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Foliar application of acibenzolar-S-methyl and protection of postharvest rock melons and Hami melons from disease

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Abstract

A pre-flowering foliar spray of the plant activator acibenzolar-S-methyl at 50 mg/L a.i. combined with a fruit dip in guazatine at 500 mg/L a.i. at harvest substantially decreased disease in stored melons. Major diseases occurring on rock melons and Hami melons were caused by *Fusarium* spp., *Alternaria* spp., *Rhizopus* spp. and *Trichothecium* sp. The treatment with acibenzolar-S-methyl alone was significantly effective in reduction of the disease severity in many but not all situations. The fungicide guazatine alone significantly decreased infection by *Fusarium* spp. but had a lesser effect on that caused by *Alternaria* spp. and *Rhizopus* spp.

Introduction

Twenty postharvest diseases have been reported in melons (Snowdown, 1990). The main postharvest diseases in rock melons are Fusarium, Rhizopus, Alternaria and Cladosporium rots (Ramsey and Smith, 1961). At room temperatures, the first two are more serious, but in cool storage, Alternaria and Cladosporium rots are more significant. Little information is recorded, however, for Hami melons.

Currently there are no effective control measures against Rhizopus and Alternaria rots. Control of other diseases is dependent on the application of protectant fungicides such as guazatine (Wade and Morris, 1983). Further research is required because the use of fungicides is becoming restricted due to concerns for the environment and health, as well as the increased cost of developing new fungicides. Withdrawal of some fungicides, such as benomyl that was used in the postharvest treatment of melons and captan for control of postharvest diseases, is a clear signal for this requirement (Janisiewicz, 1991).

Systemic acquired resistance (SAR) is attracting much attention for minimisation of plant diseases, and has been demonstrated in several plant families (Hammerschmidt and Kuć, 1995; Kessmann et al., 1994). Biotic inducers of systemic resistance in the cucurbit family include locally infecting fungal pathogens (Caruso and Kuć, 1979; Martyn et al., 1991). Synthetic activators such as dichloroisonicotinic acid and acibenzolar-S-methyl have been reported to cause SAR in cucurbits and other crop species (Métraux et al., 1991; Friedrich et al. 1996; Görlach et al., 1996).

This paper explores the effect of the activator acibenzolar-S-methyl as a foliar spray in melons on the susceptibility of the fruit to postharvest pathogens during storage, and of its potential along with a protective fungicide to provide a new control system against postharvest disease. The potential was first established on a farm in Australia, and then assessed as a practical control measure under conditions in China.

Materials and methods

Australian trials

Benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (CGA 245704; acibenzolar-S-methyl) formulated

as 50% a.i. in wettable granules (Bion WG50) was obtained from Novartis Crop Protection Australasia. Guazatine formulated in liquid was provided by Rhone-Poulenc Rural Australia.

The isolates of *Fusarium* sp. and *Alternaria* sp. from the diseased rock melons were cultured on potato dextrose agar (PDA) plates at 20 ± 2 °C for 72 h. The susceptibility of fungal mycelia growth to the chemical acibenzolar-S-methyl was tested using the technology described by Ishii et al. (1999).

The experiments were carried out on a farm at Mildura, Victoria, Australia, from 1997 to 2000. Methyl bromide was used to fumigate soil before planting. Little foliar disease was noticed on the melon crops. Rock melon cultivar Eldorado was used for experiments during the whole 3-year test, and the cultivar South Cross was also used in the second season 1998/1999.

The plots, each consisting of approximately 90 plants of the cultivars Eldorado or South Cross in 1997/1998 and 1998/1999, and 150 plants of Eldorado in 1999/2000 were randomly selected for the experiments. Some plants were sprayed with Bion WG50 at 25 or 50 mg/L a.i. before blossom. The others were used without being sprayed as controls. Each treatment was replicated three times with each plot used as one replicate. Melons of Eldorado were harvested 8 weeks after treatment, and of South Cross, after a week of extreme heat during growth, at 7 weeks after treatment when they were fully mature.

Melons were sorted for uniform size and absence of obvious injuries, and then washed for 1 min in a solution providing 100 mg/L available chlorine. Half of the melons from each of the field regimes were either placed in cartons as controls or dipped in guazatine 250 or 500 mg/L a.i. for 1 min. Melons were then placed in cartons, six per carton and two cartons per replicate. Three replicates were thus established for each of the four treatments. The cartons containing Eldorado then were kept at 2–8 °C for 3 weeks in the first two seasons and for 4 weeks in 1999/2000, except that those of the cv. South Cross then were held for a further 2 days at room temperature.

