Enhanced disease management in melons using natural defense activators

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MEDIA SUMMARY

Foliage diseases and fungal rots are a major cause of crop losses in melons. Current treatments do not provide sufficient protection under high disease pressure or during prolonged transport of fruit to market. Overuse of fungicides has led to the development of resistance to control by many of the major pathogens. In addition, the domestic and international markets are placing pressure on farmers to reduce reliance on fungicides due to health and environmental concerns.

This project examined the efficacy of using a range of activators of natural plant defence to protect melons from fungal rots such as Fusarium, Rhizopus and Alternaria during the postharvest period. These chemicals not only help maintain vine vigor and defence against common preharvest pathogens such as powdery and downy mildews, but we also show that the preharvest sprays increase the shelf life of the melon, negating the need for postharvest fungicide dipping. Preharvest applications of the defence elicitors BTH (Bion®, Syngenta Australia) ReZist® (Stoller Australia) and Silica as Silikamajic (Flairform WA), protected rockmelon fruit from postharvest disease significantly better than the control treatment. BTH provided a level of postharvest disease control at least as good as fungicide dipping for the major melons diseases. The plant defence elicitors did not adversely affect the quality of the melons in terms of yield, marketability, Brix°, flesh firmness, and colour and rind colour. The outcomes of this research indicate that induced resistance can be used as part of postharvest disease control strategy. Ongoing research is focused on how induced resistance strategies can be incoroporated into integrated pest management programs, such as with generally regarded as safe (GRAS) chemicals to control postharvest disease for the melon industry.

TECHNICAL SUMMARY

Introduction

The Australian cucurbit industry is growing rapidly with watermelons, cantaloupes and pumpkins making up the bulk of production. Australia has developed a strong export market for melons to Hong Kong, Singapore and New Zealand, and has the potential for substantial development. However, expansion of the industry is constrained by inadequate attention to fruit maturity, as well as postharvest losses and quality deterioration during long distance transport and storage.

The Problem

Fungal fruit rots caused by *Rhizopus*, *Alternaria, Geotrichum* and *Fusarium* spp. are the major diseases causing postharvest losses. Control of these diseases is dependent on postharvest treatment with fungicides dips such as guazatine, and the provision of suitable packaging and storage conditions. However, the treatments do not provide sufficient protection under high disease pressure or during lengthy/adverse transport and storage conditions. With continuing pressure for the withdrawal of postharvest fungicides (benomyl is already withdrawn), and development of resistance to fungicide treatments, new options are needed to reduce reliance on fungicide use and maintain quality during transport and marketing.

The Research

Plants protect themselves from disease through a range of natural defence mechanisms, with varying success. Recent research has shown that a range of chemicals which boost the natural defence mechanisms in plants may reduce disease losses in melons. Plants treated with activators of natural defence undergo systemic acquired resistance (SAR) and have enhanced protection against infections by viruses, bacteria, fungi and nematodes. Systemic acquired resistance (SAR) has been intensively investigated for disease control in many crops. However, there are very few reports considering SAR for postharvest disease control. In a preliminary project in 1998, it was found that preflowering application of one such 'defence booster', BTH, alone or in combination with the postharvest fungicide guazatine, reduced postharvest rockmelon fruit losses. An investigation of SAR and the potential for use in rockmelon postharvest disease control was conducted in this study.

Project Aim

The aim of this project was to:

a) Investigate whether induced resistance has the potential to reduce postharvest disease in rockmelons
b) Screen a range of plant defence elicitors for control of pre and postharvest diseases
c) Determine appropriate application rates of elcitors and timing of preharvest application
d) Assess impact of defence elicitors on yield, quality and storage of melons.

Project Outcomes

Initially a range of SAR elicitors were screened in glasshouse trials, followed by field trials at the University of Sydney Farms, NW Victoria at Mildura, Chinchilla in Queensland and at Humpty Doo in the Northern Territory.

- On the basis of relevant literature we screened the following chemicals to determine whether they elicitied natural defence in melon plants: 2,6-dichloro isonicotinic acid (INA), β-aminobutyric acid (BABA), Bion® (also known as

BTH or acibenzolar-S-methyl) from Syngenta Australia, Silica as SilikaMajic (Flairform, WA) and ReZist®, a product from Stoller Australia.

In the first study:

- Applications of the defence elicitors BTH and ReZist protected rockmelon fruit from all postharvest disease significantly better than the control treatment.
- Melon fruit were assessed for natural infection 14 days after harvest at 15°C and both BTH and ReZist protected fruit from *Alternaria alternata* and *Colletotrichum* spp. signigicantly better than the control treatment.
- Postharvest infection of melon fruit by *Fusarium equi*setti was significantly controlled by BTH with ReZist being less effective.
- The combination of using the plant defence elicitors BTH and ReZist incombination with postharvest guazatine dipping of fruit in general provided better control than elicitor alone or guazatine alone.
- Application of plant defence elicitors BTH and ReZist stimulated increased activity of the pathogenesis related protein chitinase compared to the control treatment, confirming that induced resistance had occurred and that chitinase could be used as a marker for SAR in melon plants.
- Preharvest application of BTH and ReZist® had no phytotoxic effects on the growth of plants as measured by leaf area, fruit yield, and sugar content of treated fruit.

In the second study:

- Preharvest application of BTH (Bion®) (50 ppm applied once 3 weeks before harvest), ReZist® (44ppm applied weekly through the growing season) and Silica (500 ppm applied weekly through the growing season) significantly reduced posharvest disease incidence and severity on rockmelon fruit stored for 5 weeks at 5ºC.
- Storing melons for 5 weeks at 5ºC represents the outside limit for which rockmelons can be stored. Hence marketability after this extended storage period was quite low ranging between 32 and 69%. The most marketable fruit were from the ReZist and BTH treatments.
- In this trial both ReZist and BTH gave good control of Fusarium rots as well as Alternaria rots.
- Application of SAR elicitors resulted in the significant increases in activity of pathogenesis related proteins such as chitinase and peroxidase confirming that they are potential markers for systemic acquired resistance in rockmelons.
- The timing of elicitor treatment did not appear to affect the level of protection of fruit against disease, since one, two or there applicxations of BTH provided good protection against postharvest disease
- The plant defence elicitors did not adversely affect the quality of the melons in terms of Brix°, flesh firmness, and colour and rind colour.

INTRODUCTION

The limited shelf-life of rockmelons has always been a problem to the industry in Australia. The high internal sugar and water content means that melons are highly susceptible to postharvest pathogens. The major postharvest diseases reported for rockmelons include Fusarium Rot, Alternaria Rot, Rhizopus Rot and Cladosporium Rot. With the exception of Rhizopus rot, all the causative pathogens are present in the field, preharvest. Economically feasible improvements to field management and postharvest practices are required to contain these pathogens.

Traditional control of diseases, both pre and postharvest predominantly include the use of fungicide sprays and dips. Heavy reliance on chemical inputs results in increased production costs and adverse impacts on the ecosystem, raising serious concerns for human and animal health. As a consequence, concerns for the environment have focussed efforts on seeking alternative agricultural practices for long term sustainability of agriculture.

Here, we describe the application of a novel range of chemicals that boost the natural defence responses of the melon vine. These chemicals not only helped maintain vine vigour and defence against prevalent preharvest pathogens such as powdery mildew, but we also show that the preharvest sprays increase the shelf life of the melon, reducing the need for postharvest fungicide dipping.

Five trials were conducted in total beginning with screening of activators in glasshouse trials. This was followed by 4 field trials at the University of Sydney, NSW, Mildura Victoria, Chinchilla in Queensland and Humpty Doo in Northern Territory. This report documents in detail the trials from the University of Sydney and Mildura, as they both demonstrate the usefulness of defence elicitors for protection of melons from disease during the postharvest period. The outcomes of the other trials from Chinchilla and Humpty Doo were compromised by the poor quality of the fruit (i.e. cracked or overmature) and poor temperature management during transport and storage. No clear differences between treatments could be measured for these trials and results from these trials are therefore not reported here.

LITERATURE REVIEW
Postharvest disease management

Postharvest losses of fresh fruit and vegetables are an inevitable part of the supply chain once products are harvested. Postharvest losses are commonly caused by natural senescence, physiological and microbiological damage, and mechanical injury (Salunkhe and Desai, 1984a).

Decay caused by disease is the major source of vegetable and fruit losses after harvest (Wilson et *al*., 1994). Melons are particularly susceptible to postharvest diseases due to ease of pathogen entry via the abscission zone around the peduncle at the base of the mature fruit and the high sugar content, making it attractive to pathogens. There are over twenty diseases attacking melons after harvest (Snowdon, 1991) and of these diseases, Alternaria rot, Cladosporium rot, Fusarium rot, and Rhizopus soft rot are the major postharvest diseases for rockmelons in Australia (Morris, 1977; Wade and Morris, 1982).

Figure 1: A natural infection of Fusarium rot on rockmelon

Figure 2: Growth of Rhizopus rot on rockmelon

Figure 3: Natural infection of Alternaria rot on rockmelon fruit

A break in the cool chain or a delay in the product reaching the consumer, can mean deterioration in quality, particularly caused by disease. Disease control after harvest is usually achieved by a combination of refrigeration and other treatments, such as fungicide application or dipping in hot water (Morris and Wade, 1983; Fallik et *al*., 2000)
Low temperature not only maximizes shelf life by reducing the product metabolism, but it also slows down the development of any pathogen that may be present (Sommer, 1982). However, the minimum range of temperatures that inhibit fungal growth is variable, depending on species. The recommended storage temperatures for melons are between 0 - 10°C depending on the cultivar and type of melon (Salunkhe and Desai, 1984b). Lower temperatures cause chilling injury, limiting the use of low temperature to control disease. Honeydew melons will store for 3 - 4 weeks at 7^{o}C, but will suffer chilling injury at less than 5^{o}C (Edwards, 1989). Rockmelon, which is the most chilling resistant melon type, has the optimal storage temperature of 2 – 5^{o}C (Morris, 1992). However, the temperature that can inhibit most major fungi on rockmelons is approximately 2^{o}C or less. Therefore, application of chemicals has traditionally been combined with temperature management to extend the shelf life of melons.

The netted surface of rockmelon fruit provides an ideal host environment for pathogens. Disinfection of the surface of melon prior to storage significantly reduces postharvest rots caused by pathogens (Halloran et *al*., 1999). Protective chemicals are commonly applied by dipping the fruit postharvest. Treatment of fruit with chlorine at 1000 ppm showed only moderate inhibition of surface fungi on cantaloupe. However more effective fungides were identified by Wade and Morris (1982). They dipped cantaloupes in guazatine, benomyl, fenaminosulf and captan for 1 minute and the results showed that these fungicides effectively halted *Fusarium solani*, *Geotrichum candidum*, *Alternaria alternata* and *Cladosporium* spp. growth. It was also found that a mixture of guazatine and benomyl restricted the growth of *Alternaria* spp. and *Fusarium* spp. on honeydew melon. Imazalil at 2000 ppm was effective against *A. alternata* and *Fusarium* spp. of rockmelon (Aharoni et *al*., 1992).
Alternatives to fungicides to control postharvest diseases have also been investigated. Sanosil -25 (a disinfectant containing 48% hydrogen peroxide and silver salts) effectively reduced Alternaria and Fusarium rots on rockmelons (Aharoni et *al*., 1994). Sodium bicarbonate significantly reduced the growth of *A. alternata*, *Fusarium* spp., and *Rhizopus stolonifer* on potato dextrose agar as well as on melon fruit (Aharoni et *al*., 1997). In addition, controlled atmosphere of 10% CO_2 and 10% O_2

showed a reduction in disease incidence on rockmelon (Arahoni et *al*., 1993). Dipping fruit for 2 min in hot water at 52^{o}C protected rockmelon against *Alternari*a, *Fusarium*, *Rhizopus*, and *Mucor* species (Teitel et *al*., 1989). Furthermore, treatment of cantaloupes with the combined action of a hot water rinse and brushes at 59^{o}C for 15 seconds significantly controlled *A. alternata* and *F. solani* (Fallik et *al*., 2000).

Plant natural defences and their application in disease control

In nature, plants are vulnerable to attack by many different organisms, however, generally they manage to overcome the attack and survive. Plants are able to protect themselves from disease with constitutive (passive) defence and induced defence (active) strategies (Agrios, 1997; Lucas, 1998).

Many plants possess key physical structures that play a role in minimizing pathogen invasion. The host primary cell wall is the first static barrier to pathogen entry. The cell wall is composed of complex carbohydrates such as pectin and cellulose and is made more impregnable against disease with the addition of cutin, suberin, waxes, lignin, silicon, and calcium (Ride, 1983). The water repellent characteristic of waxes on leaves and fruit prevents the formation of water film that may encourage fungi and bacteria deposition and development. Dense hair on the plant surface has a similar function and contributes to disease prevention. The thickness of cuticle layer itself hinders the direct penetration of pathogens. Fungi can penetrate the host plant by producing enzymes that degrade the cell walls, but in resistant hosts, the pathogen may then be stopped by a new set of plant defence strategies.

Preformed inhibitory compounds, called phytoanticipins (Van Etten et *al*., 1994), such as saponins, cyanogenic glycosides, glucosinolates, phenols, cucurbitacins, exist in healthy plants and act as deterrents to pathogens (Bar-Nun and Mayer, 1990, Grayer and Harbone, 1994, Osburn, 1996). Prusky and Keen (1993) found that the preformed compounds 5-12-cis- heptadecenyl resorcinol in the peel of mango and 1-acetoxy-2-hydroxy-4-oxoheneicosa-12,15-diene and 1-acetoxy-2,4-dihydroxy-n-heptadeca-16-ene in the peel of avocado could help protect these fruit against *Collectotrichum gloeosporioides*. Furthermore, hydrolytic enzymes such as chitinase and glucanase in plant surface cells help degrade cell wall components of many fungi, resulting in disease resistance. However, the activity of these enzymes is usually low in healthy plant and increases dramatically when the plant is attacked by microorganisms (Agrios, 1997).

Despite constitutive barriers, pathogens manage to defeat initial host defences and penetrate through cell walls, stomata or wounds to infect plants. However, during the infection process, fungal avirulence gene products or compounds released from the pathogen as well as plant cell wall components may, act as elicitors of host defences. These elicitors are recognized by specific receptor proteins encoded by resistance genes on the plant cell surface. The plant defence system will then be activated, resulting in different defence responses (Ebel and Scheel, 1997). These defence responses are either expressed locally or systemically.

Hypersensitive reaction

The hypersensitive response (HR) is usually described as the rapid death of plant cells, and is correlated with the restriction of pathogen penetration (Goodman and Novacky, 1994; Agrios, 1997). A characteristic of HR is the failure of membrane

integrity of the infected host cells and the accumulation of phenolic oxidation products (Goodman and Novacky, 1994), resulting in one or many brown dead cells at the infection site. The occurrence of HR may or may not be limited to cells that are physically attacked by the pathogens (Heath, 2000). A wide range of pathogens can cause HR, but the presence of an avirulence gene in the pathogen or products which are recognized by the plant's corresponding resistance gene, is required to elicit an HR response (Morel and Dangl, 1997).
HR is preceded by the generation of active oxygen species (AOS) called the oxidative burst, which includes the superoxide radical ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\cdot}$) (Baker and Orlandi, 1995; Bolwell and Wojtaszek, 1997). Generation of AOS plays an important role in the signal system to trigger plant defence mechanisms (Lamb and Dixon, 1997). Each one of the active oxygen species can be toxic (Baker and Orlandi, 1995) and they may contribute to the cell wall enforcement by mediating the accumulation of cell wall structural proteins (Brisson et *al*., 1994) as well as papillae formation (Thordal-Christensen et *al*., 1997).
The resultant host cell death from HR plays an important role in preventing the invasion of obligate parasites, which need living host cells for nutrition. The rapid death of living host cells may be sufficient to prevent the invasion of the pathogen. However, many pathogens are necrotrophic and they are also prevented from spreading through the host by the cell death around the site of infection. In this scenario, the hypersensitive response is not alone, but is accompanied with oxidative responses, the accumulation of toxic compounds and cell wall lignification (Lucas, 1998).

Phytoaelexins

Phytoalexins are low molecular weight, antimicrobial compounds that are induced after infection (Ebel, 1986; Van Etten et *al*., 1994) and most flowering families are capable of responding to pathogen infection by synthesizing phytoalexins (Kuc, 1995). Phytoalexins accumulate in the areas of necrosis, especially necrosis corresponding to HR (Hammerschmidt and Nicholson, 1999). Mayama and Tani (1982) as well as Ebel (1986) found that phytoalexins accumulated in tissues located in the vicinity of the original infection sites but not necessarily in the cells that the pathogen invaded. Phytoalexins are synthesized via the shikimate, acetate-malonate, acetate-mevalonate biosynthetic pathways (Kuc, 1995), resulting from new transcription and translation of involved genes (Hammerschmidt and Nicholson, 1999).
The role of phytoalexins in disease resistance is substantial. The phytoalexins luteolinidin and 5-methoxyluteolinidin, which were found in sorghum, inhibited germination and appressorium formation of conidia *Colletotricum sublineolum* (Lo et *al*., 1996). Similar results were found in cotton, where the phytoalexins desoxyhemigossypol and hemigossypol showed antibacterial activity by inhibiting *Xanthomonas* multiplication in defined liquid medium (Abraham et *al*., 1999). Daayf et *al*. (1997) indicated that phytoalexins in cucumber could prevent powdery mildew infection.

Cell wall modifications

The plant cell wall is a constitutive first barrier to most pathogen attack. However, this barrier is not sufficient to prevent pathogen invasion (Schäfer, 1994). The modification of cell walls after pathogen infection re-inforces the strength of the cell

wall (Brisson et *al*., 1994). For example, reinforcement at sites of attempted pathogen penetration include oxidative cross-linking of proteins and various phenolic compounds including hydroxycinnamic acid amides (Grant and Mansfield, 1999). Host cell wall deposition of lignin and callose occurred in response to pathogen infection (Benhamou et *al*., 2000; and Jeun et *al*., 2000) and the change in cell wall architecture impeded pathogen penetration (Benhamou and Belanger, 1998; Stadnik and Buchenauer, 1999). Similarly, lignin in papillae and silicon accumulation at the site of appresorium of *Colletotrichum lagenarium* restricted fungal growth on leaves of cucumber (Stein et *al*., 1993).
Cell wall modification also helped minimise susceptibility to pathogen cell wall degrading enzymes (Stermer and Hammerschmidt, 1987; Matern et *al*., 1995). According to Bradley et *al*. (1992) the oxidatively cross-linked hydroxyproline and proline-rich cell wall glycoproteins which developed rapidly after fungal elicitor treatment, decreased protoplast release (Brisson et *al*., 1994), facilitating resistance of the plant cell to pathogen invasion and enzymatic degradation.
Cell wall alterations also interrupt the flow of nutrients to pathogens and/or diffusion of toxins to host cells (Ride, 1978). For instance, the sealing of plant cell walls retards leakage of cytoplasmic components and therefore, limits available nutrients for biotrophic pathogens. This confers an increase of resistance to such pathogens. In addition, dispersal of toxins and hydrolytic enzymes to sensitive cell walls would be hindered. It is thought that products of polymerisatation of the cell walls might cause adverse effects on the pathogen membrane and inactivate pathogen enzymes and toxins (Hammond-Kosack and Jones, 1996).

