Managing mildews: prevention using systemic acquired resistance (SAR) in greenhouse and field grown cucurbits

Jenny Jobling
The University of Sydney

Project Number: VG05034

VG05034

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetables industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry.

ISBN 0 7341 1686 1

Published and distributed by:

Horticultural Australia Ltd

Level 7
179 Elizabeth Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

E-Mail: horticulture@horticulture.com.au

Know-how for Horticulture™

Horticultural Australia Ltd Final Report

VG05034 Managing mildews: prevention using systemic acquired resistance (SAR) in greenhouse and field grown cucurbits

Prepared by Jenny Jobling, University of Sydney

`

HAL Final Report

VG05034 Managing mildews: prevention using systemic acquired resistance (SAR) in greenhouse and field grown cucurbits.

Project Leader: Dr Jenny Jobling AHR, PO Box 3114 Bundeena NSW 2230 Australia
jenny@ahr.com.au

Collaborators: Dr Robyn McConchie, Faculty of Agriculture, Food and Natural Resources, University of Sydney, NSW 2006
r.mcchonchie@usyd.edu.au

Dr Anowarul Bokshi, Faculty of Agriculture Food and Natural Resources, University of Sydney NSW 2006
a.bokshi@usyd.edu.au

This project was a levy funded project.

AHR
PO Box 3114 Bundeena NSW 2230 Australia
phone +61 2 9527 0826 **fax** +61 2 9544 3782

Table of Contents

`

Media Summary

Powdery mildew causes serious problems for many horticultural crops, especially when it covers the leaves of the plant and as a result the plant doesn't produce fruit which means a reduced return for the grower.

This project aimed to search for new ways to control powdery mildew in cucumber and zucchini crops. The strategy investigated incorporated integrated pest management and safe chemicals that boost the plant's natural defence systems. These chemicals induce systemic acquired resistance (SAR) in plants so that they are ready to fight a pathogen when it attacks. Systemic acquired resistance could allow growers to minimise the number of chemical applications in a season, without compromising crop yield.

The results showed that the disease control using this method was excellent for cucumbers. However the response was not as dramatic for zucchini. The results also showed that good agronomic practices were critical with healthy plants responding much better to the program than those that were compromised by such things as lack of water or nutrients.

At the moment, many of the chemicals used in these trials are not registered for use on Horticultural crops in Australia. However, it may be possible to apply for minor use permits for these chemicals (Bion®, Rezist® and Milsana®) to allow this work to continue. If more Australian efficacy data is collected then the case for registration may be able to put to APVMA in the future.

`

Technical Summary

The efficacy of Bion®, Milsana® and Rezist® along with conventional fungicides was evaluated for the control of powdery mildew of cucumber and zucchini.

All the chemicals significantly reduced powdery mildew of both the crops in glasshouse as well as in field conditions.

Bion® and Rezist® induced increased activity of the chitinase enzyme in the plants but Milsana® did not. Induced chitinase activity is an indicator for the induction of a systemic response in the plant. Bion® and Rezist® were confirmed as inducers of SAR whereas; the mode of action of Milsana® is still not clearly understood.

The results showed that powdery mildew in cucumber can be effectively controlled by two applications of either of the chemicals under low disease pressure or when disease symptoms occur in the later stages of crop development. Under high disease pressure and with the early appearance of powdery mildew the rotational application of the chemicals was the most successful control method.

It is important to note that multiple applications of Bion® on cucumber, adversely affected plant growth which resulted in lower fruit yield. This effect was prevented when Milsana® and Bion® were applied in a weekly rotation. In fact this treatment controlled powdery mildew much better than the conventional fungicide used.

The results for zucchini were not as significant as they were for cucumbers. The application of Bion® and Rezist® increased the chitinase activity in zucchini but the level of visual disease control was highly variable between replicates. As a result the treatment effects were not statistically significant. More work is required on zucchini.

Among the three chemicals Bion® provided consistent and long lasting protection from powdery mildew. In comparison the protection from Milsana® and Rezist® was less consistent and persisted for a shorter time. The most promising treatment was the use of Milsana® and Bion® in weekly rotation.

`

Introduction

Powdery mildew is a destructive disease of greenhouse and field-grown cucurbits in Australia (Lathem and Priest, 1989). The disease is currently controlled by using fungicides (Kelly, 2007). There are many problems associated with the use of synthetic fungicides. For example, in recent years there has been an increase in pathogen resistance to commonly used synthetic fungicides (McGrath, 2001). The major group of systemic fungicides has been reported to develop resistant strains of powdery mildew within a few years of field use (O'Brien, 1994). There is also increasing community concern for threats to human health and the unpredictable economic or environmental consequences resulting from the use of synthetic fungicides (Kirrane *at al*., 2005; Schirra *et al*. 2000). As a result there is an increased research effort investigating alternatives for synthetic fungicides.

The use of the systemic resistance of plants induced by the application of chemical elicitors is emerging as a promising alternative for disease control in plants (Hammerschmidt and Yang-Cashman, 1995). Systemic resistance acts by stimulating local and systemic accumulation of defence-related compounds that are responsible for increased disease resistance of the plant (Raskin, 1998). A number of chemical elicitors such as Bion®, Rezist® and Milsana® are being tested for use against various crop diseases. Some of these elicitors are registered for commercial use in field crops.

There are number of benefits for using chemical elicitors for the control of plant diseases but at the same time there are some adverse effects which limit their commercial use. For example it has been reported that treatment with Bion® can cause early senescence, stunting in plant growth and a compromised yield (Bokshi *et al* 2006; Heil *et al*, 2000). More work is required to investigate why this variable response occurs and how it might be minimised under commercial conditions.

Many researchers are working in the area investigating systemic acquired resistance. Agostini *et al*. (2003) reported that Rezist® is a chemical elicitor which partially suppresses plant disease by inducing systemic resistance. Another disease control option being investigated is Milsana® which has been reported to induce systemic resistance without any adverse effect on plant growth and yield (Konstantinidou-Doltsinis *et al*. 2006).

The objectives of this study were to (i) evaluate the efficacy of chemicals elicitors against powdery mildew with systemic induction of antifungal enzymes, (ii) to explore options for improving the consistency of disease control using elicitors of SAR and (iii) to evaluate these control methods under commercial field conditions. In this report, we provided the first field data for the use of Bion®, Milsana® and Rezist® under commercial conditions for the control of powdery mildew on cucumber and zucchini crops.

`

Materials and Methods

Chemicals:

Bion® was provided by Syngenta, Basel, Switzerland, Milsana® from P J Margo Pvt Ltd, Bangalore, India, Rezist® from Stoller enterprises, Houston, while the fungicide Amistar® was from Syngenta, Australia and Triad 125® from Farmoz Pty Ltd, Australia.

1. *Glasshouse Screening of SAR Elicitors for powdery mildew control on cucumber*

Cucumbers, cultivar Lancelot were grown in 5 litre pots containing commercially prepared potting mix. Five seeds were planted in each pot and there were 4 pots per treatment representing 4 replicates.