Chinese trials

Crops of Hami melons, cv. Early Yellow Hami, were grown and treated in a similar way in Xinjiang province, China, except that the melons were harvested at full maturity 7 weeks after spraying of foliage, and the storage was on 5 cm thick straw layers at room temperatures of 26–29 °C for 9 days.

Each melon was inspected after storage and results recorded as percentage of total melons firstly with any symptoms and secondly with specific types of rot. Fungi associated with rots in Hami melons were isolated, established in pure culture and used to re-inoculate healthy melons, which were kept at 20–25 °C for 48 h. Fungi that caused rots were identified from microscopic inspection of mycelium and spores.

Estimates of disease severity were based on the numbers and areas of lesions according to the key:

0 – No symptom.
1 – One lesion less than 1 cm in diameter.
2 – One lesion between 1 and 3 cm or two lesions each with an area less than 2 cm.
3 – One lesion larger than 3 but smaller than 5 cm, or two lesions each of them larger than 2 but smaller than 3 cm.
4 – One lesion > 5 cm or more than 3 lesions.

The percentage data were transformed to arcsines and analysed. Disease severity = rating of symptoms/number of melons assessed. Least significant differences (LSD) were used to assess effects of treatments.

Results

Acibenzolar-S-methyl had no activity against mycelial growth of the fungi tested *in vitro* (data not shown). Application of acibenzolar-S-methyl to rock melon foliage before flowering had a major effect on disease in fruit produced on a farm at Mildura, Australia. There was a significant decrease in the incidence of disease after low temperature storage of the fruit (Table 1). A postharvest application of guazatine to the fruit had a great effect, but the use of each application in succession almost prevented disease.

In this experiment, Alternaria rot was a major disease, occurring on the sides of rock melons that had touched the ground during crop growth. These sides were soft and fragile when the fruit were removed from storage and yielded typical symptoms of Alternaria rot 2 days later, where no treatments had been applied. In contrast, comparable sides were firm after the application of both treatments in succession, but less frequently after one treatment alone. Fusarium rot mainly occurred on the stem end scars of melons which received no treatments and its incidence was

Table 1. Effect of acibenzolar-S-methyl (Bion WG50) and guazatine on disease in rock melons cv. Eldorado after storage[1]

Treatment	Percentage[2] of fruit		
	Diseased	Infected by	
		Alternaria	*Fusarium*
Control	88.5 a	55.6 a	50.0 a
Bion	55.6 b	32.5 b	26.6 b
Guazatine	21.8 c	21.6 c	0.0 e
Bion + guazatine	2.0 d	2.0 d	0.0 e

[1]Bion WG50 (50 mg/L a.i.) was applied as a spray to foliage in 1997/1998 before flowering. Guazatine (500 mg/L a.i.) was applied as a fruit dip after harvest. The melons were stored at 2–8 °C for 3 weeks.
[2]The figures followed by different letters within the first column and separately in the last two columns were significantly different at 5% by LSD.

significantly decreased by pre-flowering application of acibenzolar-S-methyl and prevented by a postharvest dip of fruit in guazatine alone (Table 1). Similar effects of successive treatments in almost preventing disease were obtained when the experiment was repeated at the same site later in the season 1998/1999.

Successive treatments greatly minimised disease at the same site with a different cultivar in the second season (Table 2). The incidence of Alternaria rot was high, and it was not well controlled with either acibenzolar-S-methyl or guazatine alone.

When tested on the cultivar Eldorado in the third season, acibenzolar-S-methyl foliar spray alone did not significantly reduce the incidence of disease mainly caused by *Alternaria* spp. but it greatly decreased disease severity (Table 3, Figure 1). The successive treatments of acibenzolar-S-methyl foliar spray and guazatine postharvest dip gave a good control of the disease at 25 mg/L of acibenzolar-S-methyl with 250 mg/L of guazatine. Increase of the concentration of acibenzolar-S-methyl to 50 mg/L and guazatine to 500 mg/L completely prevented disease.

