European Journal of Plant Pathology **108:** 41–49, 2002.

Induction of systemic acquired resistance in pepper plants by acibenzolar-S-methyl against bacterial spot disease

Roberto Buonaurio[1], Luciano Scarponi[2], Mery Ferrara[1], Paola Sidoti[3] and Angelo Bertona[3]
[1]*Dipartimento di Arboricoltura e Protezione delle Piante-Università degli Studi di Perugia-Borgo XX Giugno, 74, 06121 Perugia, Italy (Phone: +39 75 5856461; Fax: +39 75 5856482; E-mail: buonaurio@unipg.it);*
[2]*Centro di Studio sulla Chimica e Biochimica dei Fitofarmaci del CNR – Borgo XX Giugno, 72-06121 Perugia, Italy;* [3]*Syngenta Protezione Piante S.p.A., Via S. Sofia, 21, 20122 Milano, Italy*

Accepted 3 October 2001

Key words: acibenzolar-S-methyl, *Capsicum annuum*, persistence, systemic acquired resistance, translocation, *Xanthomonas campestris* pv. *vesicatoria*

Abstract

The ability of acibenzolar-S-methyl to induce resistance in pepper plants against *Xanthomonas campestris* pv. *vesicatoria* was investigated in both growth chamber and open field conditions. Growth chamber experiments showed that acibenzolar-S-methyl (300 μM) treatment protects pepper plants systemically and locally against *X. campestris* pv. *vesicatoria*. Evidence for this was a reduction in the number and diameter of bacterial spots and bacterial growth *in planta*. Systemic protection was also exerted by the acibenzolar-S-methyl acid derivative, CGA 210007, which may be produced by hydrolysis in the plant. The efficacy of acibenzolar-S-methyl was also found in open field conditions, where both leaves and fruit were protected from the disease. The highest efficacy (about 67%) was obtained by spraying the plants 6–7 times every 8–12 days with a mixture of acibenzolar-S-methyl and copper hydroxide ($2.5 + 40$ g hl^{-1} active ingredient). Persistence and translocation data obtained from the growth chamber experiments revealed a persistence of acibenzolar-S-methyl lasting five days after treatment with rapid translocation and negligible levels of acid derivative formation. Since the protection exerted by acibenzolar-S-methyl against bacterial spot disease was observed when the inducer was completely degraded, it would appear to be due to SAR activation.

Introduction

Pepper bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* is an important disease in many pepper production areas of the world (Stall, 1993), Italy included (Buonaurio et al., 1994).

Like other bacterial diseases, pepper bacterial spot is difficult to control. Chemical sprays, essentially carried out with copper compounds or streptomycin, are not completely effective since copper and streptomycin resistant *X. campestris* pv. *vesicatoria* strains frequently appear (Buonaurio et al., 1994; Stall and Thayer, 1962). The large-scale cultivation of resistant pepper cultivars, which contain the dominant resistant genes *Bs1*, *Bs2* and *Bs3*, could trigger the appearance of new bacterial races capable of overcoming the resistance (Pohronezny et al., 1992).

The use of induced resistance in plants is a promising environment-friendly strategy for controlling plant diseases, including those caused by bacteria. Induced resistance, which is expressed systemically and/or locally, is biologically activated in response to necrotizing pathogens or root-colonizing soil bacteria (Hammerschmidt et al., 2001). The best characterized systemic resistance is Systemic Acquired Resistance (SAR), which is induced by localized infections with necrotizing pathogens. It is long-lasting and effective against a broad spectrum of pathogens. In many

cases, it depends on the endogenously synthesized signal salicylic acid (SA) (Sticher et al., 1997) and is correlated with the activation of a specific set of genes (SAR genes), which include genes coding for pathogenesis-related (PR) proteins.

Besides being biologically induced, SAR can be triggered by some chemicals, including SA and its synthetic analogues, such as acibenzolar-S-methyl [benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester, CGA 245704], referred to as ASM. This compound was developed by Syngenta (Kessmann et al., 1996) and introduced in 1996 as a 'plant activator' to control wheat powdery mildew in Germany and Switzerland (Ruess et al., 1996). The acidic derivative of ASM, produced by hydrolysis in the plants, systemically protected tomato plants from *Pseudomonas syringae* pv. *tomato* attacks. Since the protection was detected even after the complete degradation of ASM and its acidic derivative, it was attributed to SAR activation (Scarponi et al., 2001). In itself, ASM has no anti-microbial activity, but has been reported to protect dicotyledonous and monocotyledonous plant species against a number of viral, bacterial and fungal diseases (Benhamou and Belanger, 1998; Cole, 1999; Friedrich et al., 1996; Görlach et al., 1996; Ishii et al., 1999; Lawton et al., 1996; Jensen et al., 1998). In *Arabidopsis thaliana* and *Nicotiana tabacum*, ASM induced the same set of SAR genes and the same spectrum of resistance induced by biological inducers (Friedrich et al., 1996; Lawton et al., 1996). Lawton et al. (1996) also showed that the induction of SAR gene expression by ASM did not require SA and they suggested that it could act as a secondary messenger analogue capable of activating the SAR signal transduction pathway independent of the SA accumulation.