Three week old seedlings with fully developed first true leaves were sprayed with the followingtreatments:

Treatments

1. Bion® 100 ppm
2. Bion® 50 ppm
3. Rezist® 44 ppm
4. Rezist® 22 ppm
5. Milsana® 1%
6. Silicate 500 ppm
7. Water

A second spray of the chemicals was applied one week later.

Bion® 100 ppm and Rezist® 44 ppm were found to be phytotoxic after the first spray and both chemicals severely damaged the foliage. For the second application these treatments were omitted.

The effects of the remaining chemicals were assessed through challenge inoculation on the treated leaves as well as histology of the leaf tissue for fungal growth and accumulation of antifungal compounds (phenolics).

One week after the second spray the 2nd and 3rd leaves were detached from the plants and placed into the Petri dish chambers. There were 12 detached leaves for each of the treatments. The detached leaves then challenged with powdery mildew and incubated at 24°C. The challenged leaves were scored every alternate day under the microscope for fungal growth and disease severity. On the 4th day after inoculation there were visible (microscopically) infection on the water control treatment. At this time leaf discs were cut from two of the challenged leaves for each treatment to perform histological studies. Cut

`

leaf discs were fixed for clearing by using the mixture of ethanol and glacial acetic acid (3:1). The cleared leaf discs were stained with toluidine blue (0.5%) and trypan blue (0.05%) for the examination of fungal growth and accumulation of phenolics in response to fungal invasion.

The other detached leaves were examined on the 12th day after inoculation under the light microscope to score the level of infection and colony development. Microscopic observation and scoring was performed on section 4 and 5 (middle sections) of the 7 sections of the leaf as shown in the Figure 1. The severity of infection and colony development was scored by using a scale of 1 to 5 where 1 = no sign of infection; 2 = only few infection with very little growth of the fungal mycelium (unbranched); 3 = few infections, fungal mycelium developed and branched around the infection site but no sporulation occurred; 4 = well established infection, abundant mycelium growth with sporulation covering about half of the area under observation; 5 = established disease with fungal colony and visible without microscope.

Leaf samples were also collected one week and three weeks after the second spray for the analysis of chitinase enzyme activity which is a marker for an induced systemic response.

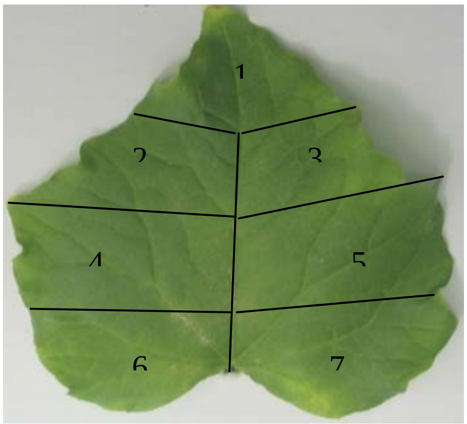

Figure 1. Sections on the leaf for scoring infection of powdery mildew using the light microscope as surface hyphae of powdery milder are not present on cucumbers.

Chitinase assay:

Third and fourth leaves of two plants were harvest one week after the second spray for analysis of chitinase activity. The chitinase activity was assayed according to Bokshi *et al.* (2005). Frozen samples of leaf (0.3 g leaf fresh weight (FW)) were ground in a mortar with liquid nitrogen after adding 1% (w/w) PVPP. The tissue was homogenised in 1 ml 50 mM potassium acetate buffer, pH 5.0 containing 1 mM EDTA. Reduced glutathione (5 mM) was added to the buffer on the day of extraction. The homogenates were centrifuged at 9000 g for 10 min at room temperature (20°C) and the supernatants were transferred to microcentrifuge tubes for enzyme or protein assays.

A 0.2 ml aliquot of the supernatant was placed in a 2 ml micro-tube with 0.1 ml 0.1 M potassium acetate buffer, pH 5.0. Tubes were equilibrated in a water bath at 37^{o}C for approx. 5 min. The reaction was started by adding 0.1 ml aqueous CM-Chitin-RBV (2 mg ml^{-1} solution; Loewe Biochemica, Sauerlarch, Germany). After 10 min, the reaction was terminated by adding 0.1 ml 2M HCl which precipitated the undegraded substrate. The tubes were cooled on ice for approx. 10 min, and then centrifuged for 5 min at 9000 g. The absorbance of the supernatant was measured at 545 nm against an acetate buffer blank.

`

Results were calculated using a linear equation derived from standard curve of the absorbance of serial dilutions of standard pure chitinase from Sigma Chemical Co., St Louis, MO, USA (product number C6137). The specific activity of chitinase was expressed as mUnits mg^{-1} of total soluble protein.

Protein determination:

Protein contents in crude extracts for chitinase assays were determined by the Bradford method (Bradford, 1976) using bovine serum albumin as standard.

2. *GRAS chemicals for the control of powder mildew of Glasshouse hydroponic cucumber*

Cucumbers, cultivar Sultana were grown using a hydroponic system and were treated with SAR and GRAS chemicals for the control of powdery mildews in glasshouse conditions. There were 18 plants per treatment in 6 replicated blocks (108 plants in total, Figure 5). There were two tanks to supply water with nutrient to the plants. One tank was for supplying the recommended nutrients only and the other was the recommended nutrients plus 100 ppm of silicate from potassium silicate (AgSil® 25A from PQ Australia Pty Ltd). At the 4th leaf stage the plants were treated with the first application of chemicals. The following SAR and GRAS chemicals were used:

1. Timorex® 1%	GRAS chemical	Spray
2. Thiovit 2%	GRAS chemical	Spray
3. Bion® 50 ppm	SAR chemical	Spray
4. Silicate 100 ppm	SAR chemical	Hydroponic
5. Amistar® 0.5%	fungicide	Spray
6. Water		Spray

At the time of first spray most of the plants were infected with powdery mildew. The chemicals were applied to the plants at 10 days intervals using a pack back air sprayer. The foliage of the Bion® treated plants were severely damaged after the second application due to phytotoxicity of the chemical. No further Bion® treatments were applied to those plants.

The development of powdery mildew was examined 10 days after the 2nd spray by checking for visible colonies on all leaves from the first true leaf upward. The disease severity on each leaf was scored by using a 1-5 scale where 1 = no visible colony; 2 = few visible powdery mildew colonies; 3 = several visible powdery mildew colonies covering up to 15% of the leaf area; 4 = powdery mildew colonies covering 16-40% leaf area and 5 = powdery mildew colonies covering more than 40% of the leaf area.

`

The firmness of the fruit was tested on 3 fruit per replication. For the firmness, skin of the fruit was peeled off at the middle of the fruit at two opposite sides and measured by using a penetrometer.

The activity of the chitinase enzyme was also measured using the method described previously.

