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Induces Resistance Against Fusarium and Pink Rots by Acibenzolar-S-Methyl in Harvested Muskmelon (*cv.* Yindi)

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Abstract

Acibenzolar-S-methyl (ASM) is a chemical activator of systematic resistance in many plants. The effect of preharvest and postharvest application of ASM was evaluated for its ability to induce resistance in muskmelon fruit. The results indicated that 50 and 100 mg L^{-1} ASM or 1 mL L^{-1} imazalil at 1 week or 1 day before harvest were effective in reducing the lesion area with 100 mg L^{-1} ASM the most effective. No treatment inhibited the infection rate. The postharvest results showed that 50 and 100 mg L^{-1} ASM, and 0.1 mL L^{-1} imazalil were effective in reducing the lesion area with 100 mg L^{-1} ASM the most effective. No treatment inhibited the infection rate. There was a clear time-dependent response of the fruit to postharvest ASM treatment, in which treatments applied 1, 3, and 5 day before inoculation provided the best results. ASM did not demonstrate any fungicide effect *in vitro* and suppressed lesion area in treated muskmelons, indicating that disease resistance was induced. The protection of ASM was associated with the activation of peroxidase (POD) in treated muskmelons.

Key words: acibenzolar-S-methyl (ASM), induced resistance, muskmelon, peroxidase (POD)

INTRODUCTION

Muskmelon (*Cucumis melo* L.) is an economically important crop in Northwest of China. The fruit are susceptible to the decay caused by several pathogenic fungi including *Alternaria alternata*, *Fusarium* spp., *Rhizopus stolonifer*, and *Trichothecium roseum* (Bi and Wang 1987). Some fungicides, such as imazalil and azxoxystrobin, showed to be quite effective in controlling most rots of melon (Aharoni *et al.* 1992; Ma *et al.* 2004). However, because of increasing concerns about chemical usage in food and the environment, there is renewed interest in nonchemical approaches to postharvest disease control (El-Ghaouth *et al.* 1998; Tian and Chan 2004).

Induction of natural disease resistance in harvested horticultural crops is being considered as a preferred strategy for disease management (Tian and Chan 2004; Terry and Joyce 2004). The chemical plant activator, acibenzolar (S-methyl benzo thiadiazole-7-carbothioate; ASM; BTH), supposedly a functional analogue of SA, has been developed for systemic induction of disease resistance in previous years (Friedrich *et al.* 1996). ASM was effective in reduction of disease in stored melons by a foliar spray treatment (Huang *et al.* 2000). The chemical has also been applied effectively as a postharvest treatment to suppress the decay in Hami melons (Bi *et al.* 2006), and the blue mould in peach and pear (Liu *et al.* 2005; Cao *et al.* 2005). ASM may act by eliciting biochemical defense reactions, including the accumulation of lignin, phenolic compounds,

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and pathogenesis-related proteins in the infected fruit. POD participates in cell wall reinforcement and involves in the final steps of lignin biosynthesis and in the cross-linking of cell wall protein (Brisson and Tenhaken 1994). The primary findings of induced resistance were that ASM induced the increase of POD activity in Hami melons (Bi *et al.* 2006), peach, and pear (Liu *et al.* 2005; Cao *et al.* 2005).

The objective of this study was to investigate the possibility of reducing decay on muskmelon fruit with preharvest or postharvest ASM treatment, and to analyse whether the induced fruit could increase peroxidase (POD) activity.

MATERIALS AND METHODS

Plant material

Uniform fruit were harvested from Minqin in Gansu Province, China, at a mean flesh firmness of 4.72 kg cm^{-2}, and a mean soluble solids content (SSC) of 10.07%. The fruit were packaged in cartons (six fruit per carton) for transport to Lanzhou in Gansu Province by truck, and subsequently stored at 22-24°C, relative humidity (RH) 50-60%.