Little attention has been given to plant-induced resistance against bacterial pathogens of the *Xanthomonas* genus. Some examples are chemically-induced resistance against *Xanthomonas axonopodis* pv. *phaseoli* in bean (Siegrist et al., 1997) and *Xanthomonas axonopodis* pv. *malvacearum* in cotton plants (Colson-Hanks et al., 2000). Furthermore, among plants of the *Solanaceae* family, tobacco, potato and tomato have been extensively used in induced resistance investigations (Ozeretskovskaya, 1995), while very few studies have been performed on pepper plants. It has been reported that DL-β-amino-n-butyric acid protects pepper plants against *Phytophthora capsici* (Sunwoo et al., 1996) and *Colletotrichum coccodes* (Hong et al., 1999).

To ascertain the possibility of using ASM as a SAR inducer in pepper crops, its efficacy in controlling bacterial spot disease by *X. campestris* pv. *vesicatoria* was investigated in connection with its persistence and translocation in the plant. Preliminary results of this study have been reported elsewhere (Buonaurio et al., 2000).

Materials and methods

Chemicals and apparatus

ASM [benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester] (CGA 245704; 50% water dispersible granule and analytical grade) and its acid derivative [benzo(1,2,3)thiadiazole-7-carboxylic acid] (CGA 210007; analytical grade) were kindly supplied by Syngenta Protezione Piante S.p.A. (Italy). HPLC grade acetonitrile and water were obtained from BDH. SPE cartridges ENV+ (sorbent mass 200 mg) were purchased from International Sorbent Technology Ltd Mid Glamorgan UK. All other reagents were of ACS grade.

A Perkin-Elmer HPLC instrument was assembled with the following modular components: Series 410 LC pumps, a Rheodyne model 7125-075 injector, a Model 235 diode array interfaced with an Omega2 analytical chromatographic workstation (version 2.50 software) and an Omega235 software upgrade kit (PE Nelson), an LC-18 column Supelcosil, 25 cm $\times$ 4.6 mm I.D. (5 μm particle size) protected by a supelguard precolumn (3 cm long) with exactly the same characteristics (Supelco Inc., Bellefonte, PA).

A GC-MS system was assembled with the following modular components: a Varian Star model 3400 chromatograph fitted with a split/splitless injector, a mass spectrometer SATURN II operating in the electron impact mode and a DB5 fused silica capillary column (30 m $\times$ 0.25 mm) coated with 0.25 μm.

Bacteria

Two bacterial strains of *X. campestris* pv. *vesicatoria* were used: the Xcv 82-8 strain, kindly provided by Prof. Dr. R.E. Stall, University of Gainsville, Florida and the DAPP-PG 234 strain, collected by us from pepper plants in Umbria (Italy). The bacterial cultures were maintained as suspension in 15% glycerol at $-80\,^{\circ}$C.

Control efficacy of acibenzolar-S-methyl and its acid derivative in growth chamber conditions

Pepper (*Capsicum annuum* L.) plants, cv. Early Calwonder were grown in sterilized compost-enriched soil in a greenhouse at 22–28 °C, under natural light conditions. About 40 days after sowing, plants were transplanted into pots and transferred to a growth chamber at 28 ± 2 °C, 60–70% RH, 65 μE m^{-2} s^{-1} illumination and 14 h light period. Plants at the 8–9th true leaf stage were used.

The effect of ASM and its acid derivative (CGA 210007) in systemically protecting pepper plants against bacterial spot disease was evaluated in two separate experiments. The first experiment was factorial designed with five replicates, where the main effects were ASM treatment at two levels (control vs treated) and leaf position at four levels (4–7th leaf). The second experiment was arranged in a completely randomized design with six replicates and three levels – control, CGA 210007 (300 μM) and ASM (300 μM). For both experiments, the adaxial and abaxial leaf surface of the 1st–3rd leaves (referred to here as basal leaves) were sprayed with either a 300 μM aqueous suspensions of ASM (64 μg ml^{-1} a.i.) or CGA 210007 (55 μg ml^{-1} a.i.), while control plants were sprayed with water. Five days after treatment, the abaxial surface of the 4–7th leaves (referred to here as apical leaves) of treated and control plants were partly infiltrated with a bacterial suspension (10^6 viable cells ml^{-1}) of *X. campestris* pv. *vesicatoria* (Xcv 82–8 strain) using a glass atomizer (Glasgerätebau Paul Ochs, Germany). To prepare the inoculum, the bacterial strain was grown on nutrient agar (Oxoid, CM3) at 27 ± 1 °C for 48 h and suspended in deionised water, the suspension was spectrophotometrically adjusted to 10^8 viable cells ml^{-1} (OD_{600} = 0.3) and diluted up to the above reported concentration. A third experiment was performed to evaluate the efficacy of ASM in protecting pepper leaves locally against *X. campestris* pv. *vesicatoria*. It was arranged in a completely randomized design with five replicates and two levels – control, and ASM (300 μM). The 7th leaves of pepper plants were treated with ASM and inoculated with the bacterium five days after the treatment.

