tests and conidial germination tests in vitro. Each strain of the fungus was cultured on potato dextrose agar (PDA) plates at 20 °C or 25 °C for appropriate period. Mycelial discs, four mm in diameter, were cut from the margins of colonies and transferred onto either PDA or Czapek agar plates supplemented with acibenzolar-S-methyl or the metabolite. These compounds were dissolved in ethanol and then added to molten PDA or Czapek agar after autoclaving. The final concentration of ethanol was 1%. After incubation at 20 °C or 25 °C for appropriate period, the diameter of each colony was measured and the EC_{50} for mycelial growth of each compound was calculated.

Conidia of *Colletotrichum lagenarium* (= *C. orbiculare*, cucumber anthracnose) were formed on PDA plates, which included 0.5% yeast extract incubated at 25 °C for one wk in the dark and for a further 1 wk under fluorescent lamps. For *Cladosporium cucumerinum* (cucumber scab), conidia were formed on PDA plates after incubation at 20 °C for two wk. Conidia of both fungi were collected and washed with distilled water (DW) by centrifugation and then suspended in two-fold diluted potato dextrose broth (PDB). Conidia of *Venturia nashicola* (the cause of scab on Japanese pear) were obtained from an orchard and suspended in DW after washing.

The conidial suspension of each fungus was mixed with a suspension of acibenzolar-S-methyl (50WG) and dropped on a glass-slide. After incubation at 20 °C or 25 °C for 24–48 h in a moist chamber, conidial

Table 1. Fungal species and strains used in this study

Species and strain	Host	Source
Cladosporium cucumerinum, CC-1	Cucumber	Japan Plant Protection Association
Colletotrichum lagenarium, C-14	Cucumber	ZEN-NOH Agricultural R & D Center
Fusarium oxysporum f.sp. *cucumerinum*, C731505	Cucumber	National Agriculture Research Center
MAFF103054	Cucumber	National Institute of Agrobiological Resources
Corynespora cassiicola, 6-1	Cucumber	Ibaraki Agricultural Center
Venturia nashicola, JS-115	Japanese pear	National Institute of Agro-Environmental Sciences
Alternaria alternata Japanese pear pathotype, Kawahara-1	Japanese pear	Tottori Horticultural Experiment Station
Botrytis cinerea, W919 7-6	Grapevine	Akita Fruit Tree Experimental Station
Didymella bryoniae, MM-1	Melon	Japan Plant Protection Association
Gymnosporangium asiaticum	*Juniperus* sp.	Akita Fruit Tree Experimental Station

germination was assessed and germ-tube length measured microscopically.

Inoculation tests on plants. (i) *Cucumber anthracnose and scab.* First leaves of two to three-wk-old cucumber plants (cv. Shin Suyo Tsukemidori), grown at 25 °C in a phytotron, were dipped in the suspensions of acibenzolar-S-methyl (50WG) at a concentration of 100 μg ml^{-1} (active ingredient: a. i.) for five sec and kept in the same condition. DW was used as a control. Zero to seven days after treatment, conidial suspensions (ca. 5×10^5 conidia ml^{-1} PDB diluted two-fold with DW) of *C. lagenarium* or *C. cucumerinum* were sprayed onto whole plants. The inoculated plants were kept at 20 °C in a dew chamber for 24 h followed by the incubation at 25 °C in a phytotron. Assessment of disease suppression by acibenzolar-S-methyl treatment was carried out seven days after inoculation.

(ii) *Cucumber Fusarium wilt.* One-wk-old cucumber plants grown at 25 °C in a phytotron were sprayed with either a suspension of acibenzolar-S-methyl (50WG) or DW as a control. Seven days later, conidial suspensions (ca. 5–10 $\times 10^5$ conidia ml^{-1} DW) of *Fusarium oxysporum* f. sp. *cucumerinum*, which were prepared from shaking culture at 28 °C in PDB for 10 days were drenched onto the cucumber plants and disease occurrence was examined one month after incubation in a phytotron.