3. *Glasshouse Screening of SAR Elicitors for powdery mildew control on zucchini.*

Zucchini, cultivar Columbia from South Pacific Seeds were grown in 8 litre pots containing commercially prepared potting mix. Four seeds were planted in each pot and there were 4 pots per treatment representing 4 replicates. Two week old seedlings with fully developed first true leaves were sprayed with following SAR chemicals:

1. Bion® 50 ppm
2. Rezist® 22 ppm
3. Milsana® 1%
4. Silicate 500 ppm
5. Water

A second spray of the chemicals was applied one week later. The development of powdery mildew from natural infection in the glasshouse was assessed two weeks after the 2nd spray using a severity scale of 1-5 where, 1 = no visible colony; 2 = few visible powdery mildew colonies; 3 = several visible powdery mildew colonies covering up to 15% of the leaf area; 4 = powdery mildew colonies covering 16-40% leaf area and 5 = powdery mildew colonies covering more than 40% of the leaf area.

The samples for chitinase assay were collected one week after the second spray by harvesting the 3rd emerging leaf of the plants. A second leaf sample was collected three weeks after the second spray by harvesting the third emerging leaf. The chitinase was assayed as described previously.

`

4. First field trial for glasshouse grown cucumbers

For this trial cucumber plants, cultivar Sultan on a commercial property were grown in a poly tunnel at Kemps Creek south west of Sydney (November 2006). The plants were grown in bags of potting mix and were watered regularly using a hydroponic growing system.

Treatments

The plants were treated with SAR chemicals for the control of powdery mildew (PM). Five week old plants at the early stage of bearing were sprayed with the following chemicals:

1. Bion® 25 ppm
2. Rezist® 22 ppm
3. Milsana® 1%
4. Fungicide (Amistar + Triad 125)
5. Water
6. Bion® (one application)
7. ReZist® (one application)

The chemicals were sprayed at fortnightly intervals. At the time of first spray the plants were growing vigorously and no powdery mildew was observed. Due to the lack of disease symptoms the fungicide treatment was not applied for the first spray application, the plants were left untreated. At the time of the second application there was a lot of powdery mildew present. The fungicide treatment plants were sprayed with Amistar® @ 0.05% (w/v). However, Amistar® did not control the powdery mildew and so for the next spray application the fungicide treatment plants were sprayed with Triad® 125 @ 0.2% (v/v).

Experimental Protocol

The experiment was set up in a poly tunnel with hydroponic cucumbers. The experiment was set up as a completely randomised design in 3 blocks. There were four treatment plants and two buffer plants (Total of 126 plants) with a buffer row either side of the experimental plants.

The plants were sprayed every 14 days and were scored for disease severity and incidence at weekly intervals.

Near the end of the growing season fruit were harvested over a 14 day period (4 harvests) to determine commercial crop yield.

Assessment of the chitinase activity of cucumbers treated with SAR chemicals

A separate experiment on the same farm in the same poly tunnel was conducted to quantify the intensity and duration of the SAR response. Plants were treated with Bion® or Rezist® either with a single or multiple spray (fortnightly). There was also a water treatment control.

`

Leaf samples that were used for the enzyme assay were taken by harvesting the 5th and 6th emerging leaves of three plants from each treatment; each plant sample was used as a replicate. Leaf samples were taken at fortnightly intervals including an initial untreated sample and the enzyme activity was measured as describe previously. At the end of the crop the plants were scored for powdery mildew development on 4th, 5th and 6th emerging leaves using the 1 to 5 scale as described for previous experiments.

5. *Second field trial with SAR and GRAS chemicals to control powdery mildews of cucumber and zucchini*

Cucumber

Cucumber cultivar Austin was grown in a polytunnel (greenhouse) using a hydroponic system. The plants were treated with SAR inducing chemicals to determine their efficacy in controlling powdery mildew. The first spray was applied to three week old plants that were at an early stage of flowering. Chemicals applied included, Bion® at 25 ppm, Rezist® at 22 ppm or Milsan® 1% and the control plants were sprayed with fungicide Triad 125 @ 0.75 ml/L with a mixture of Silica Magic® (20% soluble reactive silica) @ 100ml/100L which is approximately 200ppm of silica and this was commercial practice. One week later a second application of the chemicals was applied as described below. A third spray with the fungicide was applied three weeks later to all the treatments.

	1st spray	2nd spray	3rd spray
1.	Bion	Bion	Fungicide
2.	Rezist	Rezist	Fungicide
3.	Milsana	Milsana	Fungicide
4.	Bion	Milsana	Fungicide
5.	Milsana	Bion	Fungicide
6.	Rezist	Milsana	Fungicide
7.	Milsana	Rezist	Fungicide
8.	Bion	---------	Fungicide
9.	Rezist	---------	Fungicide
10.	Milsana	---------	Fungicide
11.	Fungicide (control)	---------	Fungicide

There were three replications per treatment, with four plants in each replicate. At the time of the first spray the plants were vigorous in growth with a few plants infected with powdery mildew.

`

The disease severity was scored by examining the leaves of the same growth stages by using a 1-5 scale where 1 = no visible colony; 2 = few visible powdery mildew colonies; 3 = several visible powdery mildew colonies covering up to 15% of the leaf area; 4 = powdery mildew colonies covering 16-40% leaf area and 5 = powdery mildew colonies covering more than 40% of the leaf area. Disease scores were recorded at weekly intervals starting one week after the first spray and continued for the following five weeks.

The induction of resistance was also evaluated by the analysis of the chitinase enzyme activity in the leaf tissue of the treated plants the method has been described previously. Leaf samples from single or multiple sprays of Bion®, Rezist® and Milsana® were collected for enzyme analysis at weekly intervals starting from day 0 for the next 4 weeks. The effects of the SAR chemicals on the crop yield were evaluated by harvesting fruit of marketable size for a two week period with harvests being done every two days.

Zucchini

A field experiment was conducted with zucchini var. 'Columbia' to investigate the induction of disease in response to chemical elicitors. The plants were grown on an 80 cm wide bed with an 80cm spacing between the beds. Seven day old seedlings were planted at a spacing of 80 cm. There were three replications per treatment with four plants in each replication. At the time of the first spray application the plants were vigorous in growth and there were visible symptoms of powdery mildew. The chemicals used in the zucchini trial were as follows:

Assessment of powdery mildew control for Zucchini

1. Bion 25 pmm
2. Rezist® 22 ppm
3. Milsana® 1%
4. Fungicide (Amistar®)
5. Water

Assessment of chitinase activity in treated Zucchini

1. Bion® 50 ppm multiple
2. Bion® 50 ppm single (first spray only)
3. Bion® 25 ppm single (first spray only)
4. Rezist® 22 ppm single (first spray only)

Assessment of powdery mildew control: The chemicals were first applied on 2 week old seedlings. The second and third sprays of the chemicals were applied 14 and 28 days after the first spray. The disease severity data was taken two and four weeks after the first spray

`

by examining the development of powdery mildew on leaves using the 1-5 severity scale as described earlier. The week 2 disease scoring was performed on three basal leaves (5th, 6th and 7th leaves) while the week 4 scoring was performed by examining the 4th, 5th and 6th emerging leaves.