Experiments with a similar design were carried out also at Xinjiang, China, but using Hami melons and postharvest storage at room temperature. Fungi isolated from diseased Hami melons were *Fusarium chlamydosporum*, *F. equiseti*, *F. semitectum*, *F. culmorum*, *F. sambucimum*, *F. oxysporum* f.sp. *cucumerinum*, *F. moniliforme* var. *subglutinans*, *Alternaria alternata*, *Rhizopus stolonifer*, *R. arrhizus*, and *Trichothecium roseum*. Minor pathogens included *Penicillium* spp. and *Geotrichum candidum*.

Fusarium rot had the highest incidence, occurring at the stem-end and cracks on the skin surface. Alternaria rot mainly occurred at places that had touched the ground during growth. Rhizopus rot was mainly on the sides of fruit. Trichothecium rots were found randomly at the stem-end, flower-end and on the sides of fruit. Either pre-flowering application of acibenzolar-S-methyl or a postharvest dip of fruit in guazatine minimised overall disease, but acibenzolar-S-methyl did not decrease Trichothecium rot (Table 4). Successive treatments were not more beneficial in minimising total disease, but they were effective against the Alternaria and Rhizopus rots.

Discussion

The experiments confirmed that postharvest diseases in rock melons include Alternaria, Fusarium and Rhizopus rots, and showed that these diseases were also responsible for loss in Hami melons in China. Guazatine gave quite effective control of Fusarium and Rhizopus rots, but was less effective against Alternaria rot, especially in prolonged storage of rock melons. No fungicide is registered in China for the protection of postharvest melons, and guazatine is the only fungicide that the Australian melon industry currently relies on for this purpose. The potential for re-enforcement of this method and a widening of its effectiveness against all major pathogens was shown by its use as a postharvest dip combined with acibenzolar-S-methyl as a preharvest foliar spray.

One spray of acibenzolar-S-methyl to leaves of melon crops prior to flowering without postharvest treatment decreased the incidence and extent of postharvest diseases to some degree. This remarkable result opens a new approach to minimisation of postharvest melon diseases, because acibenzolar-S-methyl has no direct antimicrobial action against *Fusarium* sp. and *Alternaria* sp., and it has given protection in several other plant species through induction of systemic resistance (Dann et al., 1998; Friedrich et al., 1996; Görlach et al., 1996; Jensen et al., 1998; Lawton et al., 1996; Siegrist et al., 1997). Acibenzolar-S-methyl, therefore, is hypothesised to decrease postharvest disease in melon after foliar application by activating systemic resistance, as it is reported to do in cucumber roots after foliar application (Benhamou and Bélanger, 1998).

A most interesting scientific question concerns the way in which one application of acibenzolar-S-methyl

Table 2. Effect of acibenzolar-S-methyl (Bion WG50) and guazatine on disease in rock melons cv. South Cross after storage[1]

Treatment	Percentage[2] of fruit			Disease severity	
	Diseased	Infected by		Infected by	
		Alternaria	*Fusarium*	*Alternaria*	*Fusarium*
Control	100.0 a	100.0 a	11.4 d	1.59 a	0.28 d
Bion	84.7 b	93.3 b	0.0 f	0.68 b	0.0 f
Guazatine	61.3 c	44.2 c	2.0 e	0.5 c	0.057 f
Bion + guazatine	11.5 d	2.0 e	8.4 d	0.057 f	0.11 e

[1]Bion WG50 (50 mg/L a.i.) was applied as a spray to foliage in 1998/1999 before flowering. Guazatine (500 mg/L a.i.) was applied as a fruit dip after harvest. The melons were stored at 2–8 °C for 3 weeks and a further 2 days at room temperature.
[2]The figures followed by different letters within the first column and separately in the second and third columns, and again in the last two columns, were significantly different at 5% by LSD.

Table 3. Effect of acibenzolar-S-methyl (Bion WG50) and guazatine at various concentrations on incidence of disease in rock melons cv. Eldorado after storage[1]

Postharvest treatment	Percentage of diseased melons		
	Preharvest foliar spray		
	Unsprayed	Bion 25 mg/L	Bion 50 mg/L
None	100.0 a[2]	100.0 a	98.1 a
Guazatine 250 mg/L	85.4 b	44.4 d	50.0 cd
Guazatine 500 mg/L	55.7 b	21.8 e	0.0 f

[1]Bion WG50 (25 or 50 mg/L a.i.) was applied as a spray to foliage of the rock melon Eldorado in 1999/2000 before flowering. Guazatine (250 or 500 mg/L a.i.) was applied as a fruit dip after harvest. The melons were stored at 2–8 °C for 4 weeks.
[2]The figures followed by different letters in the table were significantly different at 5% by LSD.

to foliage before flowering affects disease severity in melons harvested 7–8 weeks later and tested after a further period of storage. One possibility is that acibenzolar-S-methyl brings about systemic resistance in the foliage and thereby decreases inoculum of organisms available to infect the fruit. This seems unlikely because the foliage of control plants in the well-managed farm appeared to be clean with respect to fungal growth on surface, presenting no apparent opportunity for SAR to act at this point. Moreover, observations indicated that the principal postharvest pathogen, *Alternaria* spp., was infecting from contact points of fruit with soil, suggesting that SAR was expressed in the fruit.