(iii) *Melon gummy stem blight.* One-month-old melon plants (cv. Mrs Earl's Natsu) were sprayed with either a suspension of acibenzolar-S-methyl (50 μg ml^{-1}, a.i., [50WG]) or DW. After keeping overnight at room temperature, the plants were inoculated with the mycelial homogenates of four melon isolates (all sensitive to acibenzolar-S-methyl *in vitro*) of *Didymella bryoniae*, which were grown on PDA plates at 25 °C for 11 days. The inoculated plants were kept at 25 °C in a dew chamber for 24 h and incubated at 25 °C in a phytotron for three wk after which assessment of disease development was performed.

(iv) *Japanese pear rust and black spot.* Potted Japanese pear trees of cv. Osa-Nijisseiki were used for inoculation with *Gymnosporangium asiaticum* (Japanese pear rust) or *Alternaria alternata* Japanese pear pathotype (black spot). Young leaves were sprayed with either a suspension of acibenzolar-S-methyl (50WG) or DW and kept at 25 °C in a phytotron. Seven days later, the leaves on trees were separately inoculated with teliospore suspensions (ca. 2.5×10^5 spores ml^{-1} DW) of *G. asiaticum* or conidial suspensions (ca. 2.5–5 $\times 10^5$conidia ml^{-1} DW) of *A. alternata* Japanese pear pathotype. Teliospores of *G. asiaticum* were collected directly from the alternative host *Juniperus* sp. The inoculated trees were kept at 25 °C (rust) or 28 °C (black spot) for three days in a dew chamber. Then diseased leaves were immediately counted for black spot caused by *A. alternata*. With regard to rust, the inoculated trees were further incubated at 25 °C in a phytotron for 15 days until disease development was assessed.

(v) *Grapevine grey mold.* Young leaves of potted grapevine cv. Neo Muscut were protectively treated with a suspension of acibenzolar-S-methyl (50WG) and kept at 25 °C in a phytotron. Seven days after treatment, paper discs (eight mm in diameter) were placed on both sides of the upper leaves and conidial suspensions of *Botrytis cinerea* (ca. 5×10^5 conidia ml^{-1} of two-fold diluted PDB) were dropped onto the paper discs. After incubation at 20 °C for four days in a dew chamber, the lengths of lesions surrounding the paper discs on leaves were measured.

Field trials. In 1996, tests were conducted in an experimental orchard at the Horticultural Research Institute, Ibaraki Agricultural Center. Between 20 May and 19 June, four acibenzolar-S-methyl (50WG) sprays were applied to eight-year-old Japanese pear trees (cv. Kosui). Two trees were used for each treatment. To evaluate the efficacy of acibenzolar-S-methyl, the leaves of five randomly chosen shoots per tree were assessed for scab development on 3 July.

In 1997, further experiments were performed in an experimental orchard at the National Institute of Agro-Environmental Sciences. On 20 April and 1 May, two acibenzolar-S-methyl (50WG) sprays were applied to two-year-old Japanese pear trees (cv. Kosui). Three trees were used for each treatment. Rust development was assessed for all leaves of each tree on 23 May.

Results

In vitro *activity of acibenzolar-S-methyl.* (i) *Mycelial growth.* Inhibitory activity of acibenzolar-S-methyl against mycelial growth of the fungi tested was scarcely observed on PDA or Czapek agar plates (Table 2). EC_{50} values of acibenzolar-S-methyl for the growth of most fungi were higher than 100 μg ml^{-1}, which was the highest concentration used. However, melon isolates of *D. bryoniae*

were sensitive to this compound (EC_{50}: 2.0 μg ml^{-1} on PDA and 1.9 μg ml^{-1} on Czapek agar, respectively). Benzo(1,2,3)thiadiazole-7-carboxylic acid, the metabolite of acibenzolar-S-methyl, did not suppress the mycelial growth of fungi even at 100 μg ml^{-1} (data not shown). Interestingly, the activity was not exhibited even against melon isolates of *D. bryoniae*.