Systemic defence

When host natural defence is activated, resistance is induced not only locally at the site of infection, but may also spread systemically throughout the plant. The capability of plants to respond to an attacker by initiating local and systemic defence was first reported by Chester (1933). Systemic resistance in plants has been demonstrated via two distinctive phenomena: induced systemic resistance (ISR) and systemic acquired resistance (SAR). Systemic acquired resistance (SAR) may be synonymously used as induced systemic resistance (ISR) (Hammerschmidt et *al*., 2001; Métraux, 2001).
However, some differences between SAR and ISR are made. ISR occurs independently of the production of salicylic acid (SA) and does not involve the accumulation of pathogenesis related proteins (PRs), which are always found in SAR (Van Loon et *al*., 1998; Pieterse et *al*., 1998). ISR requires the activation of jasmonic acid (JA) and ethylene (Pieterse et *al*., 2001). Different signal perceptions lead to induced resistance in the plant along different pathways, although there is some crossover in signalling.

Induced systemic resistance in plants

Ubiquitous Rhizosphere bacteria are found on the root surface, where available nutrients are present from natural plant secretions (Wood, 1995). Certain strains of rhizosphere bacteria that stimulate growth of plants are called plant growth-promoting rhizobacteria (Kloepper et *al*., 1980). Most of the isolated strains from naturally disease-suppressive soil are *Pseudomonas* spp. and in this instance, plant growth is facilitated by inhibiting soil-borne pathogens (Pieterse et *al*., 2001). Some of these strains are able to induce the plant defence system and convey resistance to the above-

ground parts of plants. This type of induced resistance is considered as induced systemic resistance (ISR) (Kloepper et *al*., 1992; and Pieterse et *al*., 1996). ISR has been seen in many plant species such as carnation (Van Peer et *al*., 1991), radish (Leeman et *al*., 1995), tomato (Duijff et *al*., 1998), tobacco (Maurhofer et *al*., 1994) and cucumber (Wei et *al*., 1991). Resistance from ISR has been found to be effective against a wide range of plant pathogens (Van Loon et *al*., 1998). ISR in plants requires the precise recognition between the host plant and the ISR-inducing rhizobacterium. For instance, *P*. *fluorescens* WCS374r induced an ISR response in *Arabidopsis* but did not cause an induced response in radish and carnation (reviewed by Peiterse et *al*., 2001). Signal transduction leading to ISR requires jasmonic acid (JA) and ethylene (ET) responsiveness (Pieterse et *al*., 1998) and indeed, exogenous application of JA and ET has resulted in ISR in plants. The mechanism and the significance of ISR in induced broad-spectrum disease resistance are still unidentified. However, the protection of plants against disease from ISR is usually less than that of SAR (Van Loon, 1997; Pieterse et *al*., 1998).

Systemic acquired resistance in plants

Systemic acquired resistance (SAR) involves the enhancement of resistance throughout the plant from initial infection to the plant by a non-pathogen or a necrotic lesion-inducing pathogen (Agrios, 1997; Hammerschmidt and Becker, 1997). Once induced, SAR can be maintained in the plant for weeks or even months (Madamanchi and Kuc, 1991; Sticher et *al*., 1997). Kuc and Richmond (1977) and Guedes et *al*. (1980) indicated that although the level of resistance will lessen during the time of plant growth, SAR will last for the whole life time of the plants.

The SAR phenomenon has been observed in both monocotyledons (reviewed by Steiner and Schönbeck, 1995) and dicotyledons (reviewed by Deverall and Dann, 1995; Hammerschmidt and Yang-Cashman, 1995; Ozeretskovskaya, 1995) and is effective against a broad spectrum of diseases in many plant species (Kuc, 1982; Madamanchi and Kuc, 1991; Ryals et *al*., 1996; Sticher et *al*., 1997). SAR is typified by the accumulation of salicylic acid (SA) and pathogenesis-related proteins (PRs) (Ward et *al*., 1991; Uknes et *al*., 1992; Kessmann et *al*., 1994; Ryals et *al*., 1996; Sticher et *al*., 1997).

According to Conrath et *al*. (1996) and Sticher et *al*. (1997) SA is the key for the induction of SAR. The increase of endogenous SA levels has been shown to be related to the activation of genes that encode pathogenesis-related proteins and the onset of enhanced resistance (Conrath et *al*., 1996). Moreover, the role of SA was clarified in experiments using transgenic plants that can degrade SA. The NahG gene from *Pseudomonas putida* encoding salicylate hydroxylase, which can convert SA to catechol (non-inducer of SAR), was transferred into tobacco. Subsequently, NahG plants did not show SAR gene expression, nor did they accumulate SA, resulting in no SAR (Gaffney et *al*., 1993).

Work by Shulaev and his colleagues (1995) has also showed that 70% of SA accumulation was found on the upper, non-infected leaves of tobacco. The mobility of SA during SAR was also checked in labeling experiments in cucumber, in which SA production from infected sites was translocated to uninfected sites via phloem (Mölders et *al*., 1996). However, the work of Rasmussen et *al*. (1991) on cucumber with detached infected leaf trial and Vernooij et *al*. (1994) with grafted tobacco indicated that SA is not the long distance signal.

SA and 4-hydroxy benzoic acid (4HBA) increased in phloem sap along with an increase in phenylalanine ammonia-lyase in the petiole. It was suggested that the increase in SA as well as 4HBA was due to the *de novo* synthesis of the two compounds in stems and petioles in response to a translocated signal from leaf lamina (Smith-Becker et *al*., 1998). Volatile methyl salicylate (MeSA) is formed from SA after infection and can trigger defence responses by conversion to SA (Shulaev et *al*., 1997). Levels of MeSA in plant tissues are concomitant to the SA concentration after viral or bacterial infections (Seskar et *al*., 1998). Transgenic plants with NahG gene are unable to respond to MeSA, illustrating that MeSA has no direct effect on the induction of disease resistance (Seskar et *al*., 1998). MeSA may contribute to the effect of SA for intraplant signaling and plant to plant communication (Shulaev et *al*., 1997). Whether SA is itself the primary systemic signal translocated or merely transported along with the primary systemic signal remains unclear (Mauch-Mani and Métraux, 1998), but it is clear that SA plays an important role in SAR induction (Ryals et *al*., 1996).

In addition to the accumulation of SA, SAR also results in the rapid accumulation of one or more new proteins, which are not pathogen-specific. These proteins are of host origin and named pathogenesis-related proteins (PRs) (Van Loon, 1985). According to Van Loon et *al*. (1994) "*PRs are plant proteins that are induced in pathological or related situations"*. Proteins are considered as PR only if they are synthesized *de novo* upon infection, but not essentially in all pathological conditions.

The role of PR proteins in acquired resistance is based on their anti-pathogenic characteristics and/or their simultaneous accumulation in infected plants (Hammerschmidt and Becker, 1997). Fraser (1982) reported that PR proteins appeared in non-inoculated leaves, distinctly later than the manifestation of acquired resistance. Many PR proteins have anti-pathogenic activities (Linhorst, 1991; Van Loon et *al*., 1994). Chitinases (PR-3 group) in combination with ß-1,3-glucanases (PR-2 group) break down fungal cell walls and perhaps insects. It is also likely that proteinase inhibitors influence insects while the PR-8 family of chitinases, which also has lysozyme activity, may halt bacteria growth. The PR-9 plays a role in lignification and may be involved in the strengthening of the cell walls. PR-1 and PR-5 proteins are also induced strongly and supposed to affect membranes, but the specific action of these proteins has not been verified. In addition, the combination of ß-1,3-glucanase and chitinase showed synergistic effect on fungi both *in vitro* (Van Loon, 1997; Tuzun, 2001) and *in vivo* (Van Loon, 1997). Chitinase from transgenic tobacco and canola suppressed the growth of the soil-born fungus *Rhizoctonia solani* on these plants (Broglie et *al*., 1991). This evidence again verifies the role of PR proteins on disease resistance. However, some trials have shown that PR proteins do not always have effect on pathogens. Van Loon (1997) reported that chitinase showed no inhibition on many chitin-containing fungi due to a protective layer shielding the chitin in the fungal cell walls, preventing contact with chitinase.

Systemic acquired resistance and its markers on melons

Although SAR has been studied intensively in many other plant species, there are few reports concerning this phenomenon on melons. In 1977, Caruso and Kuc inoculated *Colletotrichum lagenarium* in watermelon and found that this could inhibit the growth of the disease caused by the same pathogen. Roby et *al*. (1988) found that applying an elicitor fraction from *C. lagenarium* on 21 day-old melon plants caused an increase in chitinase activity in different parts of treated plants and lessened disease symptoms by

C. lagenarium in comparison to the control plants. Smith and Hammerschmidt (1988) also reported enhancement of peroxidase activity in leaves of inoculated muskmelon, as well as watermelon seedlings, associated with the increase in systemic acquired resistance. Madi and Katan (1998) were successful in activating SAR in melon against *Rhizoctonia solani* by treating melon plants with *Penicillium janczewskii*. Recently, Smith-Becker et *al*. (2003) have shown that SAR was activated after treating cantaloupe plants with acibenzolar-S-methyl (BTH), resulting in an increase in chitinase activity and resistance to *C. lagenarium*. Huang et *al*. (2000) made use of SAR to protect rockmelon and Hami melon fruit from postharvest diseases. However, the study by Huang et *al*. (2000) did not demonstrate induction of SAR.
Clearly, SAR can be induced in melons when the plants are challenged by a pathogen, however, markers for SAR in melon plants needs to be elucidated.
As mentioned above, SA may be the major signal molecule in SAR induction through activating the synthesis of PR proteins (Uknes et *al*., 1992; Gaffney et *al*., 1993). PR proteins, therefore, should be useful markers for SAR induction because the accumulation level of the proteins is associated with the level of SAR induced. In cucurbits, the accumulation of PRs during SAR includes the enhanced activity of chitinase, peroxidase and ß-glucanase (Hammerschmidt et *al*., 1982; Métraux et *al*., 1988; Ji and Kuc, 1995; Smith-Becker et *al*., 2003).
Chitinase is a potential marker for SAR, since the level of this PR protein in non-challenged tissue is low (Smith-Becker et *al*., 2003). Chitinase is a pathogenesis-related protein in cucumber and is directly involved in defence mechanism (Métraux and Boller, 1986; Métraux et *al*., 1988; Meuwly et *al*., 1994). In cucumber, chitinase increased 100-fold in the uninfected second leaves of inoculated cucumber (Métraux and Boller, 1986).
Chitinases or endo-N-acetylglucosaminidases are the glycoside hydrolases that hydrolyze the ß-1,4-glycosidic bonds between the N-acetylglucosamine residues of chitin, found in various organisms, including fungi-Ascomycetes, Basidiomycetes and Deuteromycetes (Vidhyasekaran, 1997), insect, various crustaceans, and nematode eggs (reviewed by Punja and Zhang, 1993).
In addition to direct antifungal activity, chitinases may have a secondary role in inhibiting pathogen development following infection. Moreover, they can indirectly activate the defence response in plant by releasing elicitors through enzymatic digestion of fungal cell wall (reviewed by Punja and Zhang, 1993).

Application of SAR in plant disease control

Disease resistance in plants from the SAR response is a natural defence phenomenon with high durability, as well as a broad spectrum of disease control. Importantly, SAR is based on multiple mechanisms that make it unlikely to allow development of resistance by pathogens (Kessmann et *al*., 1996). Therefore, the potential use of SAR in controlling disease in agriculture is receiving more attention.
Transgenic plants that overexpress PR proteins with antifungal activity have recently been investigated as a method of disease control (Datta, 2002). However, in most cases, passive expression of individual PR protein genes in transgenic plants has not brought about the expected level of disease resistance. The failure to produce resistant plants to disease following alteration of a single gene shows that plant defences against diseases are dependent on a combination of multiple rather than single preformed and inducible defence mechanisms (Ye *et al*., 1995). Consequently, more pragmatic approaches have been investigated to trigger SAR and protect plants

against diseases. There are different plant receptors which receive and respond to chemical stimuli from pathogens. The interaction of these stimuli and responses has been exploited to boost the plant defence system and looks to be a promising approach to disease management. SAR can be triggered by activators (inducing agents), which are either biotic or abiotic.

Biotic activators

Pathogens themselves can be used as inducing agents to stimulate the natural defence system in plants. Tobacco mosaic virus (TMV) was first used as an inducer for SAR of tobacco by Ross (1961) against TMV and other viruses. In 1977, Caruso and Kuc used *Colletotrichum lagenarium* to activate SAR on cucumber and watermelons to protect these plants from the disease. Furthermore, Roby et *al*. (1988) showed that the use of the elicitors extracted from the mycelium of *Colletotrichum lagenarium* could increase the chitanase activity and give protection against the disease (caused by this fungus) on melons. The increase in levels of SA and chitinase activity was identified in cucumber after the plant was infected with *Pseudomonas lachrymans* (Meuwly et *al*., 1994). In the same year, Mölders et *al*. (1994) also found that the content of SA markedly increased in cucumber plants after being induced by *Pseudomonas syringae* pv. lachryman and tobacco necrosis virus. The use of *C. lindermuthianum* and *C. lagenarium* by Kuc (1982) as an activator was also successful in green bean to inhibit the growth of *C. lagenarium* and *C. lindermuthianum*, respectively. *Pseudomonas syringae* pv. *maculicola*, a non-host pathogen of potato, can induce SAR and reduce the disease symptoms on treated potato plants caused by *P. infestans* (Kombrink et *al*., 1996).

Experiments by Madi and Katan (1998) indicated that *Penicillium janczewskii* conidia applied to melon and cotton leaves can help them to avoid infection by *Rhizoctonia solani*, resulting in 100% reduction in the incidence of damping-off. In addition, tobacco necrosis virus (TNV) suspension applied to tomato activated SAR resulting in high resistance to *Phytophthora infestans* (Jeun and Buchenauer, 2001).

Based on the above observations, commercial biotic activator products derived from microbes have since been developed. For example, Harpin a bacterial protein elicitor derived from *Erwinia amylovora*, was used to stimulate SAR to protect apple against fire blight (Momol et *al*., 1999). Harpin also induced the expression of SAR genes in *Arabidopsis*, halting the growth of *Peronospora parasitica* and *Pseudomonas syringae* pv. *tomato* DC3000 (Dong et *al*., 1999). The commercial formulation of Harpin, known as Messenger®, was also applied pre and after harvest to protect apple fruit against postharvest disease-blue mold caused by *Penicillium expansum* (de Capdeville et *al*., 2003).

Another commercially available biotic activator is Milsana®, which is an extract from leaves of the giant knotweed (*Reynoutria sachalinensis* L.) (Wurms et *al*., 1999). Application of this product to wheat induced plant defences against *Septoria tritici*. Use of Milsana® to induce SAR in cucumber plants showed that it assisted in protection against powdery mildew infection (Fofana et *al*., 2002).

Abiotic activators

In addition to biological activators, the availability of many low molecular chemicals that are able to induce SAR in plants offers a very important approach for environmentally friendly disease control. The commercial use of chemical activators is more practical than biological activators since they can be easily distributed and

stored, and overcomes the problems of dealing with pathogen-derived products (Ye et *al*., 1995).
A chemical is considered to be an activator for SAR, when it meets at least three criteria: (1) no direct antimicrobial activity, (2) protection of plants against a broad range of unspecific pathogens, and (3) elicitation of the host defence mechanisms that are the same as those induced systemically after biological activation of SAR and in the tissue not catching the SAR activator (Sticher et *al*., 1997).
The application of salicylic acid (SA) at sub- antimicrobial concentrations to leaves of tobacco plants, illustrated that it could induce SAR and protect against the same range of diseases as TMV did (Ward et *al*., 1991). Pre-treatment of 2, 6 dichloro-isonicotinic acid (INA), a substance that shows no direct antimicrobial effects *in vitro* on bean, resulted in the reduction disease symptoms of anthracnose and rust fungi (Dann and Deverall, 1995). The same compound induced the defence system in soybean (Dann et *al*., 1998), and in tobacco for resistance against TMV (Kalix et *al*., 1996). Similarly, SAR in cotton plants under field conditions was activated following the application of INA and defended the plants against the disease symptoms caused by *Alternaria macrospore*, *Xanthomonas campestris* pv. *malvacearum*, and *Verticillium dahliae* (Colson-Hanks et *al*., 2000). However, both SA and INA showed phytotoxicity on treated plants, resulting in the prevention of their commercial development as plant activators (Leslie et *al*., 1996).
Another non-antifungal chemical, ß-aminobutyric acid (BABA) was observed to elicit the defence system in many plant species (Jakab et *al*., 2001). However the mode of action may not follow the SA, JA/ethylene signal pathway in *Arabidopsis* which supposed to be the main pathways for SAR or ISR respectively (Oostendorp et *al*., 2001).
The most studied resistance activator, however, is acibenzolar-S-methyl (benzothiadazole or BTH) which is the first commercial product marketed with the different trade names of BION®, ACTIGARD^{TM} and BOOST®. BTH has been regisistered in some European countries, but it has not yet been registered in Australia. BTH can activate SAR in many crops against a wide range of diseases, including fungi, bacteria, and viruses (Oostendorp et *al*., 2001).
According to Leslie et *al*. (1996) application of benzothiadazole (BTH), a non-antibiotic chemical, on tobacco plants switched on SAR, making plants resistant to *Cercospora nicotianae* (frog eye leaf spot), *Erwinia caratovora, Phytophthora parasitica* (black shank), *Pseudomonas syringae* pv. *tabaci* (bacteria wild fire), TMV, and *Peronospora tabacina* but not *Alternaria alternata* or *Botrytis cinerea*.
BTH has been shown to be an effective elicitor of SAR in cotton (Inbar et *al*., 2001), rice (Schweizer et *al*., 1999), brassica (Jensen et *al*., 1998), apple (Brisset et *al*., 2000) and pear (Ishii et *al*., 1999). In pea, the application of BTH resulted in significant increase of chitinase as well as ß-1,3- glucanase, and reduced the severity of disease symptoms on leaves (Dann and Deverall, 2000). BTH has been applied to melon plants (Huang et *al*., 2000; Smith-Becker et *al*., 2003) and cucumber (Nicole and Richard, 1998; Ishii et *al*., 1999). BTH application for controlling preharvest disease has been intensively investigated, but its application on postharvest disease control is still new.
Preharvest treated rockmelon plants with BTH 50 ppm (a.i.) reduced postharvest diseases on fruit in comparison to the control (Huang et *al*., 2000). Similar results were obtained in strawberry when preharvest BTH application at 250 – 2000 ppm (a.i.) on plants protected their harvested fruit against *Botrytis cinerea* (Terry and

Joyce, 2000). These results provide evidence that BTH is a potential SAR activator in plants to induce resistance against postharvest disease.

Conclusion

Melons are an important cash crop. Their storage life is short mainly due to disease infection. The growing regions are often far from the markets and therefore fruit are under disease pressure during transport to market. Potential for export particularly via sea, due to rising costs of air transport, as well as shortage of space, requires the extension of storage life up to 3 – 4 weeks. Therefore, one of the prerequisites for melon industry is the improvement of postharvest disease management.

Use of SAR to protect plants against disease is an important option that requires further exploration as an adjunct to, or perhaps in some instances alternative to fungicides. While there are many reports of pre-harvest use of SAR there are very few examples indicating that resistance to disease extends to the fruit postharvest. The concentration of activators and timing of application to the plant requires research since SAR resistance can be variable, depending upon crop, physiological state of plant as well as the environmental conditions under which it is applied. However, the use of elicitors of defence is only feasible if the approach does not adversely affect the growth of plants, fruit quality and yield.