Assessment of chitinase activity: Leaf samples were collected for the assessment of chitinase on day 0, 14 and 28 days after the first spray. Samples were collected from the 4th emerging leaves by cutting off a quarter of a leaf from four plants per replicate. The analysis was done as described previously.

Statistical Analysis

All data where reported was analysed using Genstat ver 10.1.0.07 (Supplied by VSN International Pty Ltd, Lawes Agricultural Trust, UK).

It is important to note that all the graphs showing disease severity in this report have no statistical analysis, only mean scores are shown. This is because score data should be analysed using ordinal logistic regression which has a probability table output. This data is very difficult to interpret and so not appropriate for a report aimed at commercial growers. The correct analysis is available and will be presented in the refereed journal article prepared reporting this work. If anyone is interested in the full data analysis please contact the report author (jenny@ahr.com.au).

`

Results and Discussion

1. *Glasshouse Screening of SAR Elicitors for powdery mildew control on cucumber*

The results of these first experiments showed the reduction of powdery mildew infection as a result of treatment with Milsana®, Rezist® and Bion® but not from silicate or water (Figure 2). Milsana® treated leaves showed no infection or colony development. There were also low levels of infection on leaves sprayed with Rezist® and Bion®. Spraying with 500 ppm of silicate at weekly intervals did not reduce the powdery mildew infection of the cucumbers.

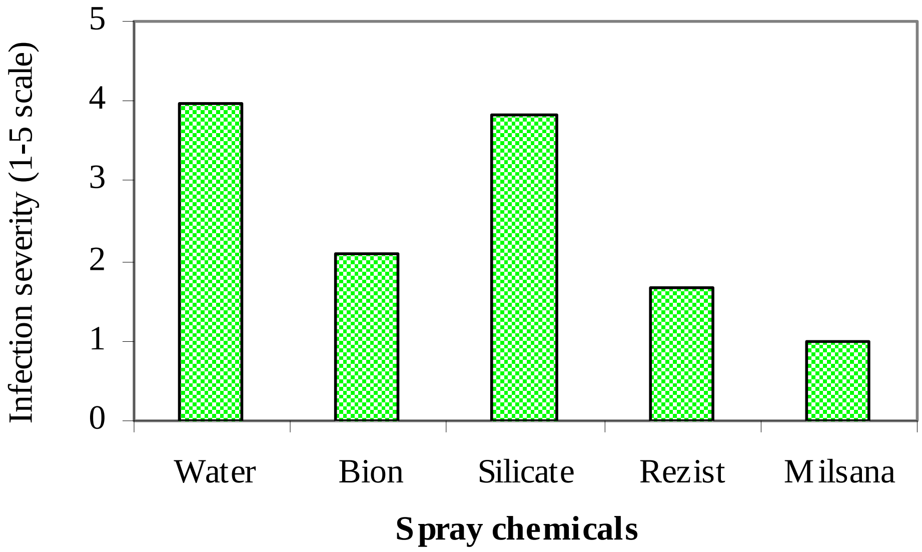

Figure 2 Severity of infection and colony development of powdery mildew on detached leaves treated with SAR chemicals.

The leaf discs were investigated under the light microscope and variations in fungal growth and accumulation of phenolics were found as a result of the application of different SAR chemicals. Fungal mycelium was found to grow vigorously in the leaves treated with water and very small amounts of phenolics were detected at the infection site. In contrast, the leaves treated with Bion® showed restricted growth of the invading fungal mycelium due to accumulation of large amounts of phenolics at the site of invasion. In the Rezist® treated leaf we noticed several visible (microscopically) sites with accumulated phenolics but could not find any germinated spores or sites with established infection. Milsana® treated leaves did not show any accumulation of phenolics or any germinating spore or infection site. This indicates that Milsana® may be a protective GRAS fungicide rather than a promoter of SAR.

`

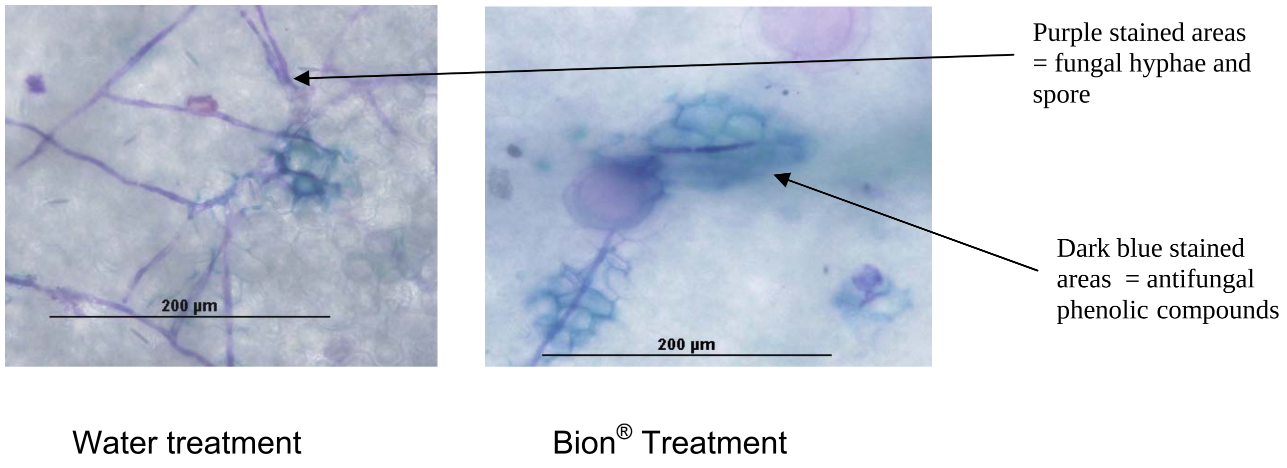

Figure 3 Leaf tissue observed under microscope for the infection and growth of fungal colony and the accumulation of phenolics in response to fungal invasion.

2. *GRAS chemicals for the control of powder mildew of glasshouse hydroponic cucumbers*

Spraying cucumber plants with the chemicals reduced the incidence and severity of powdery mildew from natural infection in glasshouse conditions. Silicate applied as a nutrient supplement as well as water spray did not affect the development of powdery mildew (Figure 4). The results suggest that the GRAS chemical Timorex® acts as a protectant against powdery mildew but can not eradicate the established fungal colony. On the other hands Thiovit® (a GRAS chemical) and Amistar® (synthetic fungicide) have shown the greatest control of the disease as protectants and these chemicals nearly eradicate the established fungal colony. The Bion® treated plants was not scored for disease development because of foliage damage from phytotoxicity of the chemical. The Bion® treatment will be repeated at a lower concentration in future experiments.

The fruit from initial harvest examined for firmness have shown variation due to treatment with chemicals (data not shown). Plants treated with Amistar® and silicate had an increased fruit firmness compared to the water treatment.