(ii) *Conidial germination and germ-tube growth.* Inhibitory activity of acibenzolar-S-methyl against conidial germination of *C. lagenarium*, *C. cucumerinum* and *V. nashicola* was null or low if any (Table 3). On the whole, this compound did not sharply inhibit the germination or germ-tube growth on a glass-slide even at 100 μg ml^{-1}. With *C. cucumerinum*, germ-tube growth was even more vigorous in the presence of acibenzolar-*S*-methyl. To the contrary, the ratio of appressorial formation was somewhat lower in *C. lagenarium* when the conidia were treated with this compound. In *V. nashicola*, germination and germ-tube growth at 100 μg ml^{-1} were retarded 62% and 78%, respectively.

Table 2. Effects of acibenzolar-S-methyl on mycelial growth of phytopathogenic fungi on culture medium

Fungal species	EC_{50}(μg ml^{-1})	
	PDA	Czapek agar
C. cucumerinum	>100	>100
C. lagenarium	>100	>100
F. oxysporum f.sp. *cucumerinum*	>100	>100
C. cassiicola	>100	>100
V. nashicola	>100	>100
A. alternata Japanese pear pathotype	>100	>100
B. cinerea	>100	>100
D. bryoniae	2.0	1.9

Disease suppression with acibenzolar-S-methyl in a greenhouse. (i) *Cucumber anthracnose, scab and Fusarium wilt*. Treatment with acibenzolar-S-methyl showed remarkable protective activity against anthracnose (Table 4). Strikingly, disease suppression with acibenzolar-S-methyl was found even when the whole cucumber plants were inoculated with the pathogen 3 h after treatment with acibenzolar-S-methyl. High control efficacy was observed on both the treated leaves (1st leaves) and untreated upper leaves. High protective activity against anthracnose was highly reproducible on treated and untreated leaves. However, phytotoxicity of acibenzolar-S-methyl (100 μg ml^{-1}) such as chlorosis, browning or mosaic formation often appeared on treated 1st leaves and very occasionally on the untreated 2nd leaves. The level of such phytotoxicity

Table 3. Effects of acibenzolar-S-methyl on conidial germination, germ-tube growth and appressorial formation of *Colletotrichum lagenarium*, *Cladosporium cucumerinum* and *Venturia nashicola* on a glass-slide[1]

Acibenzolar-S-methyl (μg ml^{-1})	Germination (%)	Germ-tube length[2] (μm)	Appressorial formation (%)
C. legenarium:			
100	73	153.9 ± 99.46	26
10	55	175.1 ± 102.83	41
1	43	158.7 ± 112.38	39
0	73	137.8 ± 102.99	63
C. cucumerinum:			
100	97	121.8 ± 34.07	
10	99	142.9 ± 45.46	
1	100	69.1 ± 24.59	
0	96	59.8 ± 21.92	
V. nashicola:			
100	29	16.9 ± 6.87	
10	40	50.9 ± 42.39	
1	69	50.9 ± 39.60	
0	77	75.4 ± 60.35	

[1]One hundred conidia were observed under microscope for each treatment.
[2]Mean and standard deviation for germinated conidia.

Table 4. Control efficacy of acibenzolar-S-methyl (100 μg ml^{-1}, a.i.) on cucumber anthracnose in a greenhouse[1]

Inoculation day after treatment	Control (%) and mean number of lesions per leaf in untreated control plots in parenthesis			
	1st leaf	2nd leaf	3rd leaf	4th leaf
0 (3 h)	−[2](105)	95.4[a](109)		
1	−(13.4)	100[a](118)	100[b](5)	
2	−(38.6)	99.1[a](106.6)	100[a](47.6)	
3	100[b](219.6)	99.6[a](226)	100[a](111.6)	100[b](21)
5	−(51.2)	100[b](65.8)	100[a](113.4)	100[a](32.2)
7	−(10.2)	100[b](8.6)	99.5[b](41.8)	100[a](15.8)

[1]Five plants were used for each treatment. Total number of leaf lesions was counted and control (%) was calculated as follows: ([Number on untreated leaves − Number on treated leaves]/Number on untreated leaves) × 100.
[2]Counting of lesions was hard to do because of the phytotoxicity of acibenzolar-S-methyl although control efficacy of the compound was obvious.
[a,b]Significantly different from untreated control at 1% and 5% level, respectively (Dunn's multiple range test).