A. UNIVERSITY OF SYDNEY FIELD TRIAL

Effect of the SAR activators Bion® (BTH) and ReZist ® on postharvest disease control in rockmelon

Materials and methods

Plant materials

This field experiment was conducted at Lansdowne Research Station, University of Sydney, Camden, NSW, Australia, from the beginning of October to the end of December 2002 with a mean of air temperature ranging from 8.6 to 27^{o}C in October 13.4 to 30^{o}C in November, and 14.9 to 29.4^{o}C in December (Australian Bureau of Meteorology).

Two months prior to transplanting, beds (1.8 m x 80 m) were prepared and base-dressed with Nitrophoska blue® (N: P: K = 12: 5.2: 14) and Superphosphate® (8.8%) at 120 kgs and 500 kgs.$^{-1}$ha respectively. Plastic mulch was used to cover beds to reduce soil surface evaporation and for weed control.

Rockmelon seedlings cv. "Eldorado" were purchased from Leppington Speedy Seedlings & Supplies Pty. Ltd., Leppington, NSW, Australia and were transplanted at a density of 0.8 m x 1.8 m. Each side of each plant was supplied with drip irrigation with emitter spacing of 30 cm on each side and with an output of 2.5 L.h^{-1}. Fertilizer was applied at regular intervals after transplanting to maintain adequate fertility in the sandy soil. After 10 days of growth, the plants were supplied with 30 kgs.$^{-1}$ha of Nitram® (34% N), and 30 kgs.$^{-1}$ha Mono ammonium phosphate® (N: P: K = 12: 61: 0). The mixture of Nitram® (34%N), Muriate of potassium® (50% K), and Nitrophoska blue® at the rates 40 kgs, 40 kgs and 75 kgs.$^{-1}$ha respectively were again added when the plants reached the age of one month. A final side dressing of Muriate of potassium® (50% K) at 45 kgs and Nitrophoska blue® at 90 kgs.0.1^{-1}ha was applied when plants were one and a half months old.

Preharvest treatment

Bion® or BTH (Syngenta Crop Protection Pty Limited, Australia) and ReZist® (Stoller, USA) containing salicylic acid (0.84%) were selected as SAR activators in this study, based on earliet glasshouse screening. The two chemicals were dispersed separately in distilled water to a concentration of 50 ppm (a.i.) for BTH and 42 ppm (a.i.) for ReZist® (according to the manufacturer's recommendation). The plants were sprayed until the beginning of run-off using a Plassay pressure sprayer with capacity of 6 litres (obtained from Arthur Yates & Co. Limited, NSW, Australia) at a flow rate of 380 mL.min^{-1}.

Treatments were applied to all plants at male flowering (28 days after transplanting) and then 2 weeks and 5 weeks after male flowering (42 and 63 days after transplanting, respectively). Buffer beds and spaces were designed to prevent contamination between treatments.

Fifteen days from transplanting, downy mildew and thrips were observed on the plants. Talstar 100EC® (bifenthrin 100 g.L^{-1}) and Mancozeb 800® were sprayed to protect plants against the pests and diseases. A week later, Fongarid 250WP® (Furalaxyl 250 g.kg^{-1}) and Terraclor® (Quintozene 750 g.kg^{-1}) were also applied to suppress the soil-borne fungus (*Fusarium* spp.) that caused stem-end rot.

Flowering occurred during the fourth week after transplanting and two weeks later fruit set was observed. Pollination was by natural vectors.

Leaf and fruit samples for chitinase assay

The third fully expanded leaves on the branches of 10 randomly selected plants from each block were collected and pooled at 0 (3 hours), 3, 7, 14, 21, 28, 35, and 42 days after the first application of treatment. At harvest, two fruit from each block were harvested, totalling eight fruit per treatment. A 4 cm^2 section of exocarp plus mesocarp (fleshy portion of the fruit) was extracted from halfway between the soil contact point and top of the fruit (equator) from each fruit, and the samples from each treatment pooled together for enzyme analysis. The endocarp and seeds were discarded. Leaf and fruit samples were placed in labelled foil packages and immediately placed into liquid nitrogen and transported to the University of Sydney for storing at -80^oC until used for enzyme assays.

Fruit harvest and postharvest treatment

At harvest (11 weeks after transplanting), 12 fruit at half-slip maturity from each block of each treatment were harvested and randomly divided into two groups for postharvest treatment. Six fruit from each block and field treatment were dipped in water for 1 minute or in the postharvest fungicide Panoctine® (guazatine, Nufarm Australia Limited, Victoria, Australia) at 500 ppm (a.i.). After dipping, the fruit were air-dried at room temperature for 1 h before placing into commercial packing boxes, lined with plastic bags with 12 x 1 cm diameter holes on each bag. The boxes were closed and placed in a cool room at 15 ± 1ºC and 75% humidity. After 14 days, disease severity was scored based on the scale of Huang et *al*. (2000):

1 = no symptom
2 = one lesion less than 1 cm in diameter.
3 = one lesion between 1 - 3 cm, or two lesions each with an area less than 2 cm
4 = one lesion larger than 3 cm but smaller than 5 cm, or two lesions each larger than 2 cm but smaller than 3 cm.
5 = one lesion > 5 cm, or more than 3 lesions.

Sugar content and fruit flesh colour

For analysis of sugar content and fruit flesh colour, two 4 cm^2 sections of exocarp plus mesocarp were collected from the equator of the same fruit that was used for enzyme samples. The mesocarp of each sample was squeezed to extract the juice and sucrose concentration (^oBrix) of the juice was determined with a refractometer (Martin et *al*., 1997). Fruit flesh colour was determined subjectively on a scale of 1 (greenest flesh) to 5 (most orange flesh) with the aid of photographic standards.

Leaf area index, plant fresh weight, and plant dried weight

At the first treatment application, one plant from each block of each treatment was randomly selected and labelled for collecting leaf area, fresh and dry weight data. Leaf area was first measured at the first treatment application and then at two-week intervals thereafter. Three to five leaves, representing the size of all leaves of the whole nominated plant, were collected and measured for area using a Leaf Area Meter (Delta-T-device, Ltd Burwell Cambridge, England). At each measurement time, the number of leaves of each size was counted on one branch and the leaf area calculated for that branch. The total plant leaf area was then estimated by multiplying branch leaf area by the number of branches. At fruit harvest, the sample plants were

cut just above the soil line and the areas of all leaves on the plant were measured using the leaf area meter. Total plant fresh and dry weights, excluding fruit, were recorded for each sample plant.

Fruit yield
All fruit in each treatment within blocks were weighed and the average yield per plant was calculated based on the total number of surviving plants on each treatment of each block.

Chitinase assay
Chitinase activity was assayed in leaf and fruit tissue following the method of Dann and Deverall (2000) with some modification. Leaf samples (0.3 g FW) or fruit samples (1 g FW) divided into separate parts of exocarp (rind: 3-4 mm depth) and mesocarp (flesh: 3-4 mm depth proximity below exocarp) were ground in a pestle and mortar with liquid nitrogen and approximately 1% w/w PVPP. The frozen powdered tissue was placed in a 2 mL centrifuge tube containing 1 mL potassium acetate buffer (50 mM pH 5.0), EDTA (1 mM) and reduced glutathione (5 mM) that was added to the buffer on the day of conducting the assay. The tube was gently inverted 3 times and centrifuged at 9,000 g for 10 min. The supernatant was used as a crude extract for the assay of chitinase activity.

Chitinase activity in the crude extracts was measured colourimetrically using carboxyl-methyl chitin linked with the dye Remazol Brilliant Violet 5R (CM-Chitin-RBV). Potassium acetate buffer (0.1 mL, 0.1 M, pH 5.0) and 0.2 mL of a crude extract were added to a microcentrifuge tube and allowed to equilibrate to 37^{o}C for ten min. The reaction was initiated by the addition of 0.1 mL aqueous CM-Chitin-RBV (2 mg.mL^{-1} solution), which was obtained from Biosys Australia, Midland, WA, Australia. The reaction was stopped by addition of 0.1 mL 2N HCl, and resulted in the precipitation of the undegraded substrate. Reaction tubes were cooled on ice for 10 min and then centrifuged for 5 min at 9000 g. Absorbance of the supernatant was recorded at 545 nm against a blank prepared similarly but adding 0.2 mL of distilled water, instead of 0.2 mL of the crude extract. An exponential equation derived from the chitinase standard curve (Appendix 1) was used for calculation of chitinase activity from absorbance values of the samples. The specific activity of chitinase was expressed as mU.mg $^{-1}$protein. All samples were assayed in duplicate.

Protein determination
Protein content of the crude extract was determined with the Bio-Rad protein assay reagent following instructions of the manufacturer (Bio-Rad Laboratories, USA) with some modification. Bovine serum albumin (BSA) was used as a standard.
Diluted reagent was prepared by filtering the mixing solution of 4 parts of distilled water and 1 part of a dye reagent concentrate (Bio-Rad® , NSW, Australia) through a Whatman # 1 filter. Four dilutions of 100, 200, 350, and 500 µg.mL^{-1} of BSA were used as protein standard solutions.
Ten microliters of each diluted crude sample extract were pipetted into a test tube containing 200 µL of the diluted reagent and vortexed for 10 seconds. After incubation at room temperature for 5 min, the absorbance of the assay solution was measured at 600 nm against a blank, which was prepared similarly but distilled water

was added instead of sample extract. The protein standard curve was used to determine the protein concentration of the samples.
All leaf extracts were diluted ten times while fruit extracts were diluted two times prior to use for protein assay. All protein assays of samples were conducted in duplicate.

Experimental design and statistical analysis
The field experiment was a randomised complete block design with 4 replicates and 3 treatments. GenStat (6th Edition) (Rothamsted Experimental Station) and Minitab release 13.31 (Minitab Inc., USA) were used to analyse the data. Chitinase activity as well as leaf area data were analysed by using REML in GenStat with the repeated measurement in time. The distribution of the discrete score data from postharvest disease assessment and fruit colour data were modelled using Ordinal Logistic Regression procedure in Minitab to provide the probability of receiving a particular score for disease incidence or fruit colour.
The data for plant fresh and dried weights, sugar content, fruit yield, fruit chitinase, and leaf disease assessment were analysed using one-way ANOVA in GenStat.

Fruit and leaf chitinase activity data, % leaf disease, and leaf area were log-transformed to normalize data before running analyses. Log-transformed data were put in graphs and the graphs were transformed to represent back-transformed values using SigmaPlot® version 8.0 purchased from RockWare Inc., USA. Statistical significance was interpreted at the level of $p \leq 0.05$.

Results

Induction of chitinase activity on plants treated with commercial SAR activators

The SAR plant activator BTH significantly amplified chitinase activity under field conditions, approximately 7 days after application, compared with those plants treated with distilled water ($p < 0.001$) (Figure 4). The same pattern of chitinase activity was observed after plants were treated a second time with BTH on day 14. However after a 3rd treatment, 21 days later, chitinase activity failed to increase significantly within 7 days. Harvest occurred 14 days after the last application of BTH.

A second SAR activator ReZist® also stimulated a significant increase in chitinase activity ($p < 0.001$) immediately after the first application, compared to the control plants (Figure 4). However, unlike BTH, ReZist® did not stimulate a second peak of chitinase activity after a second application 14 days later. From day 14 onwards, chitinase activity in the ReZist® treated plants was very similar to activity in the control plants. In addition, from day 7 to day 35 the chitinase activity of the BTH treated plants was significantly higher than either the control or ReZist® treated plants.

Fruit chitinase activity

Fruit chitinase activity was not significantly different between treatments of BTH, ReZist®, or distilled water ($p = 0.252$) (Table 1). However chitinase activity in the rind was significantly greater than the flesh ($p < 0.001$).

Table 1: Chitinase activity of rind and flesh of fruit harvested from plants treated with ReZist® (42 ppm) and BTH (Bion®) (50 ppm) or distilled water (0ppm), applied at male flowering, 2 weeks after male flowering, and 5 weeks after male flowering. Data are the transformed means of 4 replicates of pooled 2 fruit from each treatment. The back-transformed means are presented in the brackets (x)

Treatment	Chitinase activity [mU mg protein^{-1}]	
	Rind	Flesh
Control [0 ppm]	4.48a ± 0.37 (88.23)	1.16b ± 0.37 (3.19)
BTH [50 ppm]	4.86a ± 0.37 (86.49)	1.35b ± 0.37 (3.86)
ReZist® [42 ppm]	4.02a ± 0.37 (55.70)	1.11b ± 0.37 (3.03)

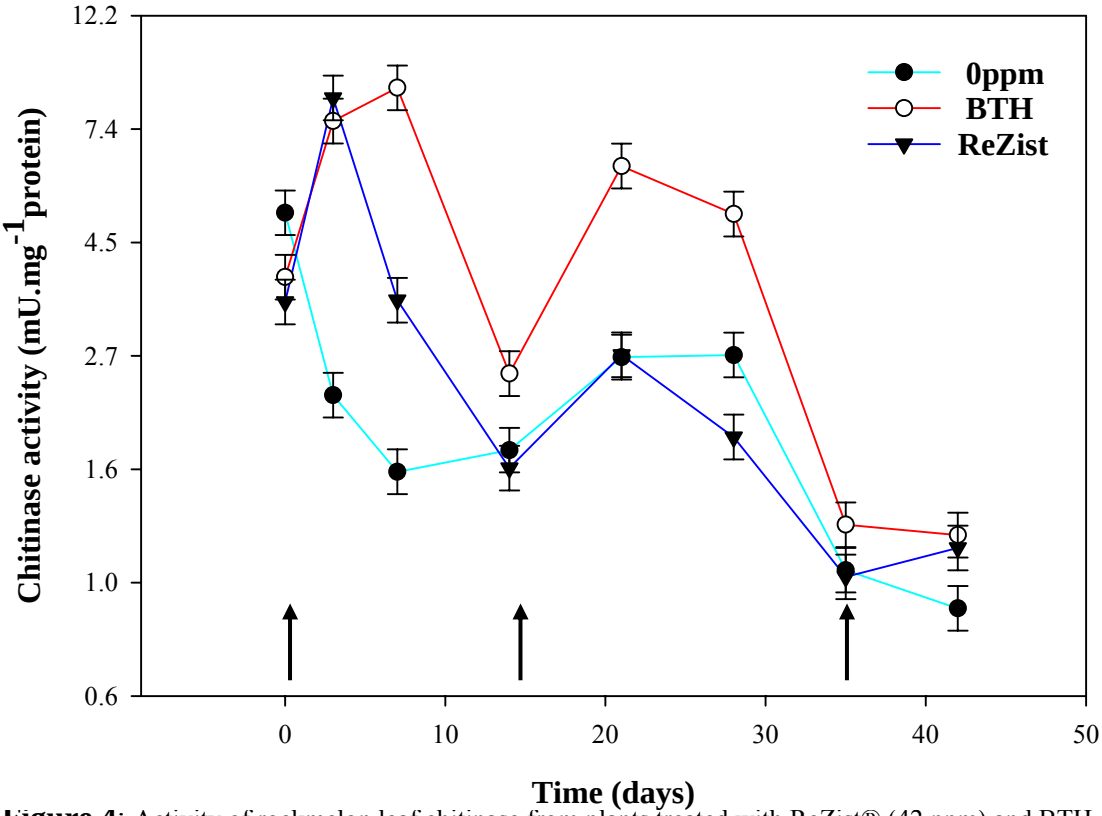


Figure 4: Activity of rockmelon leaf chitinase from plants treated with ReZist® (42 ppm) and BTH (Bion®) (50 ppm) or distilled water (0ppm), applied at male flowering, 2 weeks after male flowering, and 5 weeks after male flowering. Data are the transformed means of 4 replicates of pooled third fully expanded leaves from 10 plants. The values of chitinase activity in the Y axis are back-transformed to the original. Bars indicate standard error of the mean (vertical arrows show spraying days).

SAR protected rockmelon fruit against postharvest disease

During postharvest storage, *Alternaria alternata*, *Fusarium equiseti*, and *Colletotrichum* spp. were identified as pathogens infecting the harvested rockmelon fruit.

Overall, fruit harvested from BTH-treated plants had significantly less postharvest disease incidence ($p < 0.001$) compared to the fruit harvested from control plants (Figure 5). The additional treatment of a postharvest guazatine dip to the BTH treated fruit, had an additive effect of further reducing postharvest disease in comparison to the non-dipped treatment ($p = 0.039$) (Figure 5). Unlike the BTH treated plants, the ReZist® treated plants did not provide significantly better overall disease protection in the fruit compared with the control ($p = 0.325$) (Figure 5). However further analysis on the effects of Rezist® against individual pathogen showed some positive control (see below).

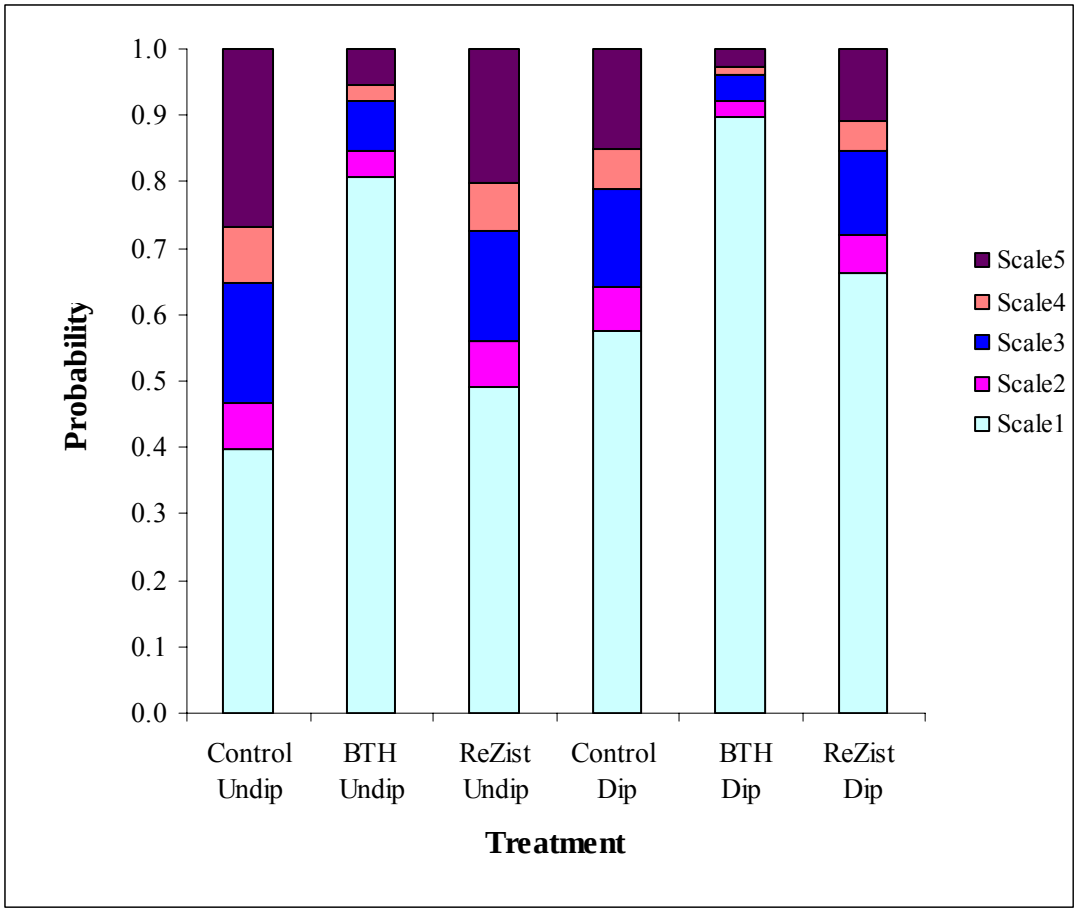


Figure 5: Probability of overall disease incidence on rockmelon fruit harvested from plants sprayed with distilled water (control), BTH (50 ppm) or ReZist (42 ppm) at male flowering, 2 and 5 weeks after male flowering and either dipped in guazatine (500 ppm) or water, then stored at 15°C. Fruit were assessed 14 days after storage using a disease rating scale 1 = no symptom, 2 = one lesion less than 1 cm in diameter, 3 = one lesion between 1 – 3 cm, or two lesions each with an area less than 2 cm, 4 = one lesion larger than 3 cm but smaller than 5 cm, or two lesions each larger than 2 cm but smaller than 3 cm, 5 = one lesion > 5 cm, or more than 3 lesions

The effects of preharvest applications of BTH, ReZist® and distilled water as well as postharvest treatment with guazatine or water on growth of each pathogen on the harvested fruit were also conducted. Fruit from plants treated with BTH and ReZist® had significantly less Alternaria rot caused by *Alternaria alternata* than those from plants treated with distilled water as control ($p = 0.020$; $p = 0.039$ respectively) (Figure 6). There was no significant difference in *Alternaria alternata* infection on fruit from plants treated with either BTH or ReZist® ($p = 0.629$). However, in both the BTH and ReZist® treated fruit, addition of the guazatine dip did not statistically reduce the number of *Alternaria alternata* infections from those fruit dipped in water ($p= 0.148$).