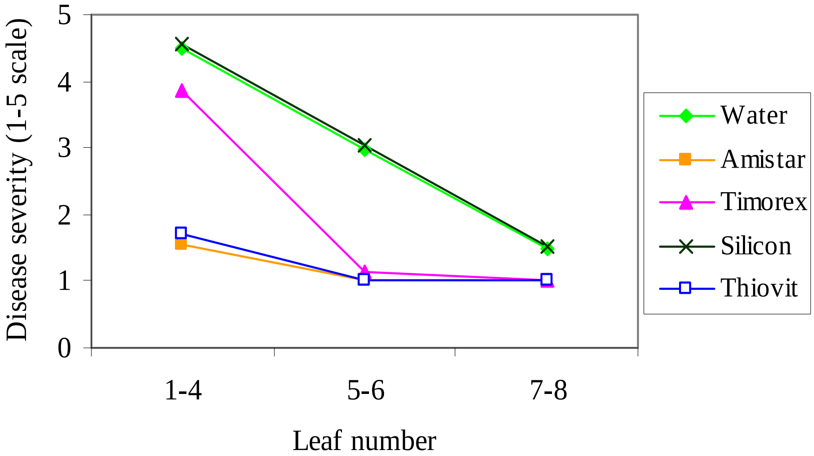

Figure 4 Powdery mildew developments on cucumber leaves treated with different SAR and GRAS chemicals. Amistar was used as the fungicide check to compare the efficacy of the treatments.

`

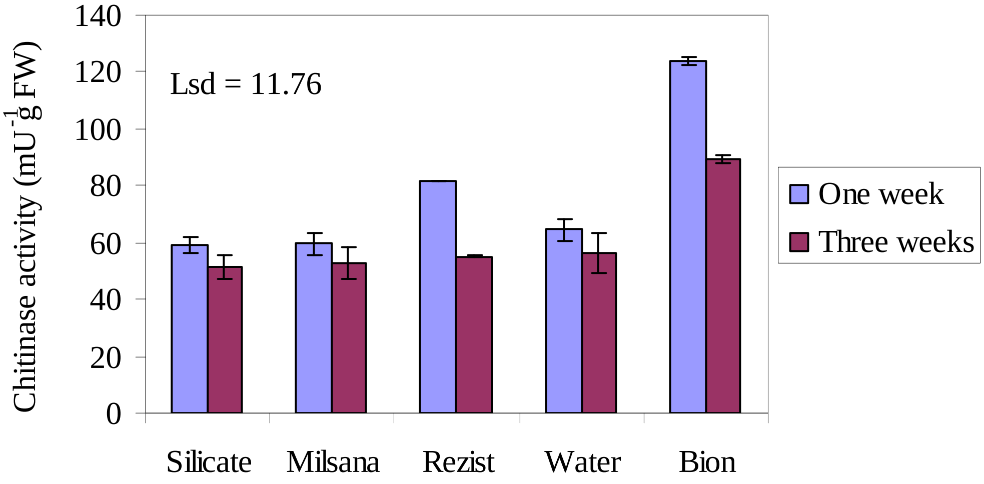

Figure 5 Chitinase activity in cucumber leaves of glasshouse grown cucumbers treated with SAR and GRAS chemicals. Leaf samples were collected one and three weeks after the second application of the chemicals (n = 8).

Chitinase activity (Figure 5) was enhanced in leaves treated with Bion® and Rezist® compared to the leaves treated with water. After three weeks the chitinase activity of all treatments dropped. The presence of fungal spores on all treatments may have stimulated a local defence response however this response was not sustained. The SAR response was sustained longest in the Bion® treated leaves. It is interesting to note that Milsana® did not show a significant increase in chitinase activity.

Conclusions Experiment 1 and 2

- Treatment with Bion®, Rezist® and Milsana® were able to reduce the severity of powdery mildew infection in cucumbers whereas treatment with silica did not.
- Bion® induced the largest increase in chitinase activity and the increase in activity was sustained for 3 weeks after inoculation. The SAR response induced with Rezist® was not as great and did not last for 3 weeks.
- Milsana® did not induce a greater activity of chitinase in cucumber leaves. There is debate as to the mode of action of Milsana®, it may only induce a local response.

3. Glasshouse Screening of SAR Elicitors for powdery mildew control on zucchini.

Figure 6 shows the severity of powdery mildew on zucchini seedlings grown in pots. The overall severity was lower in this screening trial than it was in the cucumber trial. The results show that Bion® and Milsana® reduced the severity of powdery mildew. Rezist® was less effective when applied to zucchini than it was on cucumbers seedlings (Figure 2).

`

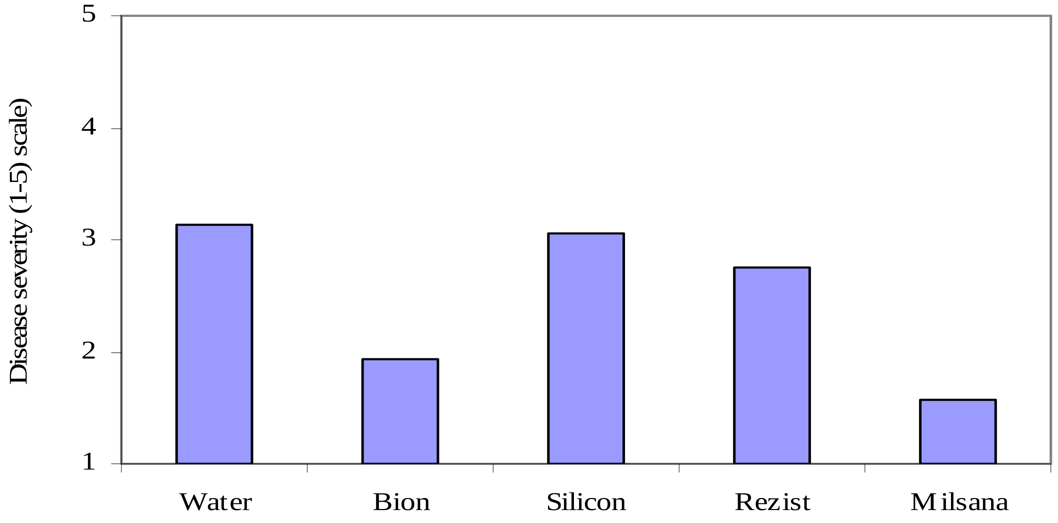

Figure 6 Severity of powdery mildew on Zucchini following the application of SAR and GRAS chemicals. Disease severity was scored two weeks after the second spray.

Figure 7 shows the corresponding levels of chitnase activity for the different treatments and the levels were very similar to those shown in cucumbers. Bion® induced the greatest increase in activity which was sustained over 3 weeks compared to Rezist® which showed an initial increase that dropped off by week 3. The sustained response induced by Bion® is likely to be one of the reasons for its efficacy in controlling the visual powdery mildew symptoms. Again Milsana® was able to reduce the severity of powdery mildew but it did not induce a heightened level of chitinase activity.