Severity of bacterial infection was evaluated by determining the infection degree, the bacterial spot diameter and bacterial growth *in planta*. The infection degree was estimated on each leaf using a 0–3 arbitrary scale (0 = no spots; 1 = 1–5 spots; 2 = 6–10 spots; 3 ≥ 11 spots); lesion diameter was determined by a stereomicroscope equipped with a micrometre scale eye-piece. Bacterial growth *in planta* was determined as described below.

Effect of acibenzolar-S-methyl on bacterial growth in planta

To determine the effect of ASM on *X. campestris* pv. *vesicatoria* growth in pepper plants, the inoculated leaves (about 1.5–2 g fresh weight), collected at 2, 4, 7 and 10 days post-inoculation from plants treated and inoculated as reported above, were ground in a mortar with sterile 0.1 M potassium phosphate buffer at pH 7.0 (5 ml g^{-1} fresh weight). Leaf homogenates were diluted tenfold and several 10 μl drops of each dilution were placed on nutrient agar (Oxoid, CM3). After 24–36 h incubation at 27±1 °C, the number of bacterial colonies were counted using a stereomicroscope.

Field trials

The efficacy of ASM in protecting pepper plants from bacterial spot disease was evaluated in three field trials carried out in Umbria (Central Italy) in 1998, 1999 and 2000 and arranged in a completely randomized block design with four replicates and a 2.5 m × 4.0 m plot size. One trial was conducted at Castiglione del Lago (Perugia) in 1998, using the pepper ecotype Topepo, while the others at Papiano (Perugia) in 1999 and 2000, using the cv. Heldor. Since the infection pressure was low in the 1998 trial, to enhance the level of infection in the 1999 and 2000 trials, some pepper seedlings were inoculated with a copper-sensitive *X. campestris* pv. *vesicatoria* strain (DAPP-PG 234) collected in 1998 from a local farm in Papiano where the infection pressure was high, and transplanted in rows between the plots. For the inoculation, pepper seedlings were air-brush sprayed with a bacterial suspension 10^8 cfu ml^{-1}. After inoculation, plants were covered with plastic bags for the first two days and kept in a greenhouse. To facilitate disease spread in the experimental fields, sprinkle irrigation was used. In all trials, treatments were ASM at different doses ranging from 1.25 to 5 g hl^{-1}, copper hydroxide at 80 g a.i. hl^{-1}, ASM + copper hydroxide at 2.5 and 40 or 80 g a.i. hl^{-1}, respectively. About one litre of each treatment per plot was sprayed on the foliage of the whole plants by the motorised knapsack sprayer F320 (Fox Motori Srl, Italy), equipped with a 2 m length boom with four nozzles (hollow cone). Four sprays were applied in the 1998 trial between 6th July

and 3rd August, seven sprays in the 1999 trial between 10th June and 23rd August and six in the 2000 trial between 30th May and 18th July. Disease symptoms were periodically assessed. When attacks on fruit were present, they were evaluated by a 0–3 arbitrary scale (0 = no spots; 1 = 1–5 spots; 2 = 6–20 spots; 3 ≥ 21 spots).

Determination of acibenzolar-S-methyl and its acid derivative

The translocation and persistence of ASM in pepper leaves were determined in plants grown in a growth chamber and treated by spraying an aqueous suspension of ASM 300 μM (50% water dispersible granule) as reported above. Samples of treated basal and untreated apical leaves were collected at 0, 6, 12, 24, 48, 72, 96 and 120 h after treatment and submitted to the extraction and determination procedure of ASM and its acid derivative according to the method of Scarponi et al. (2001) as follows. The samples (about 2 g) were ground in a mortar with liquid nitrogen and extracted with chilled acetonitrile (25 ml). The extract was centrifuged at 3100 *g* for 5 min and the supernatant evaporated to dryness in vacuum at room temperature. The residue was dissolved with 9 ml of a mixture containing potassium phosphate buffer 0.5 M, pH 3 and acetonitrile (70/30, v/v). The sample was run through the SPE cartridge previously activated with 6 ml acetonitrile followed by 6 ml of the buffer solution with acetonitrile. The cartridge was then washed with 6 ml of the buffer solution without acetonitrile. After 12 h, when the cartridge had dried, the sample was eluted with 6 ml acetonitrile. The recovered sample was evaporated under a stream of dry nitrogen at room temperature and redissolved in 1 ml acetonitrile and used for HPLC analysis.