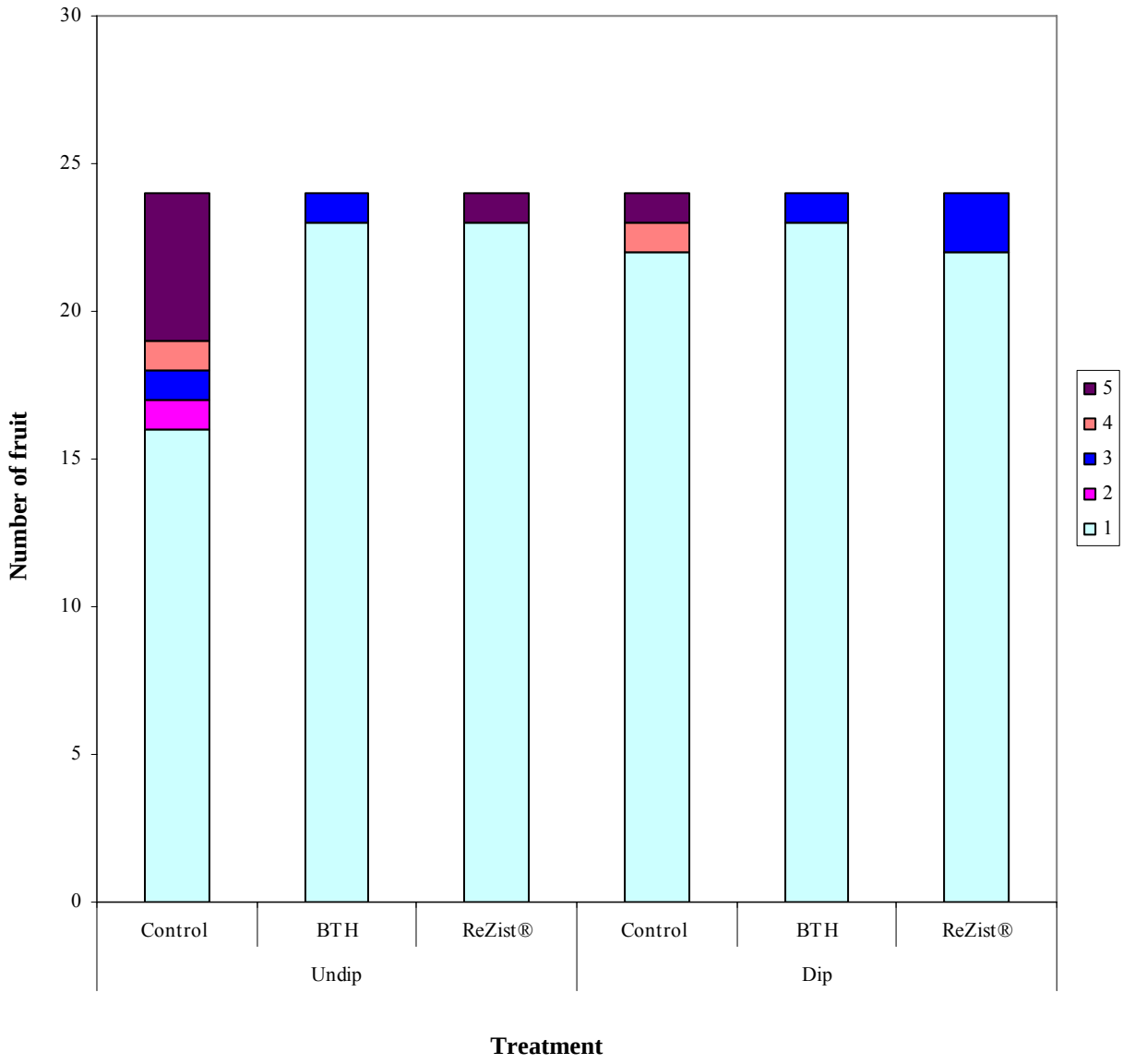


Figure 6: Incidence of *Alternaria alternata* infection on rockmelon fruit harvested from plants sprayed with distilled water (control), BTH (50 ppm) or ReZist (42 ppm) at male flowering, 2 and 5 weeks after male flowering and either dipped in guazatine (500 ppm) or water then stored at 15°C. Data are the count of number of infected fruit 14 days after storage at each scale from 1 to 5 as follows: 1 = no symptom, 2 = one lesion less than 1 cm in diameter, 3 = one lesion between 1 - 3 cm, or two lesions each with an area less than 2 cm, 4 = one lesion larger than 3 cm but smaller than 5 cm, or two lesions each larger than 2 cm but smaller than 3 cm, 5 = one lesion > 5cm, or more than 3 lesions

Fruit from the BTH treated plants had significantly less Fusarium rot caused by *Fusarium equiseti* ($p = 0.001$) and the number of lesions was not significantly further reduced by addition of the postharvest dip of guazatine (Figure 7). However, the postharvest guazatine dip significantly reduced Fusarium rot infections on fruit either harvested from ReZist® treated and controlled plants ($p = 0.040$). Fruit from the

ReZist® treated plants did not show significant postharvest resistance to *F. equiseti* in comparison to control plants (p = 0.730) (Figure 7).

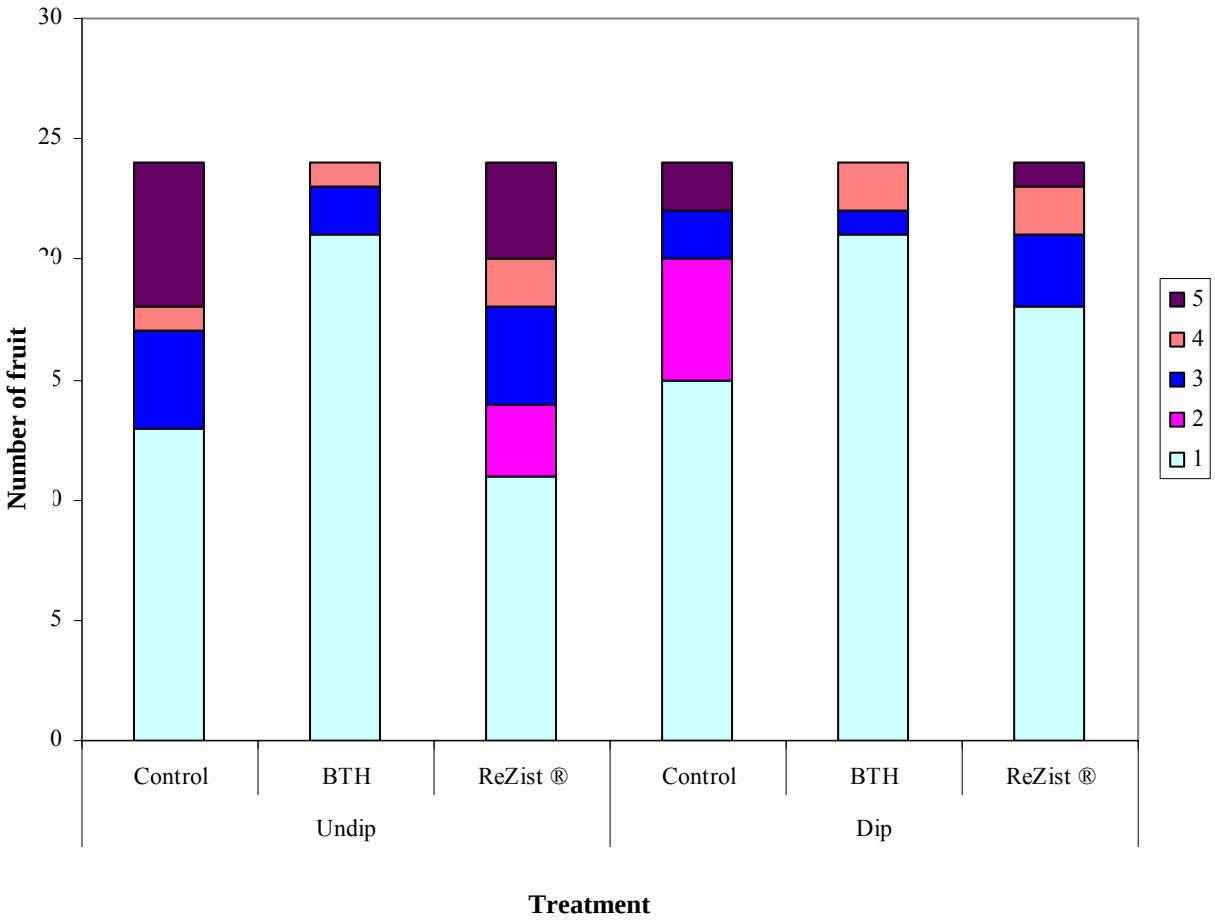


Figure 7: Infection of *Fusarium equiseti* on rockmelon fruit from plants treated with distilled water (control), BTH (50 ppm), or ReZist® (42 ppm) at male flowering, 2 and 5 weeks after male flowering and either dip in guazatine (500 ppm) or water and then stored at 15 °C. Data present the count of number infected fruit after 14 days storage at each scale from 1 to 5 as follows: 1 = no symptom, 2 = one lesion less than 1 cm in diameter, 3 = one lesion between 1 – 3 cm, or two lesions each with an area less than 2 cm, 4 = one lesion larger than 3 cm but smaller than 5 cm, or two lesions each larger than 2 cm but smaller than 3 cm, 5 = one lesion > 5 cm, or more than 3 lesions

No *Colletotrichum* spp. was found in fruit from SAR activator treated plants, however, this pathogen was found to cause rot on fruit from control plants (Figure 8). Guazatine did not significantly reduce the incidence of *Colletotrichum* spp. infection between the dipped and undipped control fruit (p = 0.282).

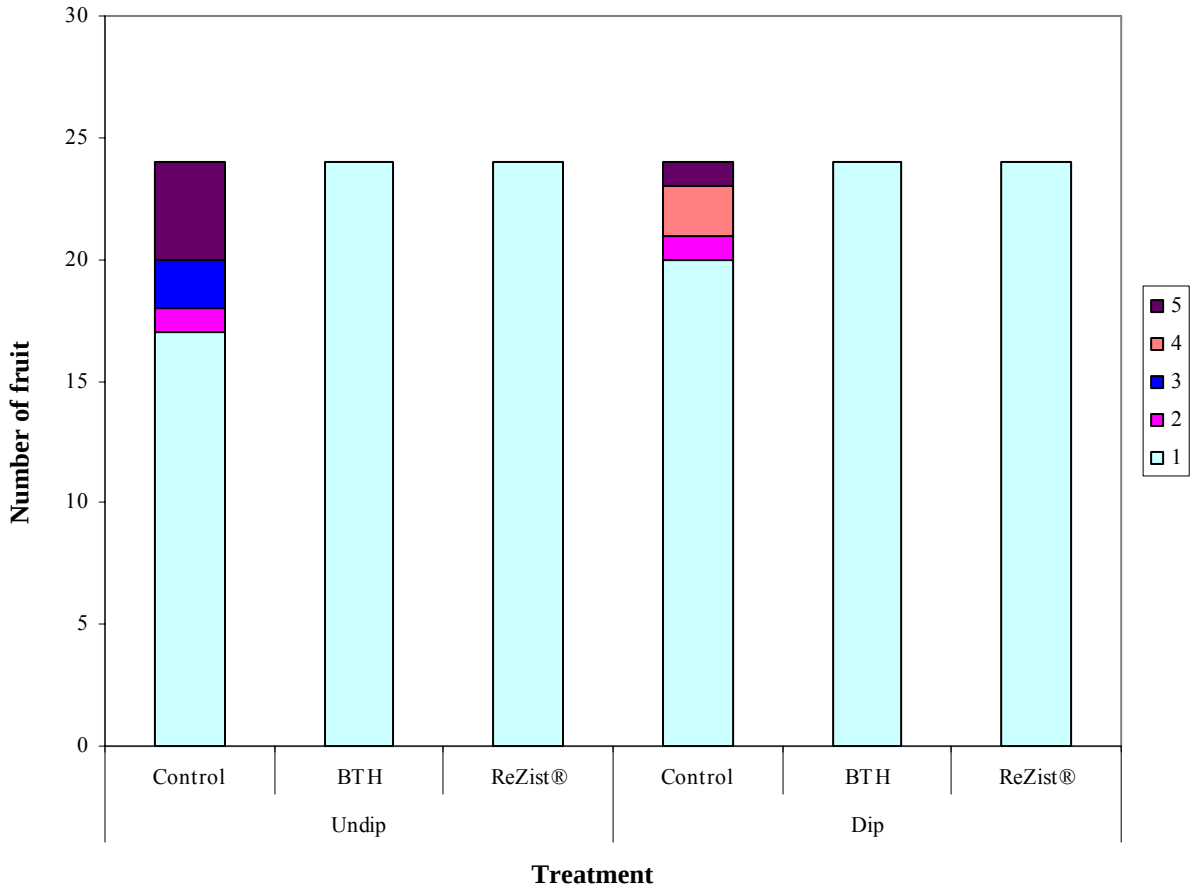


Figure 8: Infection of *Colletotrichum* spp. on rockmelon fruit from plants treated with distilled water (control), BTH (50 ppm), or ReZist® (42 ppm) at male flowering, 2 and 5 weeks after male flowering and either dip in quazatine (500 ppm) or water and then stored at 15°C. Data show the count of number infected fruit after 14 days storage at each scale from 1 to 5 as follows: 1 = no symptom, 2 = one lesion less than 1 cm in diameter, 3 = one lesion between 1 – 3 cm, or two lesions each with an area less than 2 cm, 4 = one lesion larger than 3 cm but smaller than 5 cm, or two lesions each larger than 2 cm but smaller than 3 cm, 5 = one lesion > 5 cm, or more than 3 lesions

Effects of SAR activators on growth of rockmelon

Treatment of plants with BTH and ReZist® did not significantly affect leaf area compared with the control treatment ($p = 0.098$). Furthermore, plant fresh weight as well as plant dried weight from all treatments was not significantly different ($p = 0.195$ and $p = 0.495$ respectively).

Effects of SAR activators on fruit yield of rockmelon

There was no significant difference between fruit yield from rockmelon plants treated with BTH, ReZist®, or distilled water ($p = 0.419$).

Effects of SAR activators on rockmelon fruit quality

Treatment of plants with BTH, ReZist® or distilled water did not significantly affect sugar content in fruit ($p = 0.735$). In addition, fruit colour from plants treated with BTH was not significantly different from fruit colour in those sprayed with distilled

water (p = 0.317). A similar result was found on fruit from plants treated with ReZist® (p = 0.732).

Discussion

Chitinase activity as a marker of SAR in rockmelons

The results in this study show that treatment of rockmelon plants cv. "Eldorado" with BTH increased postharvest protection of rockmelon plants and fruit from disease (Figures 5-8). Both salicylic acid (Kessmann et *al*., 1994; Rasmussen et *al*., 1995) and BTH (Friedrich et *al*., 1996; Lawton et *al*., 1996) have been shown to activate systemic acquired resistance in many plant species. For example, enhanced activity of chitinase by BTH or salicylic acid application was found in pea (Dann and Devarall, 2000), tomato (Inbar et *al*., 1998), and rose (Suo and Leung, 2001). The significant reduction in disease in BTH-treated plants and fruit in our experiments suggest that BTH and ReZist to a lesser extent successfully induced SAR in the rockmelon plants and fruit. The induction of SAR in BTH treated plants is supported by the increased activity of the PR protein chitinase in the leaves. The pattern of increased chitinase activity within 7 days of each BTH application and the subsequent decline in activity indicates that the level of chitinase activity was directly linked to BTH application. In this study, enhanced chitinase activity has therefore been shown a useful marker for induction of systemic acquired resistance in rockmelons.

Association of chitinase activity with SAR in Cucurbitaceae has been reported by Métraux et *al*. (1988), Roby et *al*. (1988), Meuwly et *al*. (1994) and Smith-Becker et *al*. (2003). In cucumber, chitinase activity was induced one day after powdery mildew infection and peaked at 7 days (Zhang and Punja, 1994). Suo and Leung (2001) investigated the time-course of chitinase activity in rose leaves and found that chitinase activity started to increase from day 1 after BTH application and leveled off at around day 6. Smith-Becker et *al*. (2003) found that leaf chitinase activity increased significantly over 4 days after cantaloupe seedlings were treated with BTH at 100 ppm. Furthermore, these authors showed that chitinase activity was significantly higher in field grown BTH treated leaves 14 days after application. These results support our findings that in rockmelon, chitinase is an inducible PR protein and pattern of activity in response to BTH application is similar.

Timing of activator application

The duration of defence induction as well as its effectiveness against disease over time is an important consideration for the application of SAR in crop protection. Guedes et *al*. (1980) challenged cucumber with *Colletotrichum lagenarium* to investigate the induction of systemic acquired resistance and showed that the best time for inducing systemic acquired resistance in plants is a week before flowering. The authors hypothesised that in cucumber, the signal for resistance may be not be produced, or that plant cells may not respond to the activated signal during fruit set, resulting in little or no induction of SAR at the time of flowering. They further state that the plant's hormonal balance is altered during flowering and fruit set, thus lowering the level of resistance response in activator treated plants (Guedes et *al*., 1980). Therefore, in our studies, we investigated the effect of SAR activator application before, during and after flowering in rockmelon plants. Contrary to the reports of Guedes et *al*. (1980) our studies show that SAR can be induced before, at or after flowering (Figure 4). Based on the pattern of chitinase activity in previous

glasshouse experiments (data not shown), SAR induction is independent of the flowering stage of the plant. Chitinase activity was similar whether BTH was applied before male flowering, at flowering or after flowering.

Effect of SAR on harvested fruit

The most striking result of the study is that preharvest application of SAR activators can be effective in controlling postharvest diseases of rockmelon fruit. BTH treated plants provided significant postharvest control of *Alternaria alternata*, *Fusarium equiseti*, and *Colletotrichum* spp. on the rockmelon fruit (Figures 5-8). This is an important finding since these diseases pose significant problems for the postharvest handling of rockmelons.