Inoculum preparation

Fusarium semitectum and *Trichothecium roseum* were isolated from muskmelon fruit and maintained on potato dextrose agar (PDA) incubated at 25-27°C. Spore suspension (1×10^5 spores mL^{-1}) were prepared from 7-day-old culture dishes by flooding with 10 mL of sterile distilled water containing 0.05 mL L^{-1} Tween 20.

In vitro assays

The effect of ASM on the mycelial growth of pathogen was assayed using the method of Ge *et al.* (2005). ASM at 50, 100 mg L^{-1} were tested against *T. roseum* and *F. semitectum*. ASM was added into PDA after the PDA was autoclaved for 15 min and cooled to 45-50°C. An 8-mm diameter disc of mycelial mat from 7-day-old culture was placed in the center of each Petri plate. The mycelial growth was measured daily as the average diameter for a 5-day incubation period at 25-27°C. Each treatment was replicated three times and the experiment was repeated twice.

Preharvest spraying assays

Treatment The assay was a random block design with 4 treatments applied either 1 day or 1 week before harvest. The plants were sprayed with ASM at 50 and 100 mg L^{-1}, imazalil at 1 mL L^{-1} and water (as CK). Each treatment consisted of 18 plants. The trial was repeated 3 times.

Inoculation At 48 h after harvest, the fruit were disinfected with 70% ethanol and air-dried before wounding with a sterile nail, forming 9 wounds (3 mm deep and 4 mm in diameter). 9 fruit per treatment were inoculated with *F. semitectum*, while the other 9 fruit were inoculated with *T. roseum*. The wounds were inoculated with 20 μL 1×10^5 spores mL^{-1} suspension. Inoculated fruit were placed in separate cartons, covered with a plastic film and stored at 22-24°C, RH 50-60%.

Postharvest dipping assays

Treatment The study was a complete random design with 4 treatments. The fruit were dipped in 50 and 100 mg L^{-1} ASM or imazalil at 1 mL L^{-1} solutions containing 0.05 mL L^{-1} Tween 20 for 10 min. Water treatment was used as the control (CK).

Inoculation The fruit were disinfected with 70% ethanol and air-dried before wounding with a sterile nail, forming 9 wounds (3 mm deep and 4 mm in diameter). 9 fruit per treatment were inoculated with *F. semitectum*, while the other 9 fruit were inoculated with *T. roseum*. The fruit were inoculated with 20 μL 1×10^5 spores mL^{-1} suspension 1, 3, 5 or 7 day after dipping. Inoculated fruit were placed in separate cartons, covered with a plastic film, and stored at 22- 24°C, RH 50-60%.

Assessment Infection rate and lesion diameter were measured after 4 days. 2 perpendicular measurements of diameter were made, and the mean used to calculate lesion area. Infection rate and lesion area were calcu-

lated as follows:

Infection rate (%) = Infected wounds/Total wounds × 100

Lesion area = $\pi D^2/4$

POD assays Fruits without inoculation were used at the same degree of maturity (parameters tested were firmness and total soluble solids). Approximately 3 g fruit samples were taken from 0.5-1.0 cm below the skin with a stainless steel cork borer around the equator of each melon. After sampling, flesh tissues were homogenized with mortar and pestle in 0.05 M cold sodium phosphate buffer, pH 5.9, containing 5% (w/v) polyvinypolypyrolidone (PVP). Homogenates were centrifuged at 8 000 r/min for 20 min at 4°C and supernatants were assayed for enzyme activities.

Peroxidase (POD) activity was determined according to Ge *et al.* (2005). Supernatants were diluted in 0.05 M sodium phosphate buffer, pH 5.9 (1:3, v/v). This diluted extract (60 μL) was added to a 3.6 mL of 0.05 M guaiacol, then 200 μL of 2% H_2O_2 were added to reaction mixture. Oxidation of guaiacol to tetraguaiacol was taken for spectrophotometric measurements with an Opera 3000 spectrophotometer at 470 nm for 3 min at 23-25°C. POD activity was expressed as nM tetraguaiacol produced per min and per mg of proteins using a coefficient of 6 165.41.