Table 5. Control efficacy of acibenzolar-S-methyl (100 μg ml^{-1}, a.i.) on cucumber scab in a greenhouse[1]

Inoculation day after treatment	Disease severity and control (%) in parenthesis				
	1st leaf	2nd leaf	3rd leaf	4th leaf	5th leaf
0 (3 h)	26.7(33.3)	53.3[b](42.9)			
1	13.3[a](80.1)	26.7[a](71.4)	46.7[a](53.3)	66.7[b](28.5)	
2	26.7(49.9)	33.3[a](50.1)	26.7[a](73.3)	13.3[a](86.7)	
3	53.3(11.2)	33.3[a](58.4)	26.7[a](73.3)	53.3[b](42.9)	
5	60.0(−12.6)	33.3(37.5)	40.0[b](53.9)	33.3[a](66.7)	86.7(7.1)
7	40.0(14.3)	26.7(42.8)	33.3[b](54.6)	40.0[b](45.4)	73.3(21.4)

[1]Five plants were used for each treatment. Each leaf was scored based on the level of disease development using the following scale: 0, no lesions; 1, small lesions formed but no effects on leaf growth; 2, large lesions with effects on leaf growth; 3, leaf died. Disease severity (DS) was calculated as follows: ([3A + 2B + C]/3D) × 100 where A, B, and C are the number of leaves corresponding to the scores 3, 2 and 1, respectively. D is the total number of leaves assessed. Control (%) = ([DS on untreated leaves − DS on treated leaves] / DS on untreated leaves) × 100.
[a,b]Significantly different from untreated control at 1% and 5% level, respectively (Dunn's multiple range test).

seemed to be variable according to the environmental conditions, e.g. light and/or temperature conditions.

Disease suppression with acibenzolar-S-methyl treatment was also shown against scab (Table 5) although the level of control was lower than that against anthracnose mentioned above. In this case also, scab development was suppressed with this compound on the plants inoculated with the pathogen as early as three h after treatment, and the results were reproducible.

Severe *Fusarium* wilt occurred on both acibenzolar-S-methyl-treated and untreated cucumber plants (data not shown). No control efficacy of acibenzolar-S-methyl was observed even at 100 μg ml^{-1}. The fungal pathogen was successfully reisolated from the symptom confirming that the wilt was due to the disease.

(ii) *Melon gummy stem blight*. Severe symptom of blight occurred on melon plants irrespective of the treatment with acibenzolar-S-methyl. All treated plants

were dead after inoculation with the pathogen. Pycnidia were observed on diseased stems and pycnospore formation was confirmed under microscope.

(iii) *Japanese pear scab, rust, black spot and grapevine grey mold.* Acibenzolar-S-methyl showed moderate disease suppression against rust and the efficacy was more active at 100 μg ml^{-1} than at 10 μg ml^{-1}: control (%) was 75.9 and 47.6, respectively. In contrast, the efficacy of acibenzolar-S-methyl treatment was very poor against both black spot of Japanese pear and grey mold of grapevine. With the treatment at 100 μg ml^{-1}, control was only achieved 2.3% and 16.8%, respectively.

Control efficacy of acibenzolar-S-methyl in a field. In 1996, four spray applications of acibenzolar-S-methyl at 100 μg ml^{-1}showed control efficacy against Japanese pear scab equal to that of the commercial fungicide, polycarbamate, used at 938 μg ml^{-1}as a control (Table 6). At 10 μg ml^{-1}of acibenzolar-S-methyl, the efficacy was inferior.

The trials carried out in 1997 demonstrated that two spray applications of acibenzolar-S-methyl were also effective against rust on Japanese pear under field conditions (Table 7). However, the control efficacy was lower even at 100 μg ml^{-1} than that of polycarbamate used at 750 μg ml^{-1} (a.i.), which is recommended for practical use.

Discussion

These days, 'sustainable development' is a key concept when we discuss the global environment. Public pressure over pesticide residues in food and in the environment has forced policy changes in several countries.