The effects of BTH and salicylic acid on controlling field plant disease have been intensively studied, however much less work has been done on the effects of SAR activators on postharvest disease. The postharvest disease protection of fruit harvested from SAR induced plants has only been reported for strawberries (Terry and Joyce, 2000) and rockmelons (Huang et *al*., 2000). In both experiments, preharvest applications of BTH to the plant provided significant control of *Botrytis cinerea* on harvested strawberry fruit, and *Alternaria* spp. as well as *Fusarium* spp. on rockmelon fruit. However, Huang et *al*. (2000) did not attempt to link the increased postharvest disease protection with an SAR marker. In our field experiment it was clear that SAR was induced preharvest and that the fruit harvested from these plants had significantly less postharvest disease than control fruit.

The postharvest dip of guazatine at 500 ppm (a.i.) was effective in controlling Fusarium rot but not Alternaria and Colletotrichum rots in rockmelon fruit. Similar results were reported by Huang et *al*. (2000) who found that melon fruit dipped in guazatine at 500 ppm controlled Fusarium rot effectively but had lesser effect on *Alternaria* spp. Interestingly, at a concentration of 1000 ppm (a.i.) guazatine inhibited *Alternaria* spp. infection on rockmelon (Wade and Morris, 1982).

In this study, guazatine provided added disease protection to the fruit from BTH treated plants. This result supports that of Molina et *al*. (1998) who also noted the synergistic effect of the combining BTH and fungicides on *Arabidopsis*. The integration of enhanced host resistance via SAR into a reduced spray-program should reduce the amount of fungicide required for disease control (Shtienberg et *al*., 1994).

Rezist®

ReZist®, containing salicylic acid, was not as effective as an activator of SAR in rockmelon despite SA having a significant role in systemic acquired resistance and disease defence. Application of ReZist® increased chitinase levels but not to the same extent as BTH did. The difference in chitinase activity patterns after application of BTH and ReZist® may be due to induced attributes. Narusaka et *al*. (1999) investigated both SA and BTH on cucumber and reported that SA induced resistance only locally whereas BTH induced resistance both locally and systemically. Further preliminary experiments to determine rates and timing of application were conducted after this experiment to determine whether the correct rates were being applied.

Effect of SAR activators on rockmelon growth and quality

Despite the benefits in disease resistance from SAR, there has been much concern about phytotoxic effects of the activators on plants (Heil, 2001; Moore et *al*., 2003). However, results from this study indicate that application of either BTH (50 ppm a.i.)

or ReZist® (42 ppm a.i.) on rockmelon plants did not adversely affect flesh fruit colour, sugar content, fruit yield, plant fresh weight, plant dried weight, or leaf area. Similar results were also found by Terry and Joyce (2000) that application of BTH at 250-2000 ppm (a.i.) showed no apparent phytotoxic effects on strawberry plants and fruit.

Conclusion

Preharvest application of BTH on rockmelon plants induces resistance of rockmelons against preharvest disease on the plants as well as postharvest disease on the fruit. Timing of activator application does not appear to be critical however requires further verification. Also in this study, chitinase was confirmed as a marker for study of systemic acquired resistance in rockmelons. Adverse effects of the elicitors on the rockmelon plants were observed for flesh fruit colour, sugar content, fruit yield, plant fresh weight, plant dried weight, or leaf area.

ReZist® also induced resistance in rockmelon plants and protected them against postharvest disease but in this experiment to a lesser extent than BTH. However, more work was conducted the following year to elucidate the effectiveness of this activator in controlling disease in rockmelon especially on the fruit.

B. MILDURA FIELD TRIAL

Investigation of SilikaMajic® (Silica), Bion® (BTH) and ReZist® as an SAR activators to protect rockmelons against field and postharvest disease.

Materials and methods

Mr Rob Wheatley of Thurla Farms, Mildura kindly granted permission to use a plot of his farm to examine the use of these preharvest inducers. The rockmelon variety grown was Dubloon (Syngenta).

Preharvest Treatments

Table 2 lists the preharvest treatments applied to plants. Given the ambiguous results from year 1 experiments, we used three concentrations of ReZist®, 11ppm, 44ppm and 88ppm in combination with Sett (1%) (Stoller). A control of Sett (1%) was also applied weekly. Foliar sprays were applied weekly after the onset of female flowering (approximately 5 weeks before harvest). SilikaMajic (Flairform, WA) was applied at two concentrations; 500ppm and 1000ppm. A weekly spray regime was also followed. BTH was supplied from Syngenta for experimental purposes only. In past experiments single sprays of BTH at 50ppm were effective. Therefore, we had four different spraying regimes; BTH one spray five weeks before harvest, BTH one spray three weeks before harvest, BTH one spray one week before harvest and two sprays of BTH at five and three weeks before harvest of the fruits.

Table 2. Treatments and spraying times applied to melon vines after flowering

Treatment	Spraying Time
ReZist® 11ppm + Sett 1%	Weekly
ReZist® 44ppm + Sett 1%	Weekly
ReZist® 88ppm + Sett 1%	Weekly
Sett 1%	Weekly
SilikaMajic 500ppm	Weekly
SilikaMajic 1000ppm	Weekly
BTH 50ppm	Twice, 5 and 3weeks before harvest
BTH 50ppm	Once, 5 weeks before harvest
BTH 50ppm	Once, 3 weeks before harvest
BTH 50ppm	Once, 1 week before harvest
Water	Weekly
Water	Twice, 5 and 3 weeks before harvest
Water	Once, 1 week before harvest

The experiment was randomised complete block design consisting of three blocks, with 20m plots for each treatment, with approximately 40 plants in each plot.

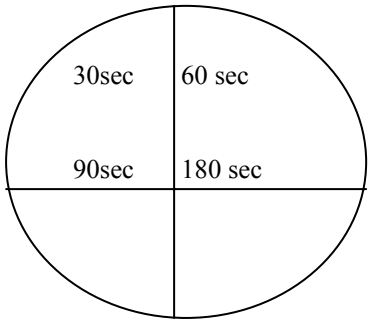
Disease Assessment
A general disease survey was conducted on the supposition that diseases in Table 3 could affect the melon crop during the growing season and postharvest.

Table 3: Possible foliar diseases of melons in the field

Disease Likely in Field	Plant Part to be Assayed	Agar Type (for isolation and identification)
Fusarium wilt	Soil Stem Roots Flowers	Soil dilution on selective Fusarium agar (SFA)
Alternaria Spot	Leaves	PDA
Powdery Mildew	Leaves	Visualization
Downy Mildew	Leaves	
Colletotricum (Anthracnose)	Leaves	PDA
Angular Leaf Spot (*Pseudomonas syringae*)	Leaves	Water Agar
Bacterial Spot (*Xanthomonas campestris*)	Leaves	Water Agar
Gummy Stem Blight (*Didmella bryonia*)	Leaves	Water Agar

Note: PDA is half strength PDA

Pathogen isolation from plant tissue
The infection site was sampled, ensuring that both living and dead material was obtained. The tissue was surface sterilized by immersing in a solution of 1% sodium hypochlorite (bleach) in 10% ethanol and then rinsing in water. A time trial of sterilizing was conducted desirable to ensure survival of fungal material.
i.e.



Pathogen isolation from soil
Fungi were isolated directly from soil using the dilution plate technique, by plating onto selective media. A uniform dispersion of 1 mL of soil suspension (between 1:50 and 1:2000 dilution in 0.05% water agar) was made across a selective medium such as MPDA (half strength PDA supplemented with antibiotics: streptomycin sulfate, 0.16g; dichloran [Allisan], 0.013g; neomycin sulfate, 0.06g)

Leaf sampling for pathogen assessment
Five leaves per plot across 3 blocks were harvested for each treatment. The leaves were graded into the 5 disease severity categories as illustrated in Table 4:

Table 4. Score for disease incidence

Intensity	**Grade**
Without Spots	1
From 1 to 2 spots	2
From 2 to 25 spots (1-5% of diseased leaf area)	3
From 5-25 % of diseased leaf area	4
>25% of leaf area diseased	5

Digital photos were taken of each plot every second week to assist in the identification and show an overview of disease severity and incidence. Plant tissue was sampled every second week and plated onto selective media while the soil samples were taken during the week of the melon harvest.

Plant and Fruit Physiology
Total leaf area, dry weight, yield
Leaf area was measured at the time of the first treatment application and then every two weeks thereafter. The third leaf of three vines were traced onto paper and measured using a leaf area meter. At the end of harvest, one plant from each plot was excised at the base, oven dried and the dry weight was recorded.
The number of harvested fruit in each treatment plot were counted and expressed as a percentage of the total number of melons including those not ready for harvest. In addition, the number of melons wasted in the field were also counted. All harvested melons were weighed to determine the yield of the melons.
Sugar Content
For analysis of sugar content and fruit flesh colour, two 4 cm^2 sections of exocarp plus mesocarp were collected from the equator of fruit on the day of harvest. The mesocarp of each sample was twisted to extract the juice and sucrose concentration (^{o}Brix) of the juice was determined with a refractometer (Martin et *al*., 1997).

Marketability of the melon

Marketability was assessed on the quality attributes sought by the retail market. These attributes included size and rind colour, in particular less than 30% browning of the rind. The melons were scored with a simple accept/reject scale and the percentage of acceptable melons was presented.

Postharvest Storage

Approximately 30 full-slip, green melons were harvested over a week period (3 harvest times) and placed immediately into cool storage (5°C). No postharvest treatments or washes were applied. Melons were packed in cardboard boxes and transported by refrigerated trucks to the Sydney Postharvest Laboratory. With minimal delay, the melons were put into cold storage (5°C) with a relative humidity of 88%. The control melons were checked weekly after three weeks. Once the control melons were displaying extensive rots, the treated melons were analysed.

A 5 point scoring system was used to determine whether there were differences in the severity of rots between treatments. A score of 1 = melons with no signs of disease; a score of 2 = melons with minimal surface hyphae present; a score of 3 melons with moderate surface hyphal growth; a score of 4 melons with multiple rots of larger than 3cm but smaller than 5cm; a score of 5 = melon with total rotting of the melon.

Further analysis was conducted to determine extent of general rots and and individual diseases such as Fusarium rot, Alternaria rot, other moulds, and attributes such as yellowing, browning and rind shrinkage.

Total soluble sugars were also measured in the postharvest fruits (see previously) and rind firmness was measured with a penetrometer.

Assaying for PR protein induction after treatments

The third fully expanded leaf on the branches of three randomly selected plants from each block, were collected at: 0 hours (same day of spray), 3 days after spray and then weekly till harvest. The leaves were removed from the plant and frozen in liquid nitrogen and stored at -80°C until processed.

Two fruit from each plot were removed from the plant weekly, and the melon rind was retained for analysis. The rind was peeled from the fruit using a vegetable peeler and frozen in liquid nitrogen and stored at -80°C until processed. The leaves and fruit were transported back to USYD labs on a bed of dry ice in polystyrene boxes.

Chitinase Assay

Leaf samples (0.3 g FW) or fruit rind samples (0.3 g FW) were ground in a pestle and mortar with liquid nitrogen and approximately 1% w/w PVPP. The frozen powdered tissue was placed in a 2 mL centrifuge tube containing 1 mL potassium acetate buffer (50 mM pH 5.0), EDTA (1 mM) and reduced glutathione (5 mM) that was added to the buffer on the day of conducting the assay. The tube was gently inverted 3 times and centrifuged at 9,000 g for 10 min. The supernatant was used as a crude extract for the assay of chitinase activity.

Chitinase activity was assayed in leaf and fruit tissue following the method of Dann and Deverall (2000) with some modification. Chitinase activity in the crude extracts was measured colourimetrically using carboxyl-methyl chitin linked with the dye Remazol Brilliant Violet 5R (CM-Chitin-RBV). Potassium acetate buffer (0.2 mL, 0.1 M, pH 5.0) and 0.1 mL of a diluted extract (1:10) were added to a microcentrifuge tube and allowed to equilibrate to 37^{o}C for ten min. The reaction was initiated by the addition of 0.1 mL aqueous CM-Chitin-RBV (2 mg mL^{-1} solution) (Biosys Australia,

Midland, WA, Australia) at 37°C for ten minutes. The reaction was stopped by addition of 0.1 mL 2N HCl which results in the precipitation of the undegraded substrate. Reaction tubes were cooled on ice for 10 min and then centrifuged for 5 min at 9000 g. Absorbance of the supernatant was recorded at 545 nm against a blank prepared similarly but adding 0.1 mL of distilled water, instead of 0.1 mL of the crude extract. The specific activity of chitinase was expressed as mU mg $^{-1}$ protein. All samples were assayed in duplicate.

Peroxidase Activity

Only the leaf samples were processed for peroxidase activity. The fruit was not analysed because natural increases in peroxidase activity occur during rind formation. Leaf tissue (0.5g FW) was ground in a mortar and pestle in liquid nitrogen and resuspended in 1mL of 0.1M Sodium Acetate buffer (pH 5) containing 1M NaCl and 1mM EDTA. Samples were centrifuged for 5 min at 9000g and the supernatant was retained.

Five µL of supernatant was added to 1mL of reaction mixture (10 mM Guiacol, 10 mM hydrogen peroxide in 50 mM Sodium acetate buffer (pH 5)). Oxidation of guiacol to tetraguiacol was monitored spectrophotometrically at 470nm for 2 minutes, taking readings every 30 seconds. Peroxidase activity was expressed as nmol tetraguaicol produced per minute per mg protein.

Protein determination

Protein content of the crude extract was determined with the Bio-Rad protein assay reagent following instructions of the manufacturer (Bio-Rad Laboratories, USA) with some modification. Bovine serum albumin (BSA) was used as a standard.

Diluted reagent was prepared by filtering the mixing solution between 4 parts of distilled water and 1 part of a dye reagent concentrate (Bio-Rad® , NSW, Australia) through a Whatman # 1 filter. Four dilutions of 100, 200, 350, and 500 µg mL^{-1} of BSA were used as protein standard solutions. Ten microliters of each diluted crude sample extract was pipetted into a test tube containing 200 µL of the diluted reagent and vortexed for ten seconds. After incubation at room temperature for 5 min, the absorbance of the assay solution was measured at 600 nm against a blank, which was prepared similarly but adding distilled water instead of sample extract. The protein standard curve was used to determine the protein concentration of the samples.

Results and Discussion

Disease Assessment

There was very little disease present in the field. The major reason for this was the lack of rainfall during the Mildura melon season. On a few plants, Alternaria Black Spot was present. Due to the lack of pathogens present in the field, it was not possible to do a randomised disease check on five random leaves in each plot. Alternatively, every plant in the plot was scored and a percentage with disease was recorded. The results are summarised in Table 5. There were no differences in disease incidence between all treatments and the control. There was no block effect evident either.

Table 5. Percentage of plants indicating disease incidence treated preharvest with ReZist®, SilikaMajic® (Silica) or Bion® (BTH) treatments as described or control treatments with water or Sett®.

TREATMENT	% plants diseased BLOCK 1	% plants diseased BLOCK 2	% plants diseased BLOCK 3
ReZist® 11ppm weekly	20	20	10
ReZist® 44ppm weekly	15	10	20
ReZist® 88ppm weekly	25	20	15
Sett (1%) weekly	15	15	20
Water weekly	20	35	20
Silica 500ppm weekly	15	25	10
Silica 1000ppm weekly	30	15	30
Water 5,3 weeks before harvest	10	30	20
BTH 5,3 weeks before harvest	30	15	15
BTH 5 weeks before harvest	3	20	30
BTH 3 weeks before harvest	15	15	20
BTH 1 weeks before harvest	5	35	12
Water, 3 weeks before harvest	20	35	10

Soil sampling of all of the treatment sites isolated *Fusarium oxysporum* and *Alternaria alternata* however symptoms on the vine and unharvested melon fruit were rarely observed. This result was interesting because Rob Wheatley had expressed concern about his transplanted plants suffering from vascular wilts. The melon plants we tested were from direct seed.

Plant Physiology

There was no significant difference between treatments for leaf area in all of the treatments (data not shown). Phytotoxicity was observed when the melon vines were treated with 88ppm ReZist®. The dry weight of the vines was measured and a score from 1 to 5 was given, with 1 being highly vigorous vines and 5 being low vigour (Table 6).

Table 6. Vine vigour (Score 1-5-) and dry weight **(g)** of plants treated preharvest with ReZist®, SilikaMajic® (Silica) or Bion® (BTH) treatments as described or control treatments with water or Sett®.

TREATMENT	Vigour Score BLOCK 1	Vigour Score BLOCK 2	Vigour Score BLOCK 3	Average Dry Weight of Vine (g) for each treament
ReZist® 11ppm weekly	3	2	2	77.73
ReZist® 44ppm weekly	2	2	3	84.20
ReZist® 88ppm weekly	3	3	2	90.50
Sett (1%) weekly	2	2	3	95.10
Water weekly	2	4	3	94.97
Silica 500ppm weekly	2	3	2	56.70
Silica 1000ppm weekly	3	3	4	78.70
Water 5,3 weeks before harvest	2	3	3	129.83
BTH 5,3 weeks before harvest	3	3	3	98.13
BTH 5 weeks before harvest	1	3	4	151.23
BTH 3 weeks before harvest	2	2	3	66.70
BTH 1 weeks before harvest	3	3	3	112.07
Water, 3X	3	3	2	64.80

Fruit Physiology

Full slip green melons from all treatments were harvested, weighed, assessed for marketability and total soluble sugars were also analysed. There were no significant differences in the average weight of the melons in all treatments ranging from 1.3 to 1.7 kg (Table 7).

Table 7. Average weight (kg) of the harvested melons from plants treated preharvest with ReZist®, SilikaMajic® (Silica) or Bion® (BTH) treatments as described or control treatments with water or Sett®.

Treatment	**Average Melon Weight (kg)** ± SE
ReZist® 11ppm weekly	1.58 ± 0.48
ReZist® 44ppm weekly	1.62 ± 0.34
ReZist® 88ppm weekly	1.67 ± 0.46
Sett (1%) weekly	1.26 ± 0.37
Water weekly	1.45 ± 0.43
Silica 500ppm weekly	1.59 ± 0.47
Silica 1000ppm weekly	1.29 ± 0.42
Water 5,3 weeks before harvest	1.35 ± 0.4
BTH 5,3 weeks before harvest	1.34 ± 0.5
BTH 5 weeks before harvest	1.67 ± 0.45
BTH 3 weeks before harvest	1.40 ± 0.36
BTH 1 weeks before harvest	1.52 ± 0.35
Water, 3 weeks before harvest	1.30 ± 0.43

Marketability was assessed on the quality attributes sought by the retail market. These attributes included size and rind colour, in particular less than 30% browning of the rind. The melons were scored with a simple accept/reject scale and the percentage of acceptable melons is shown in Table 8. The treatments that yielded the lowest marketable percentage were vines treated with Sett (1%), BTH applied twice at 5 and 3 weeks before harvest and Silica (1000ppm), which were all less than 50%. The vines treated with ReZist® (11, 44 and 88ppm), BTH applied once 1, 3 and 5 weeks before harvest and vines sprayed weekly with water showed the highest percentage

marketability. There was no significant difference in Brixº levels across treatments, ranging from 10.8-16 (Table 8).

Table 8. The effect of chemical inducers on marketability and Brix° levels of fruit harvested from plants treated preharvest with ReZist®, SilikaMajic®(Silica) or Bion® (BTH) treatments as described or control treatments with water or Sett®.