The following isocratic system was employed: mobile phase acetonitrile and water (50/50, v/v), both containing 0.06% acetic acid, flow rate 1 ml min^{-1}, injection volume 20 μl. ASM and its derivative CGA 210007, monitored at 255 nm, showed retention times of 7.89 ± 0.09 and 3.30 ± 0.07 min, respectively. To confirm the identity of the two peaks, the UV absorption spectra taken at the apex were compared with those obtained from the standard solution. Further confirmation of ASM identity was obtained by comparing the mass spectra of the samples with those of a standard solution of the compound obtained at the following GC/MS conditions: oven temperature 70 °C (1 min) ramped at 10 °C min^{-1} to a final temperature of 260 °C (5 min), carrier gas helium (1 ml min^{-1}), injector temperature 250 °C, ionization voltage 70 eV emission current 10 μA.

Results

Efficacy of acibenzolar-S-methyl and its acid derivative in growth chamber conditions

Treatment of the basal (1st–3rd) leaves of pepper plants with ASM (300 μM) caused a reduction in degree of infection and spot diameter (Table 1). These effects were statistically significant ($P \leq 0.05$) in all the leaf positions except for infection degree in the 5th leaf ($P = 0.083$) and were more pronounced in the youngest leaves. In fact, the highest efficacy (80–93%) of the inducer was observed in the 6th and 7th leaves for degree of infection and in the 7th leaf for spot diameter (Table 1). The fact that ASM did not exert the same effect in all the leaves was confirmed by ANOVA analysis of the spot diameter data, which revealed a significant ($P \leq 0.05$) interaction between treatments and leaf position (data not shown). Bacterial growth, determined at 2–10 days post-inoculation in both ASM treated and untreated leaves, dropped rapidly in treated plants (35 times lower than controls) starting 4 days

Table 1. Control efficacy of acibenzolar-S-methyl (300 μM) on pepper bacterial spot in growth chamber conditions, 21 days post-inoculation

Treatment	Infection degree[1]				Spot diameter (mm)			
	4th leaf	5th leaf	6th leaf	7th leaf	4th leaf	5th leaf	6th leaf	7th leaf
Control	2.80 b	3.00 a	3.00 b	2.80 b	0.510 b	0.590 b	0.558 b	0.706 b
Acibenzolar-S-methyl	1.40 a	1.60 a	0.60 a	0.20 a	0.364 a	0.314 a	0.288 a	0.056 a
Efficacy[2]	50	47	80	93	29	47	48	92

[1]Infection degree, 0 = no spots per leaf; 1 = 1–5 spots; 2 = 6–10 spots; 3 ≥ 11 spots.
[2](Value of untreated leaves − value of treated leaves/value of untreated leaves) × 100.
Each value is the mean of five replicates. Data followed by the same letters within each leaf position are not significantly different at $P \leq 0.05$ (χ^2 for the infection degree and Student's *t*-test for spot diameter).

post-inoculation and remained lower than in controls 7 and 10 days post-inoculation (5 and 6 times lower, respectively) (Figure 1).

Table 2 shows the effect of treating pepper plants with the ASM acid derivative (CGA 210007; 300 μM) compared to the parent molecule (300 μM) on the degree of infection, spot diameter and growth *in planta* of *X. campestris* pv. *vesicatoria*. CGA 210007 treatment caused a significant ($P \leq 0.05$) reduction in all parameters considered compared to the control plants. In addition, with ASM there was a greater reduction in the degree of infection and bacterial growth *in planta* than in CGA 210007-treated plants.

The ASM protection was also exerted at local level in the leaves (Table 3). The 7th pepper leaf treated with ASM showed a significant reduction ($P \leq 0.05$) in the number and diameter of bacterial spots and in the bacterial growth *in planta*.