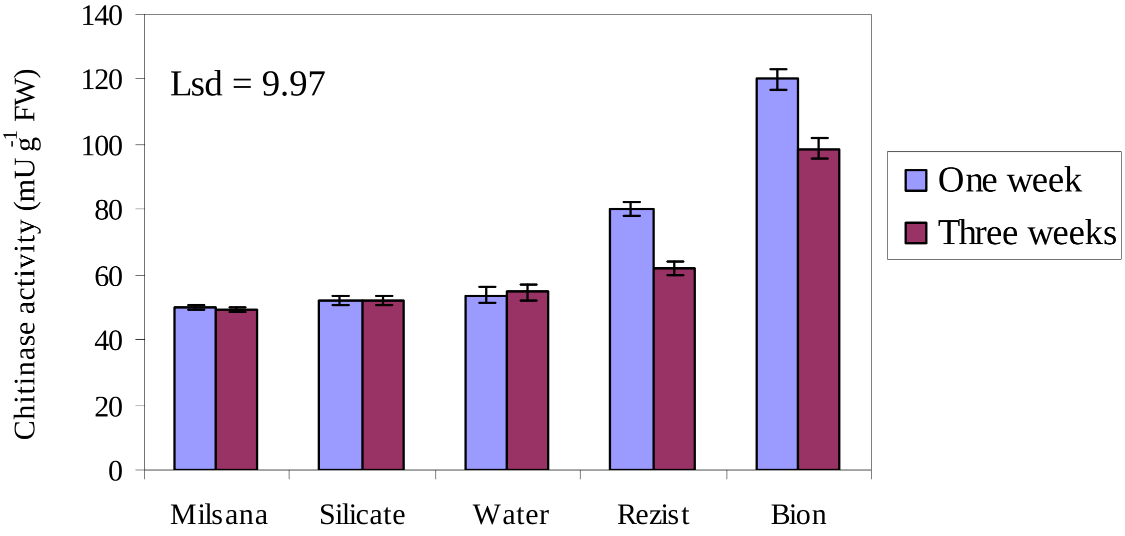

Figure 7 Chitinase in leaf tissue of Zucchini treated with SAR chemicals. Leaf samples were collected after one week and 3 weeks following spray with chemicals.

`

Conclusions Experiment 3

- Bion® and Milsana® reduced the severity of powdery mildew in zucchini seedlings. Rezist® was less effective when applied to zucchini than it was for cucumbers seedlings.
- Bion® induced the greatest increase in chitinase activity which was sustained over 3 weeks compared to Rezist® which showed an initial increase that dropped off by week 3. The sustained response induced by Bion® is likely to be one of the reasons for its efficacy in controlling the visual symptoms of powdery mildew in zucchini.
- Milsana® was able to reduce the severity of powdery mildew but it did not induce a heightened level of chitinase activity.

4. First field trial for glasshouse grown cucumbers

The results showed that the application of Bion®, Rezist® and Milsana® significantly reduced the severity of powdery mildew compared to the water treatment (Figure 8). It is important to mention that in week 2 the fungicide treatment had high levels of powdery mildew, more than the other treatments. The fungicide Amistar® was applied but it did not control the powdery mildew and so in week 4 the fungicide treated plants again had a higher severity of powdery mildew than the other treated plants. In week 4 the fungicide was changed to Triad® 125 and at week 6 the severity of the powdery mildew on the fungicide treatment plants was significantly reduced.

The plants with the lowest disease severity were the Bion® (BTH) treated plants. The photographs show a comparison of the severity of symptoms for the 4 week spray application. The photographs show that the severity of the infection in the poly tunnel was severe and this was the result of the unsettled weather conditions experienced during the trial.

Figure 10 shows the cucumber fruit yield for each treatment and there was no significant difference between the treatments. Harvesting was done over a 2 week period in the middle of the harvesting period rather than collecting all fruit from each treatment.

`

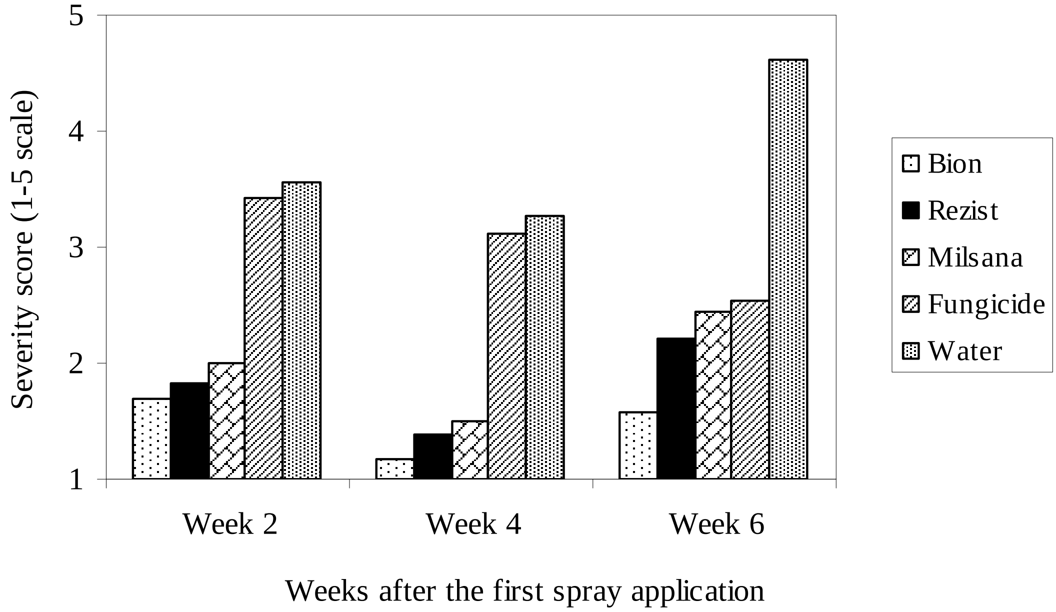

Figure 8 Severity of powdery mildew on cucumber leaves grown in a commercial poly tunnel November 2006. The effect of the chemical spray was assessed by scoring the emerging leaves. At week 2 the 9th, 10th and 11th emerging leaves were scored for the diseases severity while on week 4 and week 6 the 6th, 7th and 8th leaves were scored because of increased disease pressure. The values are the average of the three leaves per plant per replicate (n = 12).