Treatment	**% of Fruit Marketable**	**Brixº** ± SE
ReZist® 11ppm weekly	59.7	11.8 ± 0.7
ReZist® 44ppm weekly	68.6	12.2 ± 0.6
ReZist® 88ppm weekly	69	11.1 ± 1.3
Sett (1%) weekly	32.8	11.3 ± 1.2
Water weekly	52.8	12.6 ± 0.6
Silica 500ppm weekly	44.6	12.6 ± 0.5
Silica 1000ppm weekly	44.5	12.4 ± 1.0
Water 5,3 weeks before harvest	61.1	12.0 ± 0.6
BTH 5,3 weeks before harvest	67.9	10.8 ± 2.8
BTH 5 weeks before harvest	57.6	12.4 ± 1.1
BTH 3 weeks before harvest	60.8	12.4 ± 0.9
BTH 1 weeks before harvest	55.3	16 ± 0.5
Water, 3 weeks before harvest	51.2	11.7 ± 0.9

Postharvest Storage and Quality

Approximately 35-50 melons from each treatment were packed in cardboard boxes and transported back to Sydney Postharvest Laboratory (North Ryde, NSW) for storage at 5°C. The melons were scored for disease after 3 weeks as this was when control melons began to show symptoms of rots. By 5 weeks after harvest the control melons had begun decomposing due to extensive colonisation by Fusarium and Alternaria rots. The results are represented by the percentage probability of a melon scoring 1-5 (Figure 9) with each colour representing the five different scores. The statistics are based on the probability (%) of a melon falling into the score category of 1,2,3,4 or 5. In simpler terms, if a melon was given a score of between 1 and 3, the melon could still be sold to the market. Figure 9 illustrates the total moulds observed in fruit.

BTH applied once 3 weeks before harvest gave the best protection against postharvest diseases, where 65% of the melons scored 2 or below. When BTH was applied 5 weeks before harvest 54% of the melons were relatively unblemished and disease-free. Treatment with ReZist® 44ppm also showed excellent control of postharvest diseases. In general, all of the chemical inducers we used preharvest, gave significantly better control of postharvest diseases than the water only treatments. Over 80% of melons in all three water controls were severely diseased and not

suitable for sale. Sett (1%) also showed very little control of postharvest rots. Photographs of the melons are shown in Figure 10.

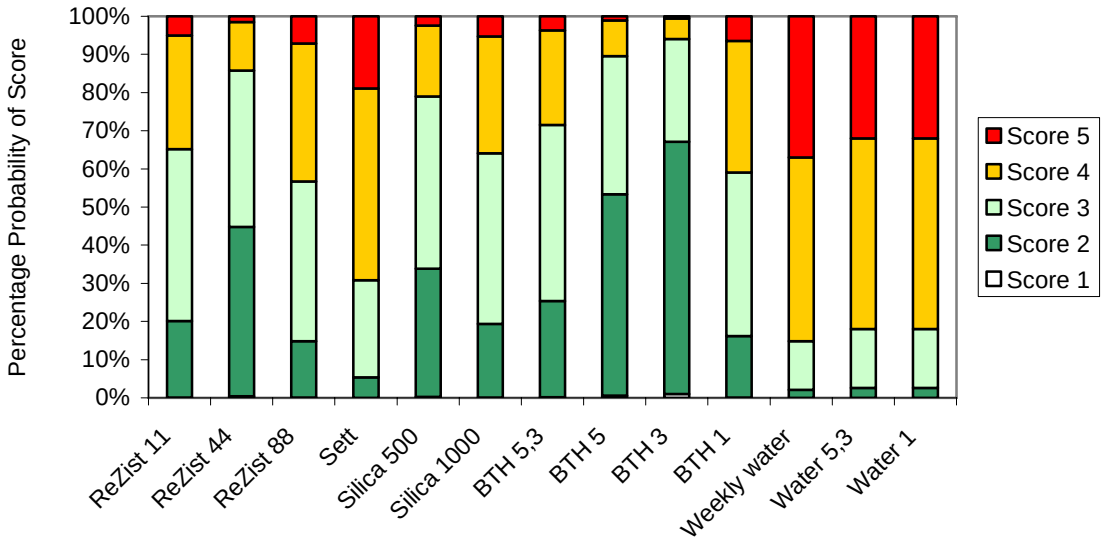


Figure 9. Disease severity of melon fruit harvested from plants treated preharvest with ReZist® at 11, 44 or 88 ppm weekly, SilikaMajic®(Silica) at 500 or 1000ppm weekly or Bion® (BTH) at 5 and 3, 3 or 1 week before harvest respectively or control treatments with water or Sett®. Melons were stored at 5°C for 5 weeks after harvest and were scored on a scale of 1- no disease to 5 – severe as described in the methods. N=35 for each treatment.

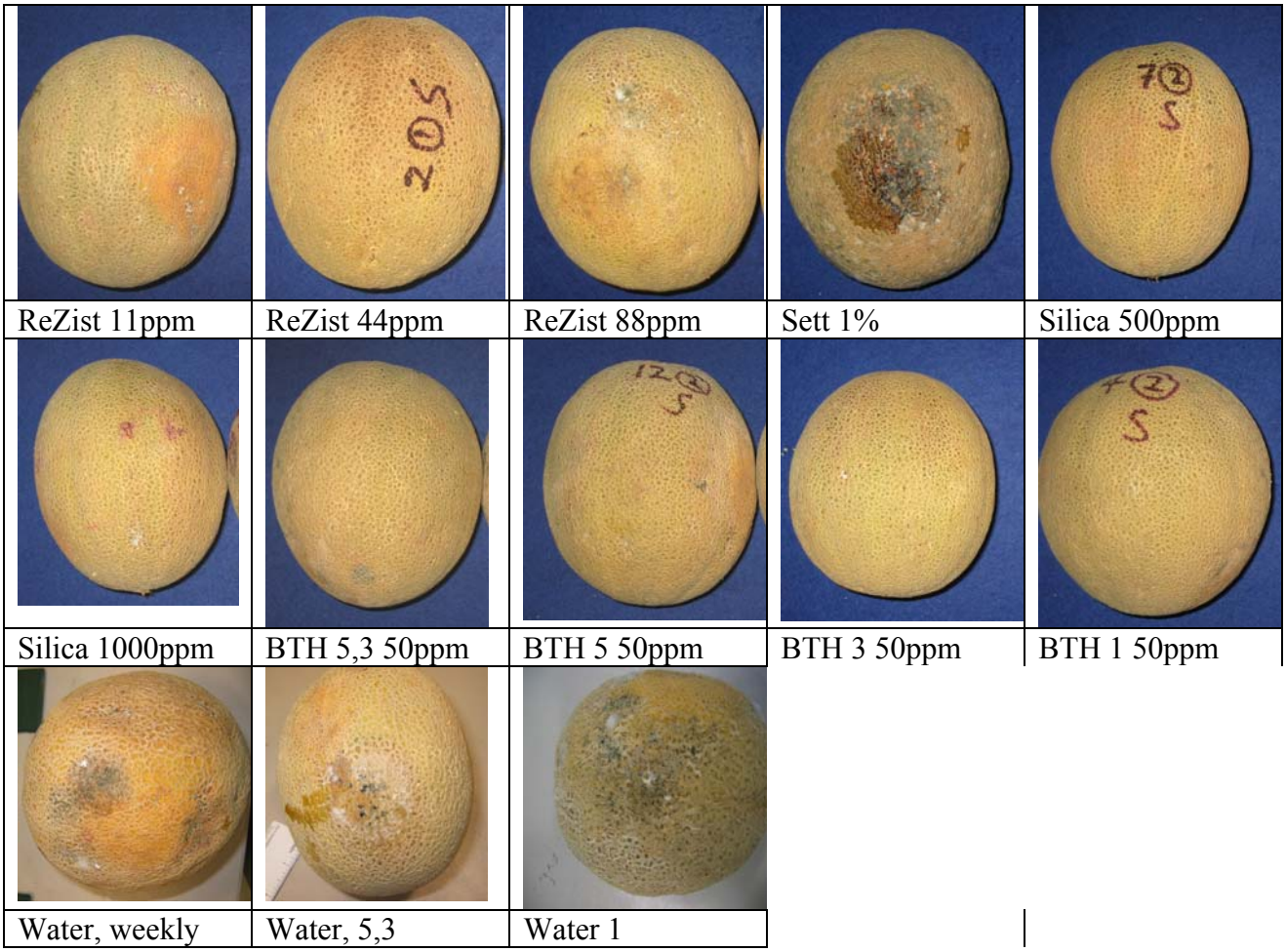


Figure 10. A melon representative of the treatment population was photographed 5 weeks after harvest (stored at 5°C). Fruit were harvested from plants treated preharvest with ReZist® at 11, 44 or 88 ppm weekly, SilikaMajic®(Silica) at 500 or 1000ppm weekly or Bion® (BTH) at 5 and 3, 3 or 1 week before harvest respectively or control treatments with water or Sett®. Melons were stored at 5°C for 5 weeks after harvest.

Assessments for control against the specific pathogens, Fusarium and Alternaria in shown in Figure 11 and 12. Figure 11 illustrates the level of control of *Fusarium* spp. on the melons with the different treatments. Consistent with the results from scoring the total moulds, BTH sprayed at 3 and 5 weeks, BTH sprayed at 5 or 3 weeks before harvest and ReZist® (44ppm) showed good control of *Fusarium* spp. where over 60% melons scored a rating of 2. The severity of Fusarium rot in the control melons was quite high with 90% of melons scoring 3 or higher. Silica sprayed at the lower concentration of 500ppm showed better control of Fusarium rot than at 1000ppm. ReZist® was not as effective when sprayed at a concentration of 11ppm or 88ppm. The preharvest treatments showed excellent control against Alternaria disease (Figure 12). BTH sprayed 3 weeks before harvest displayed the best control with 50% melons scoring 1 or total control. All of the treatments used were effective in controlling Alternaria disease in comparison to the control fruit.

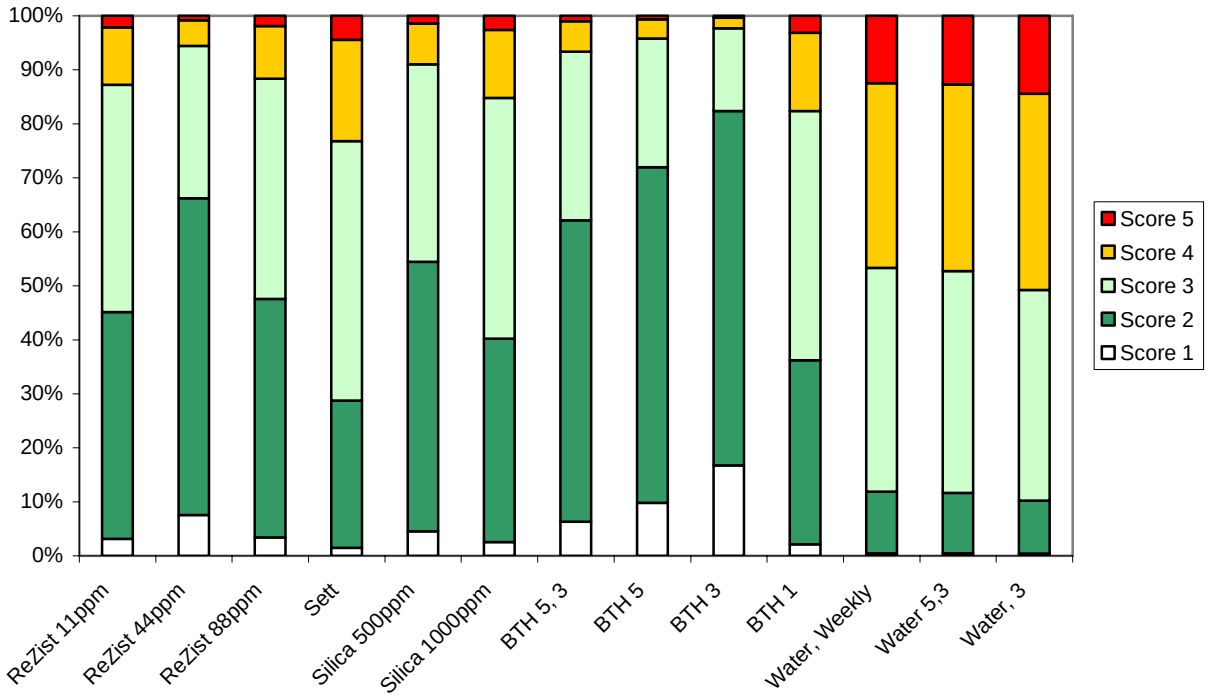


Figure 11. Disease severity of Fusarium rot in melon fruit harvested from plants treated preharvest with ReZist® at 11, 44 or 88 ppm weekly, SilikaMajic®(Silica) at 500 or 1000ppm weekly or Bion® (BTH) at 5 and 3, 3 or 1 week before harvest respectively or control treatments with water or Sett®. Melons were stored at 5°C for 5 weeks after harvest and were scored on a scale of 1- no disease to 5 – severe as described in the methods. N=35 for each treatment.

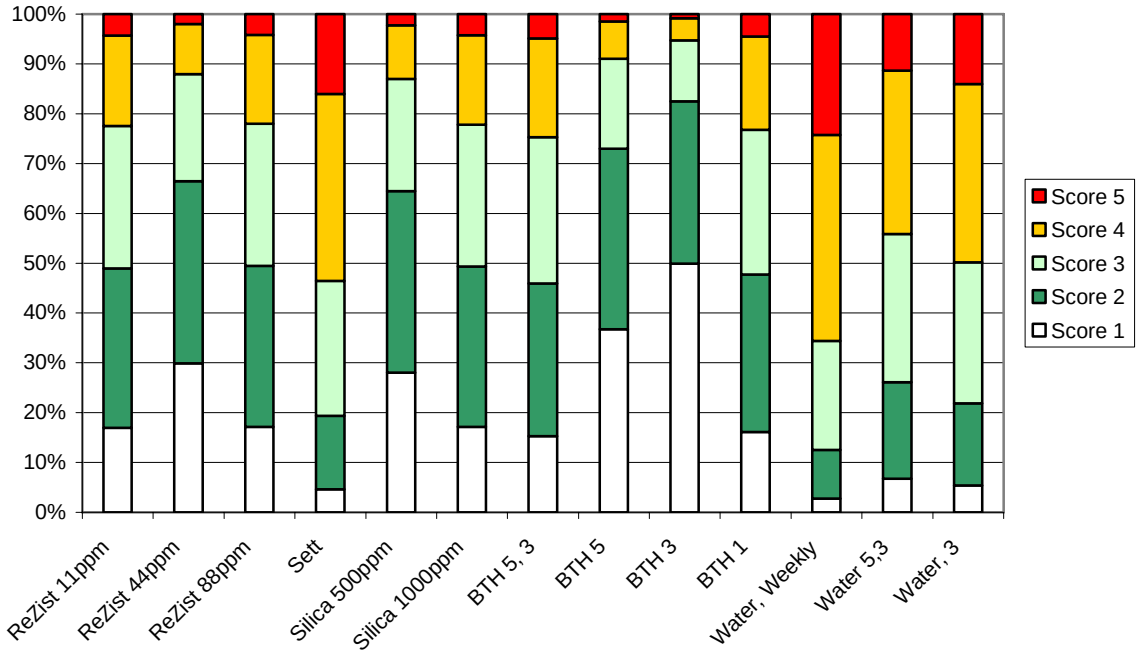


Figure 12. Disease severity of Alternaria rot in melon fruit harvested from plants treated preharvest with ReZist® at 11, 44 or 88 ppm weekly, SilikaMajic®(Silica) at 500 or 1000ppm weekly or Bion® (BTH) at 5 and 3, 3 or 1 week before harvest respectively or control treatments with water or Sett®. Melons were stored at 5°C for 5 weeks after harvest and were scored on a scale of 1- no disease to 5 – severe as described in the methods. N=35 for each treatment.

A foliar spray of BTH (50ppm) three weeks before harvest gave the best control against total moulds.

Postharvest Physiology

Prevention of postharvest rots is important. Equally important though is the quality of the fruit. We measured three different attributes that are important in postharvest quality, namely: fruit firmness, rind shrinkage and yellowing. Fruit firmness was measured with a penetrometer while rind shrinkage and yellowing was again based on a 1-5 score rating system. We also measured the total soluble sugars in the fruits postharvest. There were no significant differences observed in fruit firmness when the rind was penetrated with a penetrometer (Table 9). However, when the degree of rind shrinkage was measured, 80% melons treated with BTH 3 weeks before harvest showed no shrinkage compared to 70% in the controls (Figure 13). All treatments showed good control of rind shrinkage. Yellowing was measured to indicate if any of the melons were overripe. Dubloon is a green coloured melon. There were no significant differences in the degree of yellowing between treatments and the control (Figure 14). The total soluble sugar readings did not change during postharvest storage (Table 9).

Table 9. Penetrometer Readings and Brix° in melon fruit harvested from plants treated preharvest with ReZist® at 11, 44 or 88 ppm weekly, SilikaMajic®(Silica) at 500 or 1000ppm weekly or Bion® (BTH) at 5 and 3, 3 or 1 week before harvest respectively or control treatments with water or Sett®. Melons were stored at 5°C for 5 weeks after harvest.