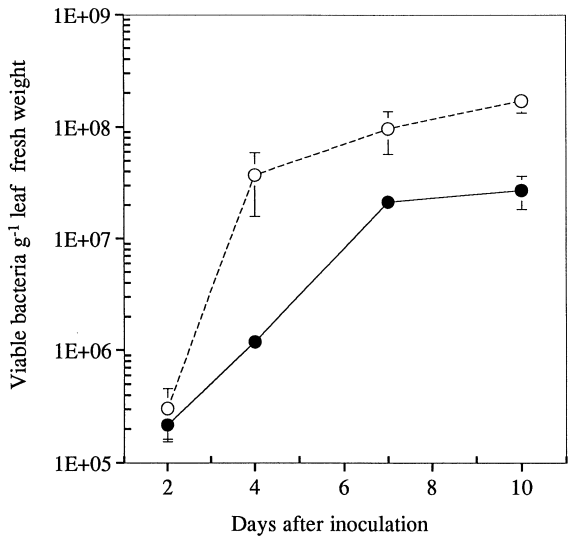

Figure 1. Effect of acibenzolar-S-methyl treatment (300 μM) on *Xanthomonas campestris* pv. *vesicatoria* growth in treated (closed circle) and untreated (open circle) pepper leaves. Data are the means of two independent experiments ± SE, three replicates for each experiment.

Table 2. Control efficacy of CGA 210007 or acibenzolar-S-methyl (300 μM, a.i.) on pepper bacterial spot in a growth chamber, 14 days post-inoculation

Treatment	Infection degree[1]	Spot diameter (mm)	Bacterial growth *in planta* (log cfu g^{-1} fresh leaf weight)
Control	1.97 c	0.36 b	9.48 c
CGA 210007	1.43 b	0.24 a	9.20 b
Acibenzolar-S-methyl	0.81 a	0.20 a	8.88 a

[1]Infection degree, 0 = no spots per leaf; 1 = 2–5 spots; 2 = 6–10 spots; 3 ≥ 11 spots.
Each value is the mean of six replicates. Data followed by the same letters are not significantly different at $P \leq 0.05$ (χ^2 for the infection degree and Duncan's multiple range test for spot diameter and bacterial growth *in planta*).

Efficacy of acibenzolar-S-methyl in field conditions

Results of the 1998 trial, characterized by a very low level of *X. campestris* pv. *vesicatoria* infection, are reported in Table 4. ASM alone or in combination with copper hydroxide, sprayed four times, significantly protected pepper plants against leaf bacterial infections

Table 3. Effect of acibenzolar-S-methyl (300 μM, a.i.) treatment of pepper leaves on local protection against *Xanthomonas campestris* pv. *vesicatoria*, 10 days post-inoculation

Treatment	Infection degree[1]	Spot diameter (mm)	Bacterial growth *in planta* (log cfu g^{-1} fresh leaf weight)
Control	2.8 b	0.506 b	8.62 b
Acibenzolar-S-methyl	1.2 a	0.236 a	7.29 a

[1]Infection degree, 0 = no spots per leaf; 1 = 2–5 spots; 2 = 6–10 spots; 3 ≥ 11 spots.
Each value is the mean of five replicates. Data followed by the same letters are not significantly different at $P \leq 0.05$ (χ^2 for the infection degree and Student's *t*-test for spot diameter and bacterial growth *in planta*).

Table 4. Control efficacy of Acibenzolar-S-methyl alone or in combination with copper hydroxide on pepper bacterial spot in the 1998 open field trial (104 days after transplanting)

Treatment[1]	Doses (g a.i. hl^{-1})	% of leaf surface infected	Efficacy[2]
Control	—	0.185b	0
Copper hydroxide	80	0.083ab	55
Acibenzolar-S-methyl	1.25	0.007a	96
Acibenzolar-S-methyl	2.5	0.000a	100
Acibenzolar-S-methyl	5	0.000a	100
Acibenzolar-S-methyl + copper hydroxide	2.5 + 40	0.000a	100

[1]Four sprays every 8–11 days were applied.
[2](Value of untreated leaves − value of treated leaves/value of untreated leaves) × 100.
Each value is the mean of four replicates. Data followed by the same letters are not significantly different at $P \leq 0.05$ (Duncan's multiple range test).

Table 5. Control efficacy of Acibenzolar-S-methyl alone or in combination with copper hydroxide on pepper bacterial spot in the 1999 open field trial (99 days after transplanting)

Treatment[1]	Doses (g a.i. hl^{-1})	Leaf infection		Fruit infection	
		% of leaf surface infected	Efficacy[2]	Infection index[3]	Efficacy[2]
Control	—	3.73 a	0	0.899 b	0
Copper hydroxide	80	3.60 a	3	0.608 ab	32
Acibenzolar-S-methyl	1.25	3.13 ab	16	0.533 a	41
Acibenzolar-S-methyl	2.5	2.51 bc	33	0.433 a	52
Acibenzolar-S-methyl	5	1.81 cd	51	0.358 a	60
Acibenzolar-S-methyl + copper hydroxide	2.5 + 40	1.15 d	69	0.300 a	67

[1]Seven sprays every 12–14 days were applied.
[2](Value of untreated leaves − value of treated leaves/value of untreated leaves) × 100.
[3]Infection index, 0 = no spots; 1 = 1–5 spots; 2 = 6–20 spots; 3 ≥ 21 spots.
Each value is the mean of four replicates. Leaf and fruit infection data followed by the same letters are not significantly different at $P \leq 0.05$ (Duncan's multiple range test).