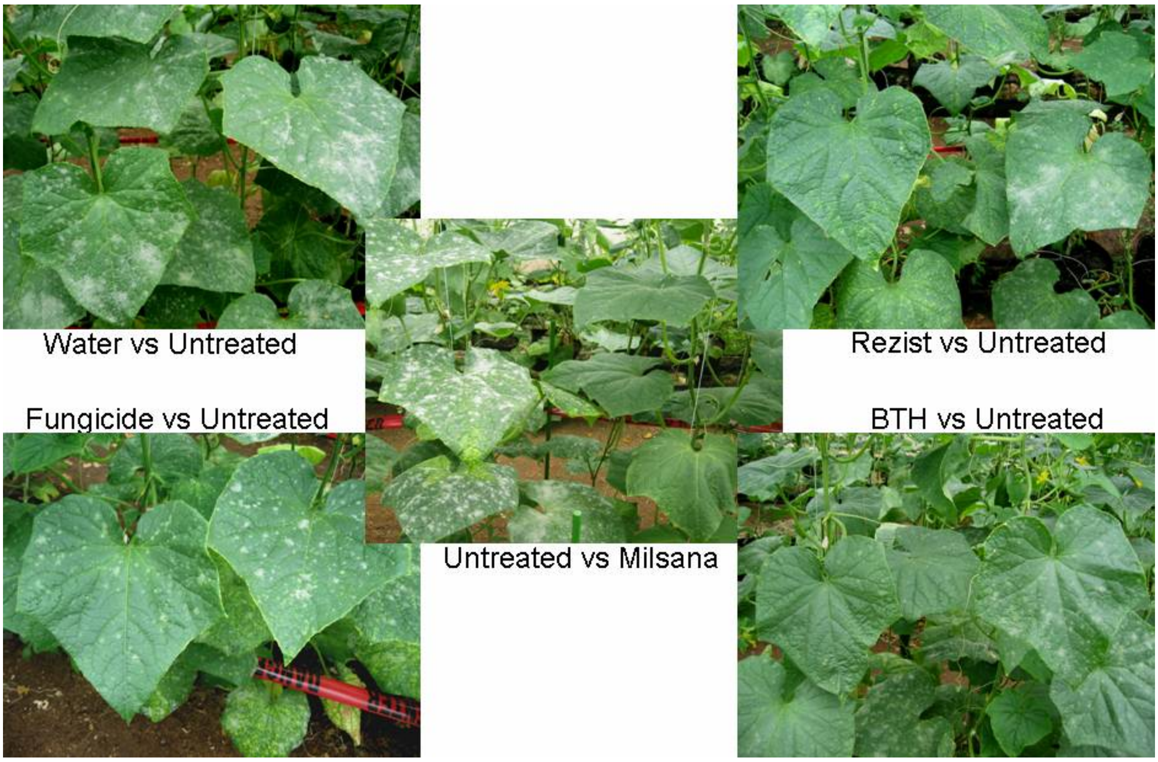

Figure 9 Severity of the powdery mildew on cucumber plants sprayed with SAR and GRAS chemicals. Pictures are the two adjacent plants of two different treatments (as labelled).

`

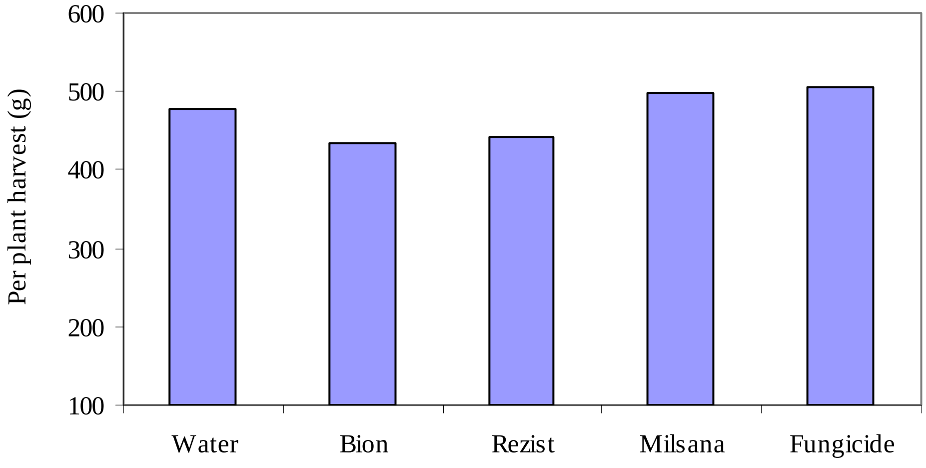

Figure 10 Effect of spray chemicals on the yield of cucumber. The harvest data was taken from four successive harvests in the middle of the harvesting period of the crop.

Figure 11 shows the severity of powdery mildew for plants treated with a single application of either Bion® or Rezist® and multiple fortnightly applications. The results show that more than one application of Bion® or Rezist® is required to ensure a significant reduction in the severity of powdery mildew present on the plants. One application reduces the severity but multiple applications reduce the severity even further. As expected the severity of the disease was highest on the older leaves (leaf 6) compared to the younger leaves (leaf 4).

Multiple applications of Bion® reduced the severity of powdery mildew in this trial more than the application of the fungicide and part of the reason for this was that Amistar® was not effective in controlling powdery mildew in this trial. This result highlights the need to develop alternative powdery mildew control strategies and that SAR is one option to consider.

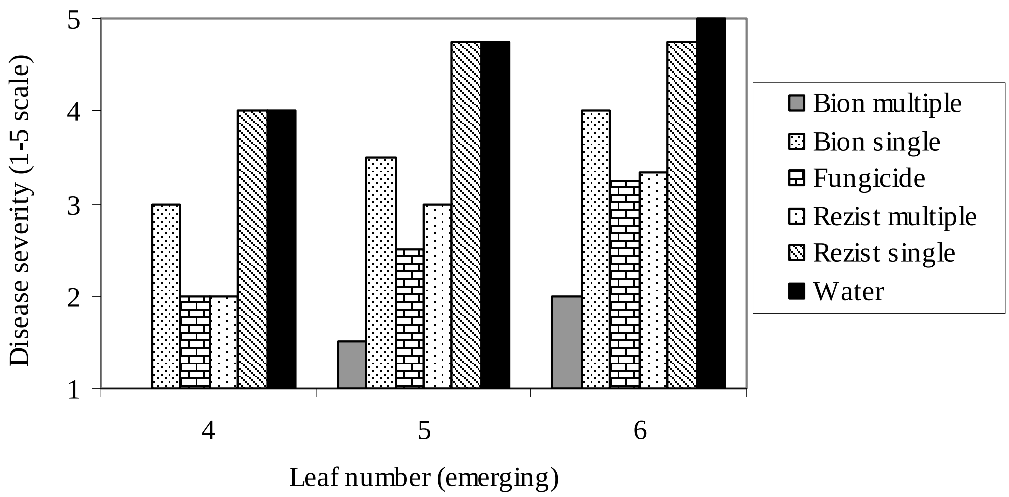

Figure 11 Severity of powdery mildew on cucumber leaves treated with SAR chemicals for different times. Scoring of disease severity performed six weeks after the 1st spray of the chemicals.

`

Chitinase activity: There was a significant difference in the chitinase activity of the different SAR treatments (Fig. 12). The chitinase activity was much greater for leaves treated with Bion® and Rezist® compared to the water treatment which indicates that this two chemicals do in fact induce SAR in cucumbers.

It is interesting to note that a single application of either Bion® or Rezist® induced an increase in chitinase activity but the activity was not sustained. For a persistent heighten chitinase or SAR response more than one application of the elicitors is required.