Treatment	**Penetrometer Readings (N)** ± SE	**Brix°** ± SE
ReZist® 11ppm weekly	49 ± 0.94	12.68 ± 1.9
ReZist® 44ppm weekly	4.04 ± 0.72	12.3 ± 1.1
ReZist® 88ppm weekly	4.5 ± 1.31	13.22 ± 2.26
Sett (1%) weekly	2.84 ± 0.58	12.77 ± 1.78
Water weekly	2.7 ± 0.54	11.87 ± 2.09
Silica 500ppm weekly	2.8 ± 0.67	11 ± 3.5
Silica 1000ppm weekly	2.63 ± 0.45	11.38 ± 2.98
Water 5,3 weeks before harvest	2.84 ± 0.63	155 ± 1.2
BTH 5,3 weeks before harvest	3.19 ± 0.87	13.18 ± 1.49
BTH 5 weeks before harvest	61 ± 0.57	12.92 ± 2.35
BTH 3 weeks before harvest	3.06 ± 0.57	12.37 ± 1.76
BTH 1 weeks before harvest	3.19 ± 0.88	12.03 ± 2.4
Water, 3 weeks before harvest	2.58 ± 0.39	12.65 ± 1.64

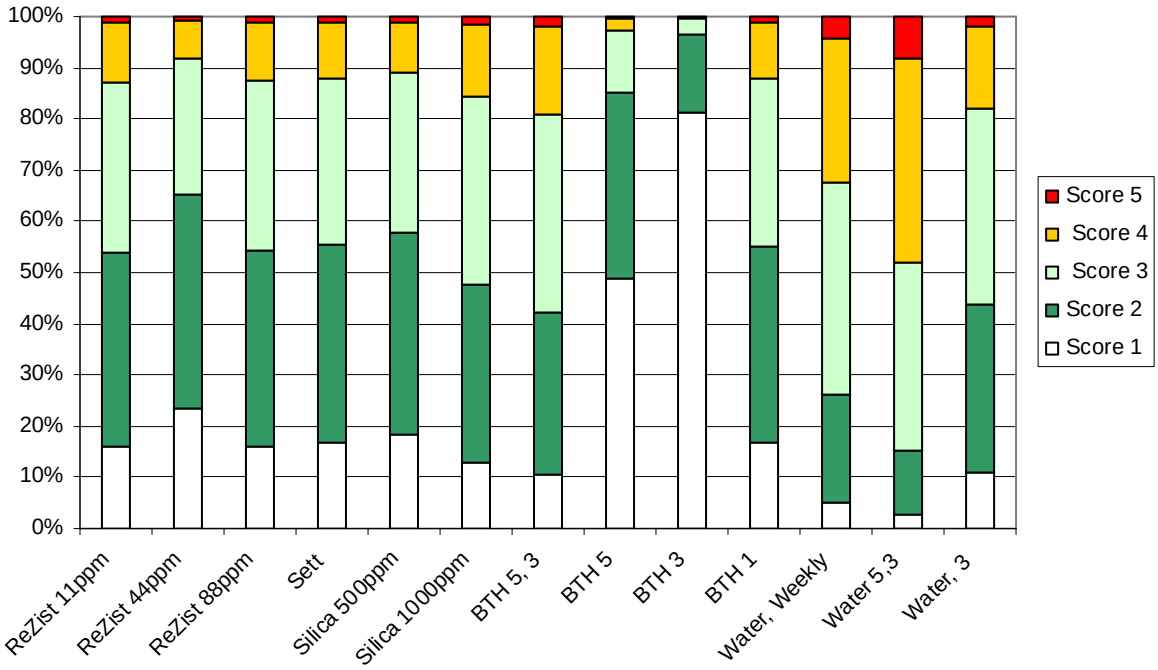


Figure 13. Degree of rind shrinkage in melon fruit harvested from plants treated preharvest with ReZist® at 11, 44 or 88 ppm weekly, SilikaMajic®(Silica) at 500 or 1000ppm weekly or Bion® (BTH) at 5 and 3, 3 or 1 week before harvest respectively or control treatments with water or Sett®. Melons were stored at 5°C for 5 weeks after harvest. N= 35

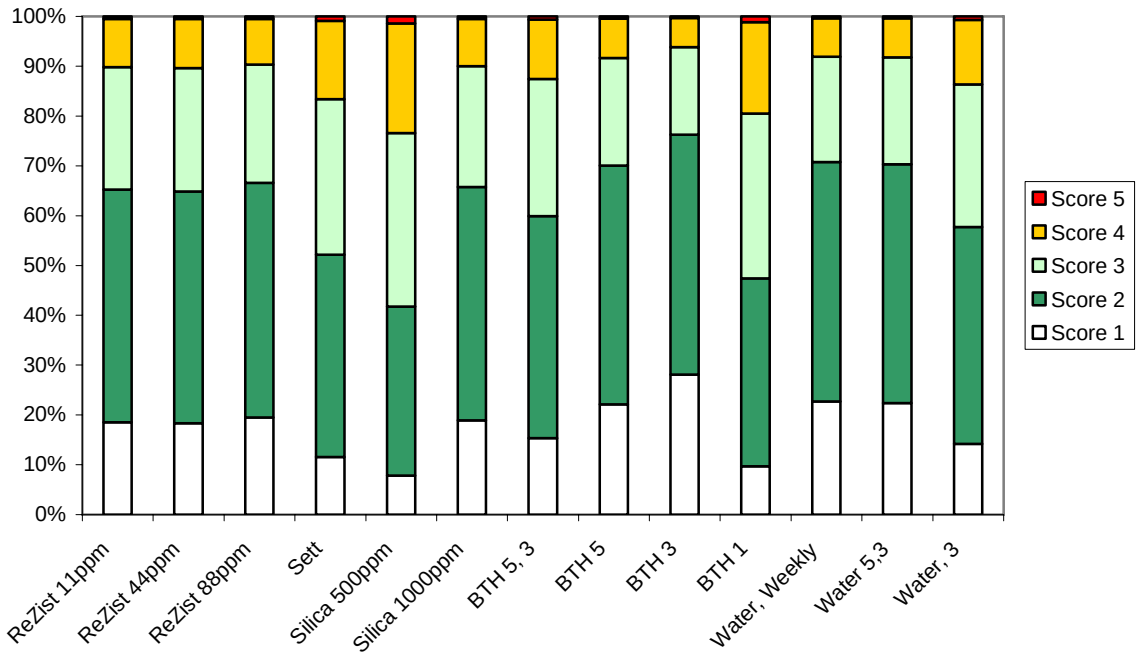


Figure 14. Degree of yellowing in melon fruit harvested from plants treated preharvest with ReZist® at 11, 44 or 88 ppm weekly, SilikaMajic®(Silica) at 500 or 1000ppm weekly or Bion® (BTH) at 5 and 3, 3 or 1 week before harvest respectively or control treatments with water or Sett®. Melons were stored at 5°C for 5 weeks after harvest. N= 35

Enzyme Analysis

Two key pathogenesis related (PR) enzymes were analysed, chitinase and peroxidase to identify markers of SAR. Leaves were collected every three days throughout the five week trial. Fruit were collected once a week, prior to the weekly spray. Fruit were not analysed for peroxidase activity. Peroxidase normally increases in activity during the natural fruit development processes, particularly when the fruit start to net. There would be no way of telling whether increases in peroxidase were due to the treatments applied or natural processes.

ReZist ®

Chitinase Activity

The level of chitinase activity detected in water-treated control leaves remained relatively steady ranging between 2 and 5mU/min/mg protein throughout the trial (Figure 15). Treatments of 11ppm and 88ppm ReZist® did not differ significantly from the water-treated control chitinase activities throughout the trial. In contrast, the 44ppm ReZist® treatment resulted in an increase of chitinase activity from 3.5 to 10mU within 3 days, then declining back to 5mU at day 6. The second ReZist® (44ppm) application did not increase the levels, however a third spray at 13 days saw the chitinase levels increase to 10mU. A similar application/enzyme induction pattern was observed when the fourth spray was applied at 21 days. Similar to the second

application at 6 days, increases in chitinase activity were not observed. The fifth and final application at 28 days saw the highest levels of chitinase activity in the leaves, peaking at 18mU. Leaves were also collected at 35 days (3 days before the harvest of the fruit). The chitinase levels had declined back to around 8mU however this activity was still higher than that observed in the water- treated controls (2.5mU) and the Sett only control (5.2mU).

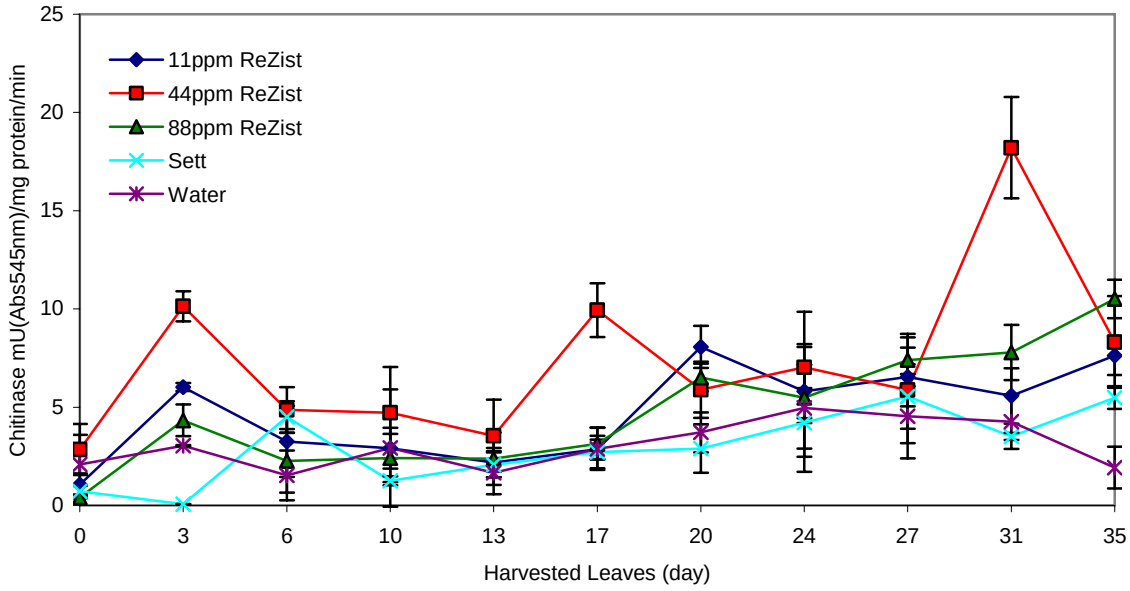


Figure 15. Activity of chitinase in melon leaves harvested from plants treated preharvest with ReZist® at 11, 44 88 ppm weekly, or control treatments with water or Sett® applied weekly. N=3

Activity of melon leaf chitinase from plants treated with ReZist® at 11, 44 and 88ppm, Sett (1%) or water applied weekly. Leaves were harvested at 3 and 6 days after treatment.

It was previously established in our lab that the bulk of enzymic activity was specific to the melon rind. Chitinase levels were much higher in the fruit rind compared to the leaf assays. However the pattern of expression of chitinase was not as clear when looking at the application-induction relationship. Applications of ReZist® (44 and 88ppm) and Sett (1%) induced initial increases of chitinase activity within the first week of application (Figure 16). These levels were much higher than those observed in fruit treated with 11ppm ReZist® or the water-treated control. The second application saw steady increases in the fruit treated with 88ppm or 11ppm ReZist®. Consistent with the observations made with the leaf enzyme assays, the alternate weekly application of 44ppm ReZist® showed little induction of activity. Upon the third application of ReZist® (44ppm), the chitinase levels steeply rose from 25-40mU. Increases were observed in all ReZist® treatments, excluding the water-treated control. Again, a drop in activity was observed between all treatment sprays at day 21 and 28. The fifth and final application saw all treatments increase in chitinase levels to around 35mU. Interestingly, melons treated with Sett (1%) dropped in

chitinase activity from 37mU to 15mU. There was also a steady increase in chitinase levels in the water-treated control melons. After 20 days, chitinase levels steadily rose from 15mU to 36mU. This is the same level that the treated melons were showing.

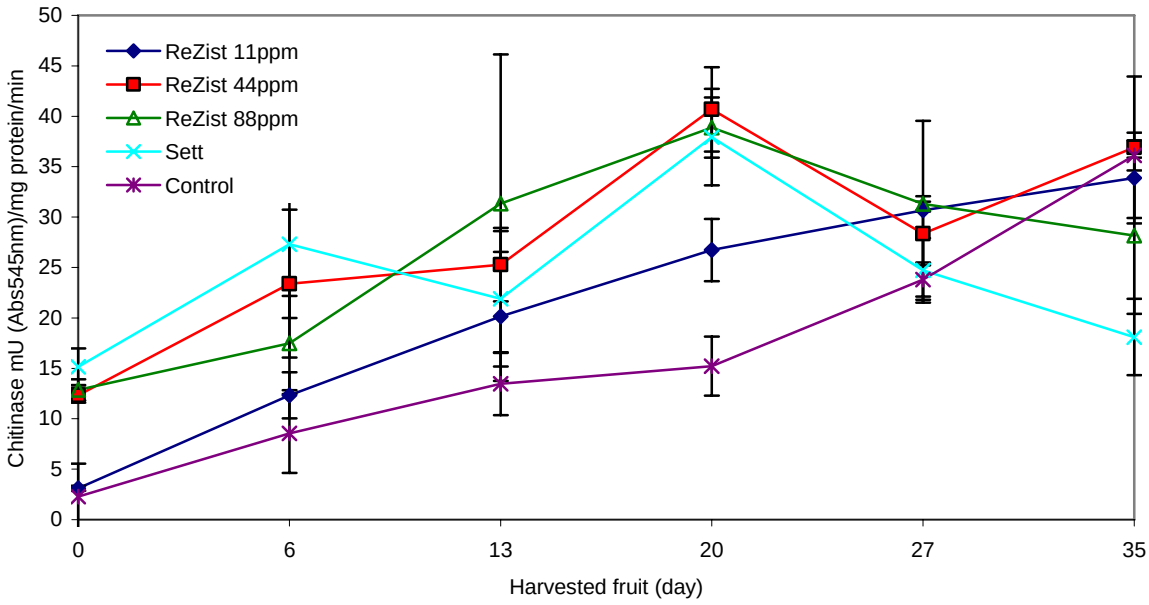


Figure 16. Activity of chitinase in melon fruit harvested at different maturity from plants treated preharvest with ReZist® at 11, 44 or 88 ppm weekly or control treatments with water or Sett® applied weekly. N=3

Activity of melon rind chitinase from plants treated with ReZist® at 11, 44 and 88ppm, Sett (1%) or water applied weekly. Melons were harvested 6 days after treatment. Melons differed in size and maturity during the course of the trial. Chitinase activities were also measured in the fruit rinds, 5 weeks postharvest. Although very high levels of chitinase activity were detected (25-43mU), there were no significant differences between treatments or the water-treated controls (Figure 17).

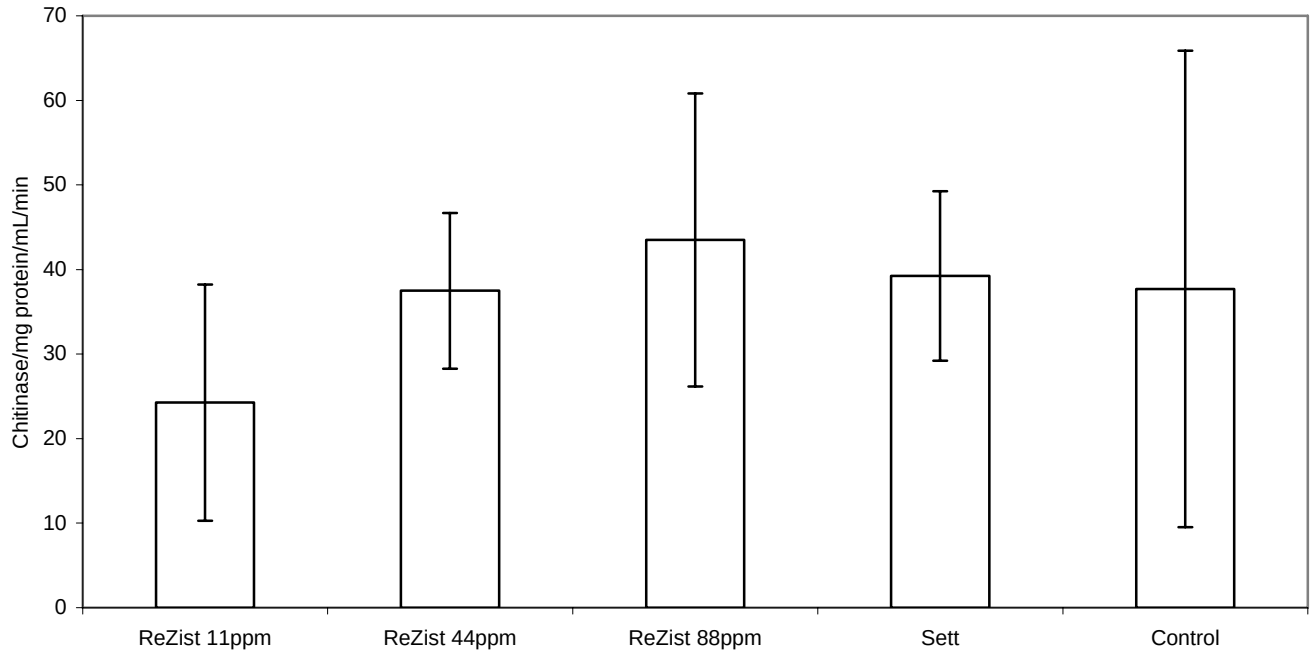


Figure 17. Chitinase activity in melon fruit harvested from plants treated preharvest with ReZist® at 11, 44 or 88 ppm weekly or control treatments with water or Sett® applied weekly. Melons were stored at 5°C for 5 weeks after harvest. N=3

Peroxidase Activity

Peroxidase activity was measured in the leaves treated with ReZist®, Sett and water every three days during the trial. After the first application of ReZist® a small, but transient burst, was observed in all treatments when measured three days later (Figure 18). After application of the second spray, a much larger peak in activity was observed in the leaves treated with ReZist® 44ppm, 88ppm and 11ppm with activities of 30,18 and 15 nmol respectively. Water-treated control peroxidase levels were 2 nmol on day 10. Peroxidase levels dropped to basal levels between each spray. When the third treatment was applied, the peak in peroxidase activity shifted from 3 to 6 days after the application. Leaves treated with 44ppm ReZist® increased again to just over 30 nmol. There was no increase in activity when treated with 11ppm ReZist®. Interestingly, in the leaves treated with 88ppm ReZist®, a huge peak in activity (54 nmol) was observed 6 days after the third spray. This activity dropped rapidly to 11 nmol after three days despite being treated a fourth time. An additional peak in peroxidase activity was observed in leaves treated with 44ppm ReZist®, peaking at 45 nmol at 6 days after the fourth spray. This activity dropped to 22 nmol and remained steady despite a fifth and final treatment. A small increase in peroxidase activity was observed in all samples in the last week before harvest.

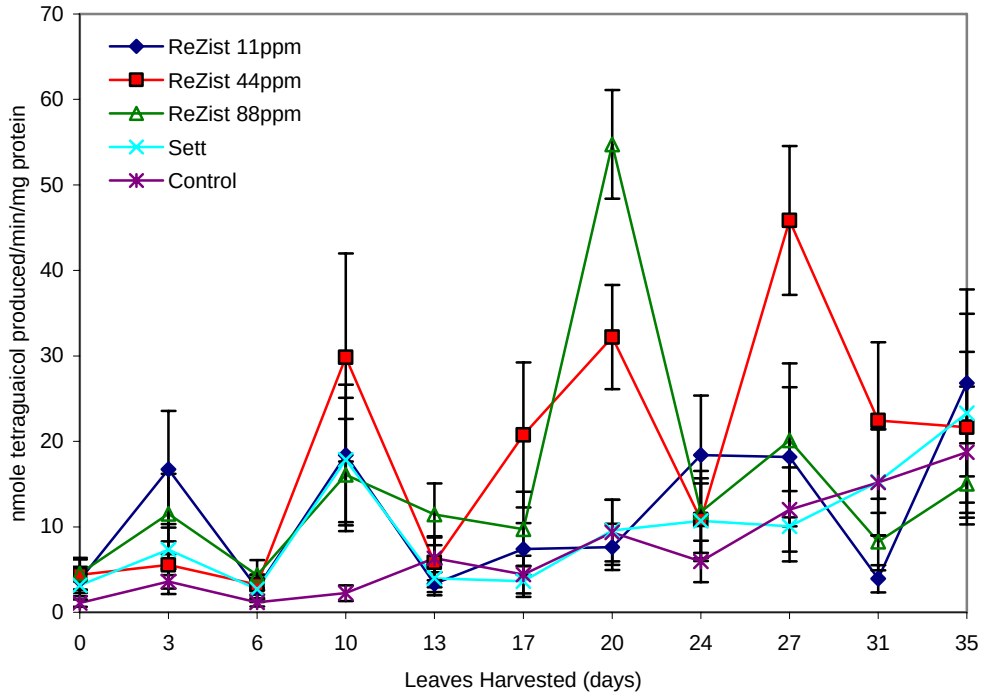


Figure 18. Peroxidase activity (measured by the oxidation of guiacol to tetraguiacol (470nm)) in melon leaves harvested from plants treated preharvest with ReZist® at 11, 44 or 88 ppm weekly, or control treatments with water or Sett® applied weekly. N=3

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Silica Treatments

Chitinase Activity

Two concentrations of silica, 500ppm and 1000ppm, in the commercial formulation SilikaMajik, were applied as a weekly foliar spray. Chitinase levels doubled in leaves within 3 days of the first application from 3.5 mU to 7 mU and by 6 days to 8.2 mU when treated with 500ppm Silica (Figure 19). Leaves were slower to respond to the higher concentration of 1000ppm with increases observed only after 6 days of application. Surprisingly, the chitinase levels in leaves treated with 500ppm did not follow any treatment-activity trends. Upon the second and third applications, chitinase levels declined in activity to 6 mU after the second spray and 4 mU after the third spray. These levels, however, were still higher than that observed in the water-treated control which remained at between 2 and 4 mU for the duration of the trial. Chitinase activity was much higher in the leaves treated with 1000ppm Silica. Approximately six days after each of the first three sprays, chitinase activity peaked at around 8 mU. After the fourth spray, chitinase activity was at its highest level in the leaves (14 mU) before declining to 10 mU just prior to fruit harvest.