(96–100% efficacy). By contrast, copper hydroxide alone was not significantly effective.

In the 1999 trial, seven spray applications of ASM alone or in combination with copper hydroxide were effective in protecting plants against leaf and fruit *X. campestris* pv. *vesicatoria* attacks, while copper hydroxide (80 g a.i. hl^{-1}) did not significantly protect plants from the disease (Table 5). Applications of ASM + copper hydroxide (2.5 + 40 g a.i. hl^{-1}) gave the highest efficacy (69 and 67 for leaf and fruit infections, respectively). A significant linear regression ($y = 3.475 - 0.341x$; $R^2 = 0.976$) was found between different doses of ASM (1.25; 2.50; 5.00 g a.i. hl^{-1}) and the percentage of leaf surface infected. The same analysis was not significant when fruit infection was examined.

Table 6 gives the results of the trial carried out in 2000, in which six applications were performed. The data confirm that the highest efficacy (68%) is obtained applying the mixture ASM and copper hydroxide (2.5 + 40 g a.i. hl^{-1}). Bacterial spot symptoms were not observed in fruit in the 1998 and 2000 trials.

Table 6. Control efficacy of acibenzolar-S-methyl alone or in combination with copper hydroxide on pepper bacterial spot in the 2000 open field trial (78 days after transplanting)

Treatment[1]	Doses (g a.i. hl^{-1})	% of leaf surface infected	Efficacy[2]
Control	—	15.22c	0
Copper hydroxide	80	7.41b	51
Acibenzolar-S-methyl	2.5	6.05ab	60
Acibenzolar-S-methyl + copper hydroxide	2.5 + 40	4.92a	68
Acibenzolar-S-methyl + copper hydroxide	2.5 + 80	5.58ab	63

[1]Six sprays every 8–11 days were applied.
[2](Value of untreated leaves − value of treated leaves/value of untreated leaves) × 100.
Each value is the mean of four replicates. Data followed by the same letters are not significantly different at $P \leq 0.05$ (Duncan's multiple range test).

Acibenzolar-S-methyl persistence and translocation

The reproducibility and accuracy of the analytical method were ascertained by using untreated plant samples spiked with four different amounts of ASM ranging from 10 to 100 μg g^{-1} fresh leaf weight. The good recovery levels, ranging from 91% to 102%, confirmed the suitability of the analytical procedure in pepper plants.

Data on ASM residues in both treated basal and untreated apical leaves are reported in Figure 2. ASM residues decreased in basal and apical leaves as time elapsed, with an abrupt fall between 6 and 12 h becoming negligible 120 h after treatment. The residue levels of the ASM acid derivative were constantly lower than the detection limit of the analytical method.

ASM translocation to apical leaves was rapid but not relevant. In fact 2.95 μg g^{-1} f.wt. of the compound, corresponding to a 4.0% of relative translocation, the maximum translocation level, were found in the untreated apical leaves as early as 6 h after treatment. It then decreased becoming negligible 48 h after treatment.

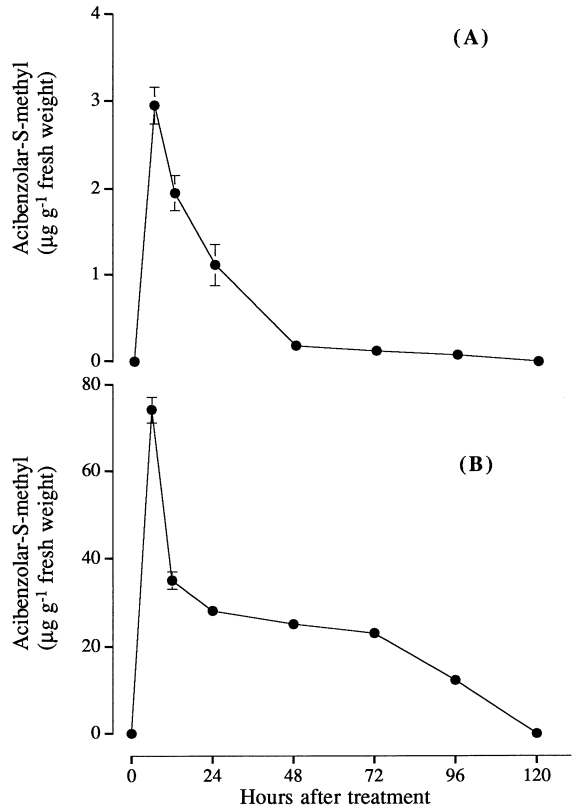

Figure 2. Trends of acibenzolar-S-methyl residues in untreated apical (A) and treated basal (B) pepper leaves. Data are the means of three determinations ± SE.