Protein determination Protein content in the crude enzyme extracts was determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as the standard protein. The enzyme tested was performed with a minimum of three tissue samples replicated per treatment and per time point. Each experiment was repeated at least three times.

Statistical analyses All statistical analyses were performed with data processing system (DPS). The means of the treatments were separated at the 5% significance level using Duncan's multiple range test.

RESULTS

In vitro assays

No difference was observed in the mycelial growth of *T. roseum* and *F. semitectum* at different concentrations of ASM when amended into PDA after 5 days at 25-27°C, but imazalil inhibited the mycelial growth (Table).

Preharvest spraying study

ASM treatment 1 week before harvest reduced the lesion area of pink rot and Fusarium rot (Fig.1). The

Table Effects of ASM on mycelial growth of *T. roseum* and *F. semitectum* in Petri dishes after 5 days incubation at 25-27°C

Treatment	Mycelial growth (mm diameter)	
	T. roseum	*F. semitectum*
CK	39.7 ± 9.64	66.5 ± 0.94
1 mL L^{-1} imazalil	13.5 ± 2.81	25.5 ± 9.2
50 mg L^{-1} ASM	38.5 ± 6.8	66.3 ± 2.66
100 mg L^{-1} ASM	38.5 ± 5.8	63.7 ± 1.4

Each value is the mean (± SE) based on 6 measurements in duplicate experiments.

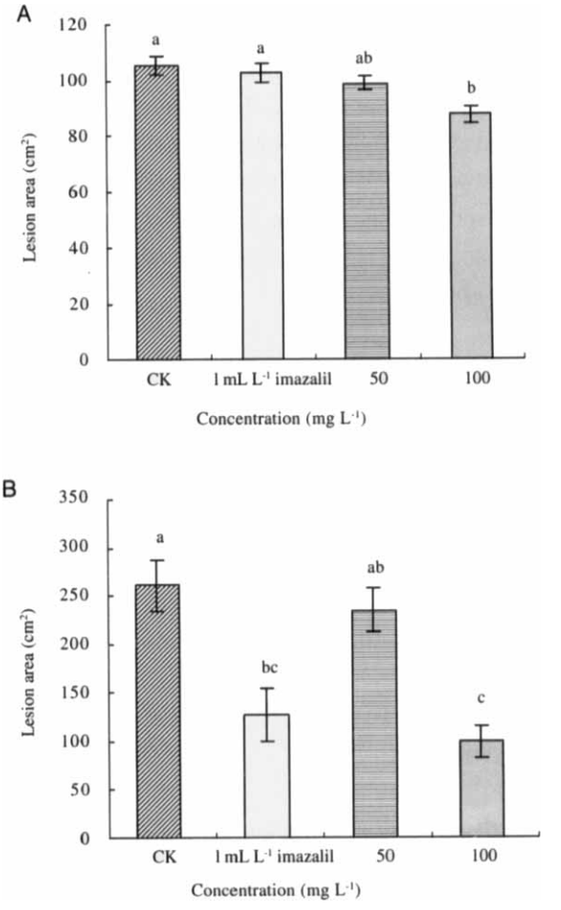

Fig. 1 Effects of ASM applied 1 week before harvest on the lesion development of pink rot (A) and Fusarium rot (B) of muskmelon stored at 22-24°C, RH 50-60% for 4 days. Means within an experiment followed by the same letter are not significantly different ($P = 0.05$). Vertical bars represent the standard error of the mean. The same as below.

concentration at 100 mg L^{-1} provided control of lesion development superior to 1 mL L^{-1} imazalil for pink rot. No differences were found in the infection rate of Fusarium and pink rots at different concentrations of ASM.

ASM application 1 day before harvest inhibited the lesion development of both pink and Fusarium rots (Fig. 2). Both ASM concentrations provided superior control of pink rot compared to 1 mL L^{-1} imazalil (Fig.2-A). However, there is no significant difference between 1 mL L^{-1} imazalil and ASM treatments on control of Fusarium rot (Fig.2-B). None of the chemical treatments reduced the infection rate of pink rot or Fusarium rot.