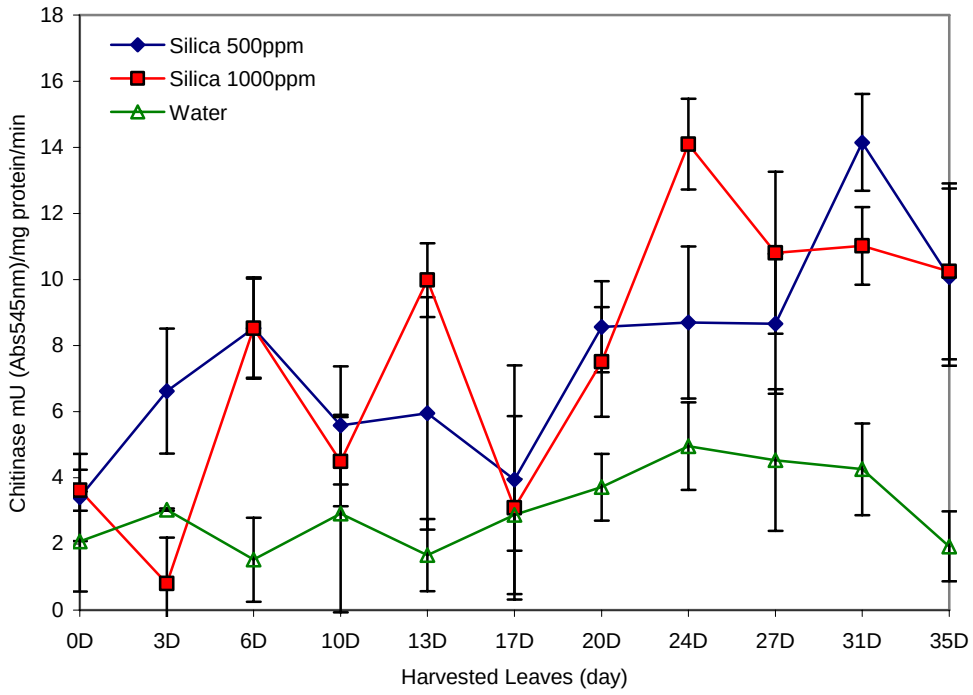

Figure 19. Activity of chitinase in melon leaves harvested from plants treated preharvest with SilikaMajic®(Silica) at 500 or 1000ppm weekly or control treatments with water Leaves were harvested at 3 and 6 days after treatment. N=3

The fruit were collected weekly and the chitinase levels were assayed in the fruit rind as described previously. Both concentrations of silica followed a very similar pattern (Figure 20). In fruit collected one week after the first applications of 500 and 1000ppm silica, chitinase activity rapidly increased to 51 and 49 mU, respectively. Water-treated control melon chitinase values were less than 10 mU at the same time. After the second spray, the chitinase activity levels dropped to the control levels. However, after the third and fourth applications, the chitinase activity levels steadily increased to around 50 mU for both concentrations.

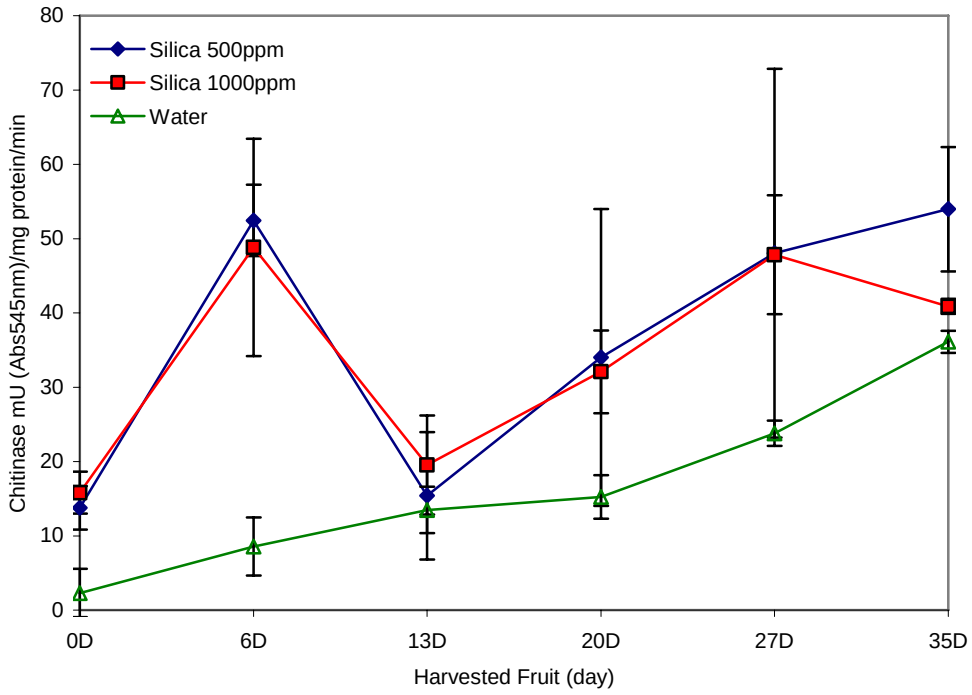

Figure 20. Activity of chitinase in melon fruit leaves harvested from plants treated preharvest with SilikaMajic®(Silica) at 500 or 1000ppm weekly or control treatments with water applied weekly. Melons were harvested 6 days after treatment. Melons differed in size and maturity during the course of the trial. N=3

Chitinase levels were checked in the fruit, five weeks postharvest (Figure 21). The chitinase activities in the fruit treated with 500ppm were significantly higher (72 mU) compared to the water-treated fruit (38mU). Fruit treated with 1000ppm were not significantly different to lower silica treatment.

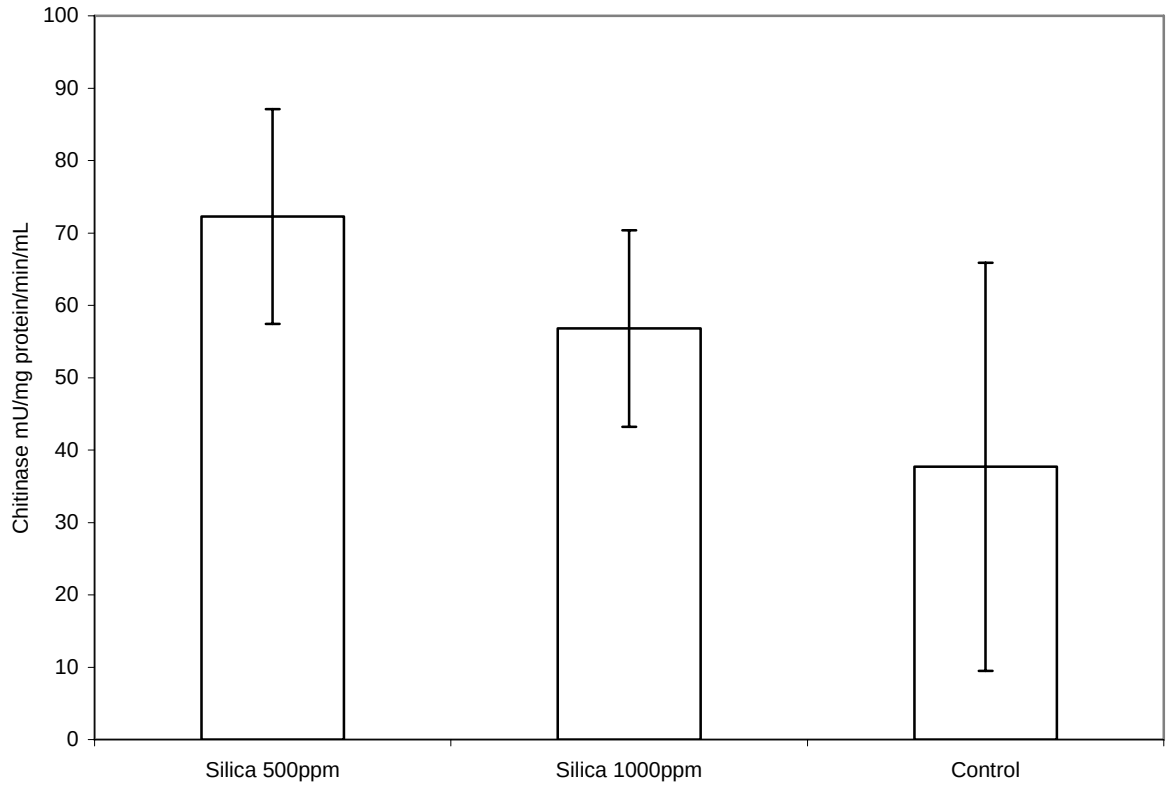

Figure 21. Chitinase activity in melons harvested from plants treated preharvest with SilikaMajic® (Silica) at 500 or 1000ppm weekly or control treatments with water applied weekly. Melons were stored for 5 weeks postharvest at 5°C (88%r.h.). The rind was assayed for chitinase..

Peroxidase Activity

In response to the silica treatments at both 500 and 1000ppm, it appeared that peroxidase activity levels increased over time and spray application (Figure 22). Treatment with 500ppm silica, peroxidase activity was increasing in small fluctuations six days after each application and then dropping slightly at three days. Over time, this drop in activity became smaller until there was a steady accumulation in activity after the fourth and fifth sprays to its highest level of 72 nmol, prior to harvest. The application of 1000ppm was slightly different in that there were no obvious fluctuations in activity after the applications, however, the peroxidase activities steadily increased to the maximum value of 52 nmol, just before harvest. The peroxidase levels were significantly higher than the control levels throughout the entire trial.

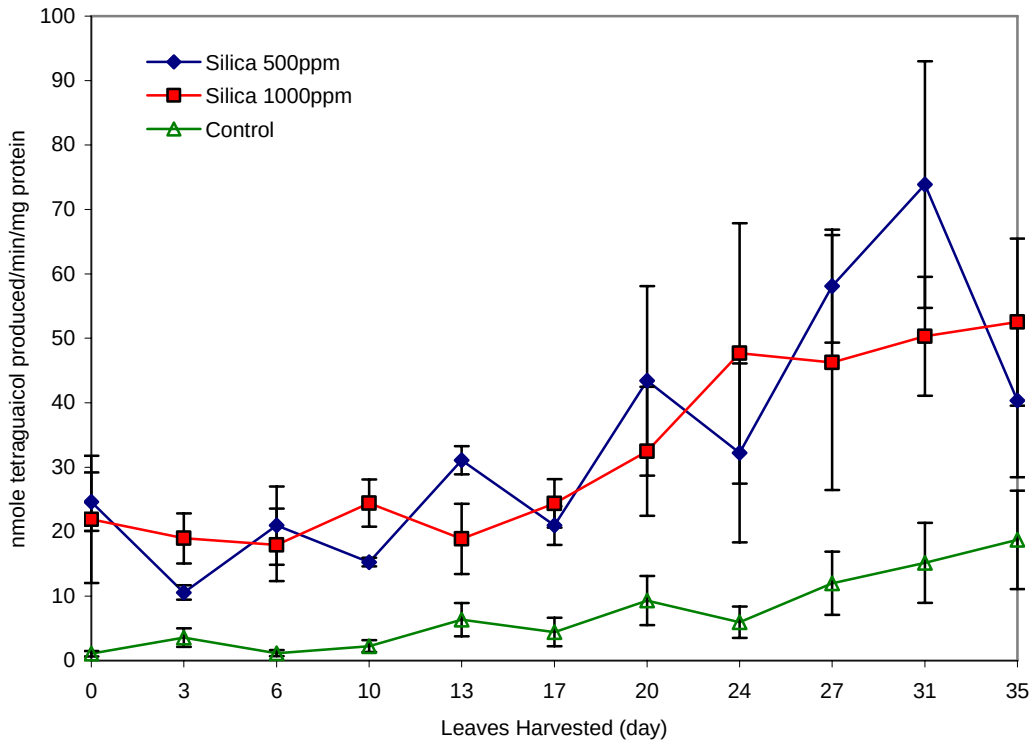

Figure 22. Peroxidase activity (measured by the oxidation of guiacol to tetraguiacol (470nm)) in melon leaves harvested from plants treated preharvest with SilikaMajic® (Silica) at 500 or 1000ppm weekly or control treatments of water applied weekly.

Conclusions

From the postharvest trial, it was very clear that preharvest sprays of BTH 3 weeks before harvest, ReZist® 44ppm applied weekly and Silica, 500ppm applied weekly, all significantly enhanced postharvest resistance to saprophytic pathogens and also enhanced melon quality. This trial provided an opportunity to measure in detail pathogenesis related proteins that are markers of enhanced defence, both in the leaves preharvest and postharvest in the fruit. Enhanced induction of the pathogenesis-related enzymes, chitinase and peroxidase were measured in the treatments that provided the best posthavest protection against disease. Enzyme activities were much higher in these treatments than the controls. Chitinase activity plays an important role in SAR mediated disease resistance, not only because of its hydrolytic activity od myceliua but also in facilitating the production of antifungal compounds in plants, such as phytoalexins (Mauch and Staehelin, 1989). Plant peroxidases are a large group of enzymes that are also up regulated during resistance responses and have been shown to be involved in the cross-linking of cell wall proteins, production of oxygen radicals and lignification which then act as a barrier to pathogen attack. The increase in peroxidase activity we onserved in SAR treated melon plants appears to be associated with increase in defence of the melon.

GENERAL DISCUSSION AND CONCLUSIONS

Postharvest disease is a major issue facing the Australian rockmelon industry with Alternaria, Cladosporium, Fusarium and Rhizopus rots causing the greatest problem. (Morris, 1977; Wade and Morris, 1982). Rockmelons are often shipped long distances to the major markets on the east coast from northwest and northern Australia increasing the disease pressure on these fruit (Morris et *al*, 2001). Furthermore, expanding the storage life of Australian melons up to 28-35 days is necessary for export to distant markets via sea transport (Morris, 1992). This is able to be achieved if postharvest disease is under control.

Control of postharvest disease on melons in Australia relies heavily on the application of fungicides as postharvest dips. Recently, public concern in reducing synthetic fungicide use, especially in postharvest disease management, has led to research into finding alternative approaches. One of the most promising alternatives is boosting a plants' natural defence, commonly termed Systemic Acquired Resistence (SAR). Resistance from SAR has been widely reported for control of preharvest diseases; however studies of this phenomenon on postharvest disease are minimal.

Plants treated with SAR activators, trigger defence responses in plants, including the accumulation of PR proteins such as chitinase and ß-1,3-glucanase. PR proteins have received considerable attention because of their rapid simultaneous accumulation in infected plants (Hammerschmidt and Becker, 1997) and their crucial roles in warding off invading pathogens (Schroder et *al*., 1992; Van Loon, 1997; Tuzun 2001). Unlike local defence responses to pathogen infection, such as phytoalexin accumulation, cell wall cross-linking and formation of oxygen free radicals (Lamb et *al*., 1989; Neuenschwander et *al*., 1995), the induction of PR proteins is also seen in non-inoculated plant parts that develop SAR; therefore, they are often used as indicators for SAR (Hammerschmidt et *al*., 2001).

In the first study:

- Applications of the defence elicitors BTH and ReZist protected rockmelon fruit from all postharvest disease significantly better than the control treatment.
- Melon fruit were assessed for natural infection 14 days after harvest at 15°C and both BTH and ReZist protected fruit from *Alternaria alternata* and *Colletotrichum* spp. signigicantly better than the control treatment.
- Postharvest infection of melon fruit by *Fusarium equi*setti was significantly controlled by BTH with ReZist being less effective.
- The combination of using the plant defence elicitors BTH and ReZist incombination with postharvest guazatine dipping of fruit in general provided better control than elicitor alone or guazatine alone.
- Application of plant defence elicitors BTH and ReZist stimulated increased activity of the pathogenesis related protein chitinase compared to the control treatment, confirming that induced resistance had occurred and that chitinase could be used as a marker for SAR in melon plants.
- In this experiment increased chitinase activity was not detected in fruit harvest from elicitor treated plants.

In the second study:

- Preharvest application of BTH (Bion®) (50 ppm applied once 3 weeks before harvest), ReZist® (44ppm applied weekly through the growing season) and Silica (500 ppm applied weekly through the growing season) significantly reduced posharvest disease incidence and severity on rockmelon fruit stored for 5 weeks at 5ºC.
- Storing melons for 5 weeks at 5ºC represents the outside limit for which rockmelons can be stored. Hence marketability after this extended storage period was quite low ranging between 32 and 69%. The most marketable fruit were from the ReZist and BTH treatments.
- In this trial both ReZist and BTH gave good control of Fusarium rots as well as Alternaria rots.
- Application of SAR elicitors resulted in the significant increases in activity of pathogenesis related proteins such as chitinase and peroxidase confirming that they are potential markers for systemic acquired resistance in rockmelons.
- The timing of elicitor treatment did not appear to affect the level of protection of fruit against disease, since one, two or there applicxations of BTH provided good protection against postharvest disease
- The plant defence elicitors did not adversely affect the quality of the melons in terms of Brix°, flesh firmness, and colour and rind colour.

The findings of this research provide fundamental knowledge for further research and application of biocontrol approaches in rockmelon postharvest management. Further research has been conducted in other projects to assess use of SAR in conjunction with generally regarded as safe (GRAS) chemicals which will be of considerable value to the Australian melon industry.

TECHNOLOGY TRANSFER

Conference Papers

Bokshi, A.I ., Morris, S.C and R. McConchie. 2003. Systemic Acquired Resistance: An Environmentally safe way for the control of pre and postharvest diseases of melons (poster) *Australasian Postharvest Conference*, Brisbane October 1 – 3 (Abstract in 2003 proceedings: P182).

McConchie R., Nguyen P. T. , McDonald, K. Anwaral B. and Morris S.C. 2004. Systemic Acquired Resistance as a Strategy for Postharvest Disease Management on Rockmelon (*Cucumis melo* Var. *Reticulatus*) 5th ISHS Postharvest Symposium, Verona, Italy 6-11 June

Bokshi, A.I. , Morris, S.C. and McConchie, R. 2005. Environmentally safe ways for the control of postharvest diseases of melons by integrating heat treatment, safe chemical and systemic acquired resistance. Australasian Postharvest Horticulture Conference, New Zealand 2005

McConchie R., McDonald, K. Anwaral B. and Morris S.C. 2005. Systemic Acquired Resistance as a Strategy for Disease Management in Rockmelon (*Cucumis melo* Var. *Reticulatus*). 3rd International Symposium on Cucurbitaceae. Townsville 12-16th September

Industry

McDonald, K.L., Bokshi, A., Morris, S.C. and McConchie, M.R. 2003. Inducing defence responses in rockmelon. *Melon Runner* 17:42-45.

McDonald, K. (2004) Preharvest Treatments of Melon Vines with Chemical Inducers of Systemic Acquired Resistance Increases the Shelf life of Melons. *Melon Runner* 18:12-13.

RECOMMENDATIONS

The outcomes of this research indicate that induced resistance can be used as part of postharvest disease control strategy for melons. Ongoing research is focused on refining ways in which induced resistance strategies can be incoroporated into integrated pest management programs, such as with generally regarded as safe (GRAS) chemicals to control postharvest disease for the melon industry.

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