Discussion

The studies of Friedrich et al. (1996), Lawton et al. (1996), Siegrist et al. (1997), Cole (1999), Brisset et al. (2000), Colson-Hanks et al. (2000) and Scarponi et al. (2001) demonstrated that ASM can protect plants against attacks by some *P. syringae* and *X. campestris* pathovars, *Erwinia amylovora* and *Erwinia carotovora* subsp. *carotovora*. This study provides evidence that ASM is also effective in locally and systemically protecting pepper plants against attacks by *X. campestris* pv. *vesicatoria* and that, according to the above studies, the reduced disease severity is correlated with a significant decrease in bacterial growth *in planta*. The efficacy of ASM was confirmed in field conditions against fruit attacks by the bacterium. Field trials showed that 6–7 sprays of ASM + copper hydroxide ($2.5 + 40\,g\,hl^{-1}$), applied every 8–12 days, gave the highest protection. Although copper hydroxide alone did not provide a reproducible protection, when mixed with ASM it enhanced the effect of the inducer, thus enabling a reduction of the dose. The higher efficacy of ASM could also be due to its possible control of infections starting from the epiphytic population of *X. campestris* pv. *vesicatoria* present in pepper buds, an important inoculum reservoir, which is not controlled by copper sprays (Pernezny and Collins, 1997).

Persistence and translocation experiments, performed in a growth chamber, revealed that ASM was able to translocate to the untreated apical leaves. Since the trends of ASM residue curves in basal and apical leaves were very similar (see Figure 2), the decrease in the translocated compound as time elapsed seems due to degradation rather than to a reduced translocation. Although the acid derivative of ASM was able to protect pepper plants from bacterial spot disease similar to the parent molecule, its negligible formation in pepper leaves would not significantly contribute to the protective effect. Consequently, since protection against bacterial spot in peppers was observed when ASM was completely degraded, protection must be due to an induction of resistance. Since a concentration of ASM about 25 times lower was found in untreated apical leaves with respect to treated ones, it is possible that protection is due to systemic resistance rather than a local effect of the inducer.

The nature of the mechanisms responsible for the resistance induced by ASM in plant-bacterium combinations is still unknown. Some studies (Friedrich et al., 1996; Lawton et al., 1996) showed that the induction of SAR gene expression by ASM did not require SA, an endogenous plant molecule that plays an important role in the signal transduction pathway leading to SAR (Delaney et al., 1994; Durner et al., 1997). This finding suggests that ASM could act as a secondary messenger analogue capable of activating the SAR signal transduction pathway independent of SA accumulation (Lawton et al., 1996). Among the genes induced by ASM, are those coding chitinase activities that may account for the protective effect exerted by ASM in plants infected with pathogenic bacteria, including *X. campestris* pv. *vesicatoria*. It is known that some plant chitinases have lysozyme activity also and therefore can hydrolyse bacterial cell walls (Boller et al., 1983; Heitz et al., 1994). O'Garro and Charlemance (1994) reported that *X. campestris* pv. *vesicatoria* growth was drastically reduced in flower but not in leaf tissue of pepper plants inoculated with the bacterium and that this decrease was associated

with a rapid increase in chitinase activities. Lee and Hwang (1996) suggested that the high level of chitinase they found in leaf intercellular fluid in the incompatible pepper-*X. campestris* pv. *vesicatoria* interaction could account for the reduced bacterial growth in this combination. Further investigation is required to establish how ASM induces resistance to *X. campestris* pv. *vesicatoria* in pepper plants.

Acknowledgements

The authors wish to thank Dr. A. Onofri, Dipartimento di Scienze Agro-Ambientali e della Produzione Vegetale, Perugia University, Italy, for help with the statistical analysis. The work was supported in part by a grant from C.N.R. 'Diversità di microrganismi in ambiente agroambientale-alimentare. Indagini sulla biodiversità di batteri fitopatogeni' to R.B.

References

Benhamou N and Belanger RR (1998) Benzothiadiazole-mediated induced resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato. Plant Physiology 118: 1203–1212

Boller T, Gehri A, Mauch F and Vogeli U (1983) *Phaseolus vulgaris* chitinase in bean leaves: induction by ethylene, purification, properties and possible function. Planta 157: 22–31

Brisset MN, Cesbron S, Thomson SV and Paulin JP (2000) Acibenzolar-S-methyl induces the accumulation of defense-related enzymes in apple and protects from fire blight. European Journal of Plant Pathology 106: 529–536

Buonaurio R, Stravato VM and Scortichini M (1994) Characterization of *Xanthomonas campestris* pv. *vesicatoria* from *Capsicum annuum* L in Italy. Plant Disease 78: 296–299