ASM treatment 1 day before harvest provided good control of Fusarium rot compared to application 1 week before harvest (Fig.3-B). ASM applied at 50 mg L^{-1} d^{-1} before harvest significantly inhibited the lesion development of Fusarium rot compared to ASM applied 1 week before harvest (Fig.3-B). No significance was found between 1 week before harvest and 1 day before harvest on pink rot (Fig.3-A).

Postharvest assay

ASM dipping at 1, 3, 5, 7 day before inoculation significantly inhibited the lesion development of pink and Fusarium rots (Fig.4). ASM applied at 100 mg L^{-1} 1, 3, 5, 7 day before inoculation inhibited the lesion development of both pink and Fusarium rots compared to CK (Fig.4). ASM applied at 50 mg L^{-1} 1, 3, 7 day before inoculation also inhibited the growth of pink rot (Fig.4-A). ASM applied at 50 mg L^{-1} 5 day before inoculation inhibited the growth of Fusarium rot (Fig.

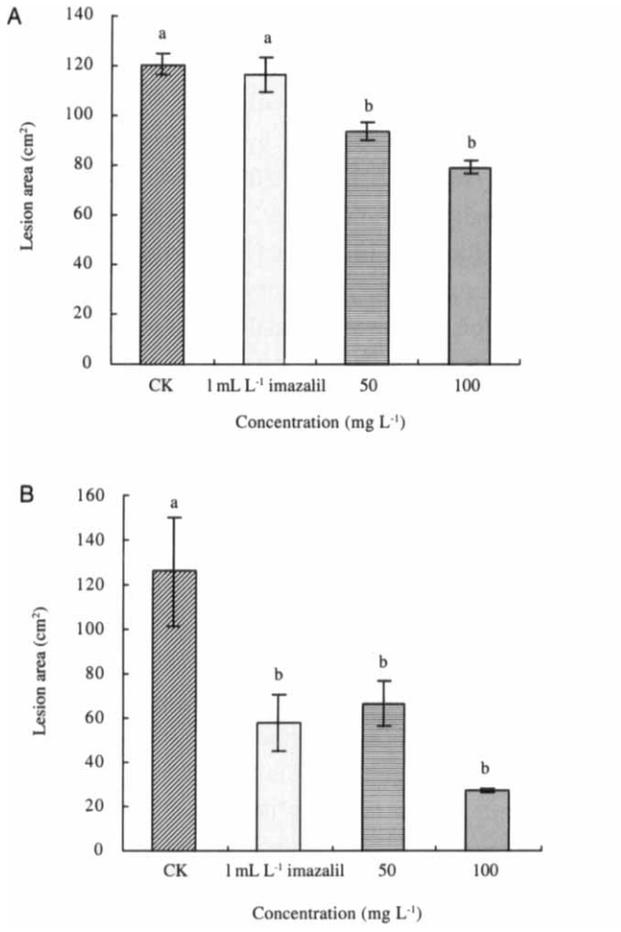

Fig. 2 Effects of ASM applied 1 day before harvest on the lesion development of pink rot (A) and Fusarium rot (B) of muskmelon stored at 22-24°C, RH 50-60% for 4 days.