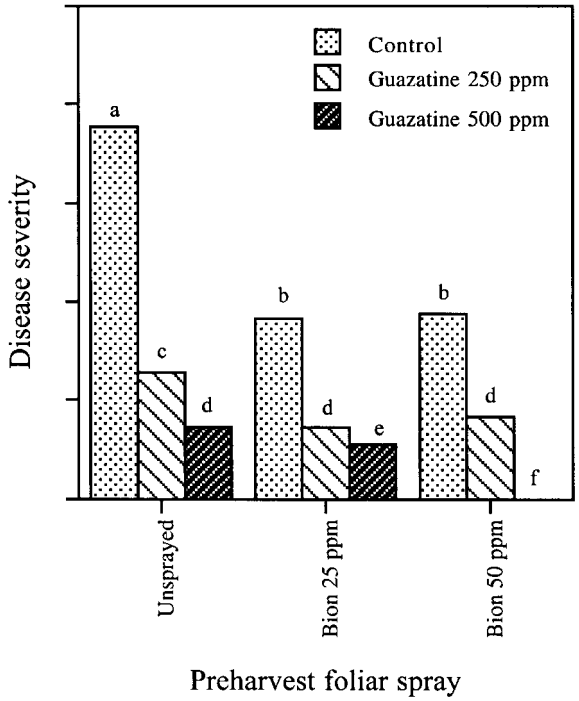

Figure 1. The combined effect of different concentrations of foliar spray and postharvest fruit dip on disease severity in harvested melons. Bion WG50 (25 or 50 mg/L a.i.) was applied as a spray to foliage of the rock melon Eldorado in 1999 before flowering. Guazatine (250 or 500 mg/L a.i.) was applied as a fruit dip after harvest. The melons were stored at 2–8 °C for 4 weeks. The figures followed by different letters above the bars were significantly different at 5% by LSD.

Table 4. Effect of acibenzolar-S-methyl (Bion WG50) and guazatine on disease in Hami melon cv. Early Yellow Hami after storage[1]

Treatment	Percentage of fruit				
	Diseased	Infected by			
		Alternaria	*Fusarium*	*Rhizopus*	*Trichothecium*
Control	73.5 a[2]	25.4 c	53.3 a	21.9 c	9.3 e
Bion	50.0 b	13.0 de	44.3 b	1.2 g	8.8 e
Guazatine	29.7 c	10.0 de	11.6 de	1.2 g	0.0 h
Bion + guazatine	23.2	4.5 f	15.7 d	0.0 h	10.0 de

[1]Bion WG50 (50 mg/L a.i.) was applied as a spray to foliage in 1998 before flowering. Guazatine (500 mg/L a.i.) was applied as a fruit dip after harvest. The melons were kept on a straw layer (5 cm thick) at room temperature for 9 days.
[2]The figures followed by different letters within the first column and separately in the last four columns were significantly different at 5% by LSD.

A strong possibility, therefore, is that acibenzolar-S-methyl or a second messenger affects fruit-generating cells in the flower so that a long-lasting change in the fruit is brought about. The time and distance over which the change is effected may require greater explanation about underlying mechanisms than that currently available from research on effects of acibenzolar-S-methyl in other plants. Rapid uptake from foliar sprays is followed by metabolic conversion in plants to a closely related product which moves up and down through the phloem (Novartis, 1997). Binding to receptor sites throughout plants is presumed to precede the known activation of genes and the accumulation of proteins which differ in characteristics between plant species (Friedrich et al., 1996; Görlach et al., 1996; Lawton et al., 1996). Consequences vary between reports, but include impediments to penetration by pathogens (Lawton et al., 1996), possible hydrolysis of components of fungal walls (Siegrist et al., 1997) and sensitisation of plant cells to react more rapidly to attempted infection (Benhamou and Bélanger, 1998).

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