Buonaurio R, Martinetti L, Bassi R, Bertona A and Scarponi L (2000) Resistenza a *Xanthomonas campestris* pv. *vesicatoria* indotta dal CGA 245704 in piante di peperone. Traslocazione e persistenza dell'induttore nella pianta. Atti Giornate Fitopatologiche, Perugia 2: 385–386

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 spot in cotton. Plant Pathology 49: 171–178

Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gutrella M, Kessmann H, Ward E and Ryals J (1994) A central role of salicylic acid in plant disease resistance. Science 266: 1247–1250

Durner J, Shah J and Klessig DF (1997) Salicylic acid and disease resistance in plants. Trends in Plant Science 2: 266–274

Friedrich L, Lawton K, Ruess W, Masner P, Specker N, Rella MG, Meier B, Dincher S, Staub T, Uknes S, Metraux JP, Kessmann H and Ryals J (1996) A benzothiadiazole derivative induces systemic acquired resistance in tobacco. Plant Journal 10: 61–70

Görlach J, Volrath S, Knaufbeiter G, Hengy G, Beckhove U, Kogel KH, Oostendorp M, Staub T, Ward E, Kessmann H and Ryals J (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 8: 629–643

Hammerschmidt R, Metraux JP and van Loon LC (2001) Inducing resistance: a summary of papers presented at the first International symposium on induced resistance to plant diseases, Corfu, May 2000. European Journal of Plant Pathology 107: 1–6

Heitz T, Segond S, Kauffmann S, Geoffroy P, Prasad V, Brunner F, Fritig B and Legrand M (1994) Molecular characterization of a novel tobacco pathogenesis-related (PR) protein – a new plant chitinase/lysozyme. Molecular & General Genetics 245: 246–254

Hong J, Hwang B, Kim C, Hong JK, Hwang BK and Kim CH (1999) Induction of local and systemic resistance to *Colletotrichum coccodes* in pepper plants by DL-β-amino-n-butyric acid. Journal of Phytopathology 147: 193–198

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, Latundedada 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

Kessmann H, Oostendorp M, Staub T, Goerlach J, Friedrich L, Lawton K and Ryals J (1996) CGA 2455704: Mode of action of a new plant activator (pp 961–966) Brighton Crop Protection Conference

Lawton KA, Friedrich L, Hunt M, Weymann K, Delaney T, Kessmann H, Staub T and Ryals J (1996) Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. Plant Journal 10: 71–82

Lee YK and Hwang BK (1996) Differential induction and accumulation of β-1,3-glucanase and chitinase isoforms in the intercellular space and leaf tissues of pepper by *Xanthomonas campestris* pv. *vesicatoria* infection. Journal of Phytopathology 144: 79–87

O'Garro LW and Charlemange E (1994) Comparison of bacterial growth and activity of glucanase and chitinase in pepper leaf and flower tissue infected with *Xanthomonas campestris* pv. *vesicatoria*. Physiological and Molecular Plant Pathology 45: 181–188

Ozeretskovskaya OL (1995) Induced resistance in the *Solanaceae*. In: Hammerschmidt R and Kuc J (eds) Induced Resistance to Disease in Plants, (pp 31–62) Kluwer Academic Publishers, Dordrecht

Pernezny K and Collins J (1997) Epiphytic populations of *Xanthomonas campestris* pv. *vesicatoria* on pepper – relationships to host-plant resistance and exposure to copper sprays. Plant Disease 81: 791–794

Pohronezny K, Stall RE, Canteros BI, Datnoff LE and Subramanya R (1992) Sudden shift in the prevalent race of

Xanthomonas campestris pv. *vesicatoria* in pepper fields in southern Florida. Plant Disease 76: 118–120

Ruess 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, (pp 53–60) Brighton Crop Protection Conference

Scarponi L, Buonaurio R and Martinetti L (2001) Persistence and translocation of a benzothiadiazole derivative in tomato plants in relation to systemic acquired resistance against *Pseudomonas syringae* pv. *tomato*. Pest Management Science 57: 262–268

Siegrist J, Glenewinkel D, Kolle C and Schmidtke M (1997) Chemically induced resistance in green bean against bacterial and fungal pathogens. Journal of Plant Diseases and Protection 104: 599–610

Stall RE and Thayer PL (1962) Streptomycin resistance of the bacterial spot pathogen and control with streptomycin. Plant Disease Reporter 16: 389–392

Stall RE (1993) *Xanthomonas campestris* pv. *vesicatoria*: cause of bacterial spot of tomato and pepper. In: Swings JG and Civerolo EL (eds) *Xanthomonas* (pp 57–60) Chapman & Hall, London

Sticher L, Mauchmani B and Metraux JP (1997) Systemic acquired resistance. Annual Review of Phytopathology 35: 235–270

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