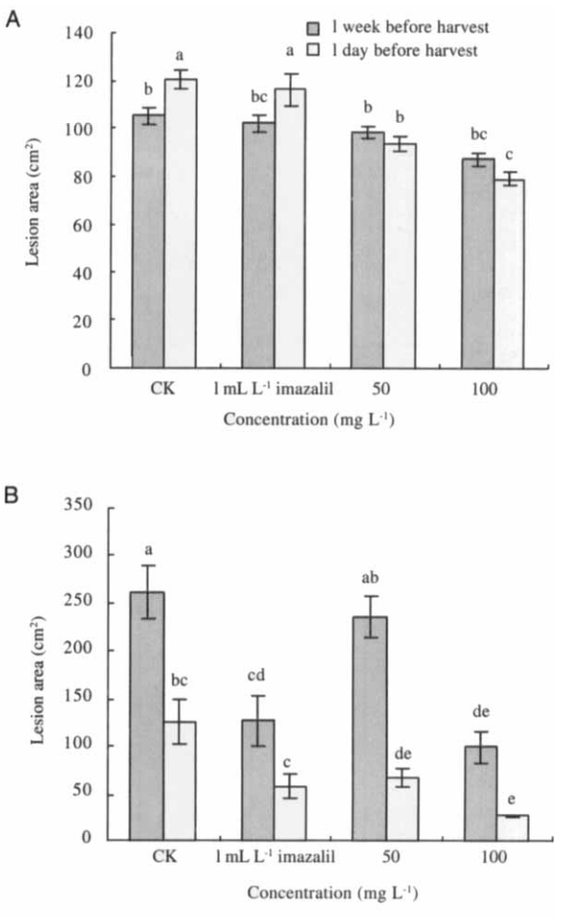

Fig. 3 Effects of ASM applied 1 week before harvest or 1 day before harvest on the lesion development of pink rot (A) and Fusarium rot (B) of muskmelon stored at 22-24°C, RH 50-60% for 4 days.

4-B). 100 mg L^{-1} ASM provided better control of pink and Fusarium rots compared to 1 mL L^{-1} imazalil 3, 5 day before inoculation (Fig.4). None of the chemical treatments reduced the infection rate of pink rot or Fusarium rot.

Effect of ASM dipping on peroxidase activity in the fruit

ASM treatment at 100 mg L^{-1} caused a more progressive and significant increase in peroxidase activity of tissues sampled compared with CK. The maximum peroxidase activity was found 8 days after treatment, which was over 2 times higher in the fruit treated with ASM than in the CK. The activation lasted at least 10 days (Fig.5).

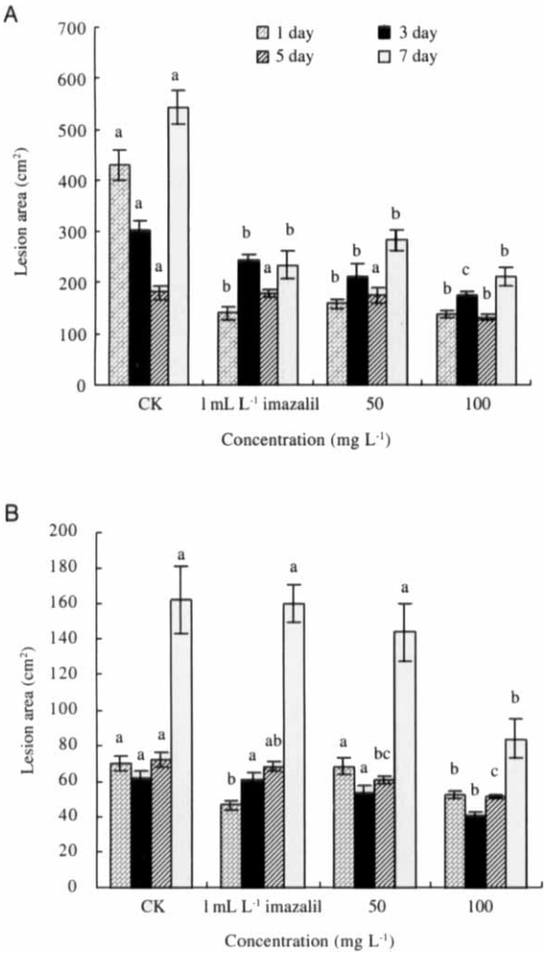

Fig. 4 Effects of different inoculation time after postharvest ASM treatment on lesion development of pink (A) and Fusarium (B) rots of muskmelon stored at 22-24°C, RH 50-60% for 4 days.