Table 6. Control efficacy of acibenzolar-S-methyl on Japanese pear scab in a field

Treatment	Number of leaves assessed	Diseased leaves (%)	Control (%)[1]
Acibenzolar-S-methyl			
100 μg ml^{-1}	191	23.0[a]	50.0
10	238	33.6	27.0
Polycarbamate			
938 μg ml^{-1}	238	20.6[a]	55.2
None	248	46.0	

[1]([Diseased leaves (%) on untreated trees − Diseased leaves (%) on treated trees] / Diseased leaves (%) on untreated trees) × 100.
[a]Significantly different from untreated control at 1% level (Dunn's multiple range test).

Table 7. Control efficacy of acibenzolar-S-methyl on Japanese pear rust in a field

Treatment	Number of leaves assessed	Diseased leaves (%) and Control (%)[1] in parenthesis	Number of lesions/leaf and Control (%)[2]
Acibenzolar-S-methyl			
200 μg ml^{-1}	219	8.8[b](49.4)	0.10[b](63.0)
100	214	9.6[b](44.8)	0.10[b](63.0)
10	244	11.7(32.8)	0.15(44.4)
Polycarbamate			
750 μg ml^{-1}	231	0.9[b](94.8)	0.01[b](96.3)
None	237	17.4	0.27

[1]([Number of diseased leaves on untreated trees − Number of diseased leaves on treated trees]/ Number of diseased leaves on untreated trees) × 100.
[2]([Number of lesions per leaf on untreated trees − Number of lesions per leaf on treated trees]/ Number of lesions per leaf on untreated trees) × 100.
[b]Significantly different from untreated control at 5% level (Dunn's multiple range test).

Moreover, applications of pesticides must be reduced so as to diminish problems of resistance in the target organisms. Most fungicides currently used have a direct mode of action on target pathogens. However, probenazole, which has been widely used for rice blast control for more than 20 years in Japan, does have no direct antifungal activity (Watanabe, 1977). Instead, this compound has been shown to induce resistance to the pathogen (Watanabe et al., 1979). In addition, pathogen-resistance to probenazole has never been encountered. Therefore, it is likely that application of this type of disease control agent will help to reduce pesticide input to the environment as well as minimize the risk of resistance.

Within this concept, the benzothiadiazole compound acibenzolar-S-methyl has recently been developed and commercialized for the control of cereal powdery mildew in Europe (Ruess et al., 1996). Several reports have already been made in which acibenzolar-S-methyl and its metabolites showed no antimicrobial activity (Kessmann et al., 1995; Friedrich et al., 1996; Ruess et al., 1996). In this paper, we used two representative culture media for fungal growth, (nutrient-rich PDA and – poor Czapek agar) as it is well known that antifungal activity of a compound is greatly influenced by the medium used. It was confirmed that acibenzolar-S-methyl was non-fungitoxic in standard mycelial growth and spore germination tests (Tables 2 and 3) although we first found an exception that the melon isolates of *D. bryoniae* were sensitive to acibenzolar-S-methyl *in vitro*. Results from further experiments also showed that 21 out of 23 melon isolates were sensitive to acibenzolar-S-methyl and four cucumber isolates were all insensitive (data not shown).

In glass-slide tests, when fungal conidia were treated with acibenzolar-S-methyl at 100 $\mu g\,ml^{-1}$, appressorial formation of *C. lagenarium*, and germination as well as germ-tube growth of *V. nashicola* were retarded to some extent. However, results from microscopic observations showed that these effects were not obvious on both cucumber and Japanese pear leaves previously treated with the compound. Fungal penetration was not strongly inhibited and infection hyphae were frequently formed under resistance-induction conditions (Ishii et al., 1998a). The data will be published elsewhere in more detail.