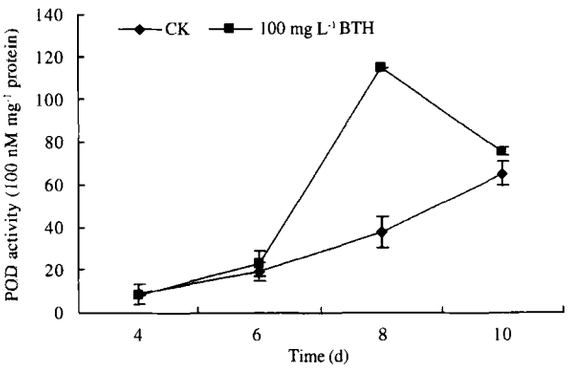

Fig. 5 Induction of peroxidase activity in muskmelon by ASM dipping. Vertical bars represent the standard error.

DISCUSSION

As shown in previous studies that ASM could activate the establishment of systemic acquired resistance (SAR) and result in an enhanced resistance against virus or fungal attack in several plants (Friedrich *et al.* 1996). It has also demonstrated that ASM could be used for control of postharvest decays in fruit, either by spraying during fruit growth or by dipping after harvest (Huang *et al.* 2000; Bi *et al.* 2006; Liu *et al.* 2005; Cao *et al.* 2005; Terry and Joyce 2000; Willingham *et al.* 2002). However, little was focused on the systemic investigation of the inhibitory effect of ASM on the muskmelon fruit disease, and the ability of ASM to enhance fruit defense responses was still not well understood (Terry and Joyce 2004). Results from the present study showed that ASM treatment provided a significant suppression of decay in muskmelon fruit. This suppression was clearly correlated with the activation of defense enzyme. Since ASM did not demonstrate any fungicide effect *in vitro*, it activated one well-known defense related enzyme. These data evidence that this chemical can induce resistance in muskmelon fruit.

The effect of ASM on induced resistance depends on the concentration and the interval between treatment and pathogen inoculation. The most effective concentration in reducing decay was 100 mg L^{-1} in muskmelon. The results also indicated that ASM treatment 1 week before harvest and 1 day before harvest at 100 mg L^{-1} provided better control of pink rot compared to 1 mL L^{-1} imazalil. Harvested fruit treated with 100 mg L^{-1} ASM 3 and 5 day before inoculation also pro-

vided superior control of pink rot compared to 1 mL L^{-1} imazalil. But for Fusarium rot, 100 mg L^{-1} ASM treatment 3, 5, 7 day before inoculation provided better control compared to 1 mL L^{-1} imazalil. The reason may be that different agents have different resistance to any kind of pathogen.

A time-dependent response indicates that treated melons are able to resist the disease if they have enough time to develop the mechanisms necessary for stopping or slowing the ingress and advance of the pathogen. Infection of tissues is not immediate after the inoculation because the pathogen may require several days to be established in infected sites. This provides additional time for the plant to respond to the inducing compounds and reaches higher levels of defense induction (Brisset and Cesbron 2000). In this study, the enhanced resistance in ASM-treated muskmelon fruit was associated with rapid induction of POD activity. The increased activity of POD has been thought to be the key component in local and systemic disease resistance (Bi *et al.* 2006). POD participates in cell wall reinforcement and involves in the final steps of lignin biosynthesis and in the cross-linking of cell wall protein (Brisson and Tenhaken 1994). Increase of POD activity is typical of the process of pathogensis, and oxidative enzymes are known as 'wound or oxidative burst enzymes' (Wojtaszek 1997). Our results showed a significant accumulation of POD in muskmelon (*cv.* Yindi), which increased the resistance against *F. semitectum* and *T. roseum* by accelerating the reinforcement of the cell wall. The fungus establishes and spreads in the intercellular space of parenchyma cells and cell wall fortification could limit their progression.

In addition, there are likely other defense mechanisms involved in the observed suppression. Other defense mechanisms could be more rapidly induced than the POD chosen in this study, and could be more effective against the pathogens (Hammerschmidt 1999).

In conclusion, results above showed that preharvest and postharvest treatment of ASM reduces decay severity and increases the activity of POD. This demonstrates that the defense response in muskmelon fruit to elicitor treatment is similar to that in other systems. Furthermore, our results suggest that the concept of induced resistance could be manipulated in harvested commodities.

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