It has briefly been mentioned that acibenzolar-S-methyl rapidly degrades and/or metabolized in rice plants (Kaji et al., 1997). In our studies, the metabolite benzo(1,2,3)thiadiazole-7-carboxylic acid was ineffective *in vitro* against the melon isolates of *D. bryoniae* (data not shown). This might explain why acibenzolar-S-methyl did not control gummy stem blight caused by this fungus on melon plants, i.e., acibenzolar-S-methyl might be rapidly converted to the metabolite in the plants although analysis data on the fate of acibenzolar-S-methyl in melon are not yet available. It is also possible that neither acibenzolar-S-methyl nor the metabolite could induce resistance to gummy stem blight in melon. Alternatively, melon plants may not have any or sufficient targets for these compounds as discussed on non-inducible immunity mutants of *Arabidopsis* (Lawton et al., 1996).

Control efficacy of acibenzolar-S-methyl against cucumber anthracnose (Kessmann and Nordmeyer, 1996) was confirmed in our experiments carried out in a greenhouse (Table 4). The effect was also observed against cucumber scab (Table 5) although control performance of the compound was higher against the former disease. Furthermore, as mentioned by Kuć et al. (1975), prior inoculation of first leaves of cucumber with *C. lagenarium* conidia induced resistance to this pathogen on upper leaves (data not shown). It is generally accepted that induction of disease resistance in plants occurs several days after treatment with whatever chemical or biological agents are used for induction (Kuć and Richmond, 1977; Kessmann and Nordmeyer, 1996). Surprisingly, however, the resistance induction against both cucumber anthracnose and scab seemed to be triggered as rapidly as three h after treatment with acibenzolar-S-methyl. Narusaka et al. (submitted) have recently carried out experiments using cucumber plants in which acibenzolar-S-methyl-treated leaves were detached at various times and whole plants subsequently inoculated with *C. cucumerinum*. The authors found the rapid expression of peroxidase, chitinase and other resistance-related genes in upper untreated leaves several h after treatment with this compound. The fact that treatment of cucumber plants with the non-fungitoxic compound acibenzolar-S-methyl resulted in suppression of anthracnose and scab in both upper and lower leaves as shown in this paper strongly suggests that this compound induces not only locally acquired resistance (LAR) but systemic acquired resistance (SAR) as well. Further research will be necessary in order to understand how acibenzolar-S-methyl triggers the induction of resistance. It should be considered that the fungi need some time until they infect the plant and this time should be added to the time between application of acibenzolar-S-methyl and SAR-expression.

As briefly reported before (Ishii et al., 1998a), critical events for the suppression of growth of infection hyphae may occur after the fungi achieved penetration, resulting in a decrease in disease development.

It would be fascinating if acibenzolar-S-methyl can be used in practice for the control of some soil-borne diseases, which are generally hard to control with conventional cultural practice. Recently, it has been reported that exogenous, foliar applications of acibenzolar-S-methyl sensitized susceptible cucumber plants to react more rapidly and efficiently against the soil-borne pathogen *Pythium ultimum* (Benhamou and Bélanger, 1998). In our studies, however, this compound never showed efficacy against cucumber *Fusarium* wilt so far tested in a greenhouse (data not shown).

Until now, there have been only a few reports, regarding chemical induction of systemic disease resistance in fruit trees (Kalix et al., 1996; Reglinski et al., 1997). In our work, neither black spot of Japanese pear nor grey mold of grapevine was controlled by acibenzolar-S-methyl. The pathogens of these diseases are saprophytic fungi. Moreover, *A. alternata* Japanese pear pathotype is a pathogen well known for its ability to produce a host-specific toxin (Nishimura, 1987) which is secreted from germ-tubes during early stages of fungal infection causing severe damage to host cells and leading to the formation of necrotic lesions. It might be difficult to expect acibenzolar-S-methyl to induce disease resistance against these types of pathogens. On the other hand, the development of rust on potted Japanese pear trees was suppressed by the treatment with acibenzolar-S-methyl in a greenhouse. The efficacy of this compound was further tested in a field and about 50% control was achieved against both scab and rust at 100 $\mu g\,ml^{-1}$ (Tables 6 and 7). In a practical sense, this level of control might not be acceptable to farmers since many excellent fungicides, e.g., sterol demethylation inhibitors (DMIs) are commercially available. However, it is still meaningful to know the mechanism of disease control with acibenzolar-S-methyl on Japanese pear because this compound has no antifungal properties against *V. nashicola* as mentioned above.

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