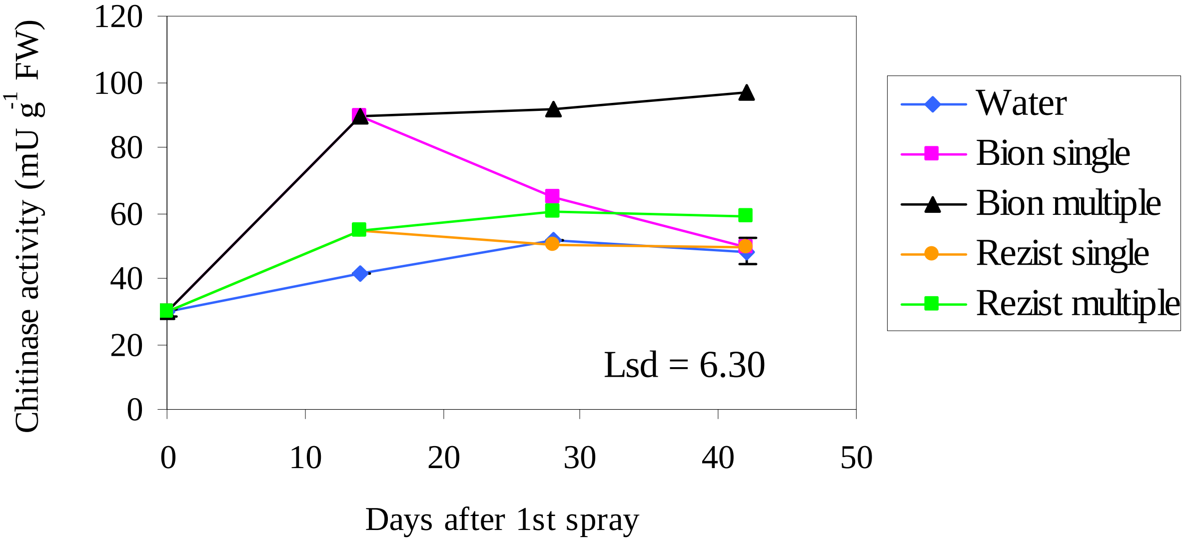

Figure 12 Chitinase activity of cucumber leaves treated with single and multiple applications of Bion®, Rezist® and water. Leaf samples were collected from each treatment prior to the fortnightly application of the treatment. The 5th and 6th leaves of each of the treated plants were collected (n=6).

Conclusions Experiment 3 – First Field Trial for Cucumbers

- The results show that the application of Bion®, Rezist® and Milsana® significantly reduced the severity of powdery mildew. The plants with the lowest disease severity were the Bion® (BTH) treated plants.
- There was no significant difference in the yields of the different treatments.
- The results show that more than one application of Bion® or Rezist® is required to ensure a significant reduction in the severity of powdery mildew present on the plants. It is interesting to note that a single application of either Bion® or Rezist® induced an increase in chitinase activity but the activity was not sustained. For a persistent heighten chitinase or SAR response two applications of the elicitors is required.
- Two applications of Bion® reduced the severity of powdery mildew in this trial more than the application of the fungicide and part of the reason for this was that the fungicide Amistar® was not effective in controlling powdery mildew in this trial.

`

5. Results and Discussion for the second field trial with SAR and GRAS chemicals to control powdery mildews of cucumber and zucchini

Results - Cucumber

The results of the experiment showed a significant control of powdery mildew by the application of one or two of Bion® or a combination of Bion® and Milsana® (Figure 13). The best control of powdery mildew was obtained by applying two sprays of Bion® or a combination treatment of Milsana® spray followed by a Bion® spray. A similar application pattern using Rezist® and Milsana® controlled powdery mildew but to a slightly lesser extent than the treatments with Bion® and Milsana®.

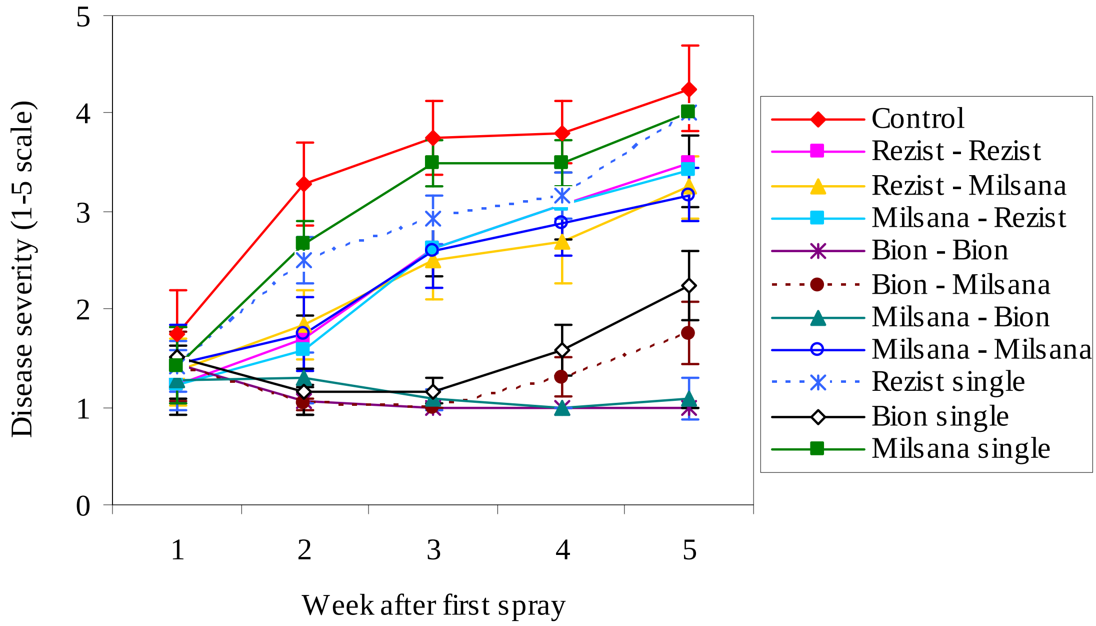

Figure 13 Severity of powdery mildew on cucumber leaves. The leaves were assessed by scoring the basal leaves during week 1, 2 and 3 after the first spray while week 4 the scoring was performed from emerging leaves and on week 5 the whole plant was given a score. The bars on the lines represent standard errors of the treatments.

The leaf samples that were analysed for chitinase were found to increase in enzyme activity within seven days of the first spray of either Bion® or Rezist® but not with Milsana® (Fig. 14). A second spray of Bion® or Rezist® further increased the activity of the enzyme. A single spray of Bion® maintained a heightened level of chitinase activity for the five weeks of assessment. The chitinase activity increased more after the second application of Bion®, however the increase was not statistically different to the level of activity reported for the single application of Bion®. The chitinase activity increased to a moderate level with Rezist®, however the response quickly declined in the following weeks after a single application. Multiple applications of Rezist® increased the chitinase activity further and the response was maintained at the heightened level for the next 5 weeks. The increase in chitinase activity in Rezist® treated leaves was less than the activity reported for the Bion® treatments.

`

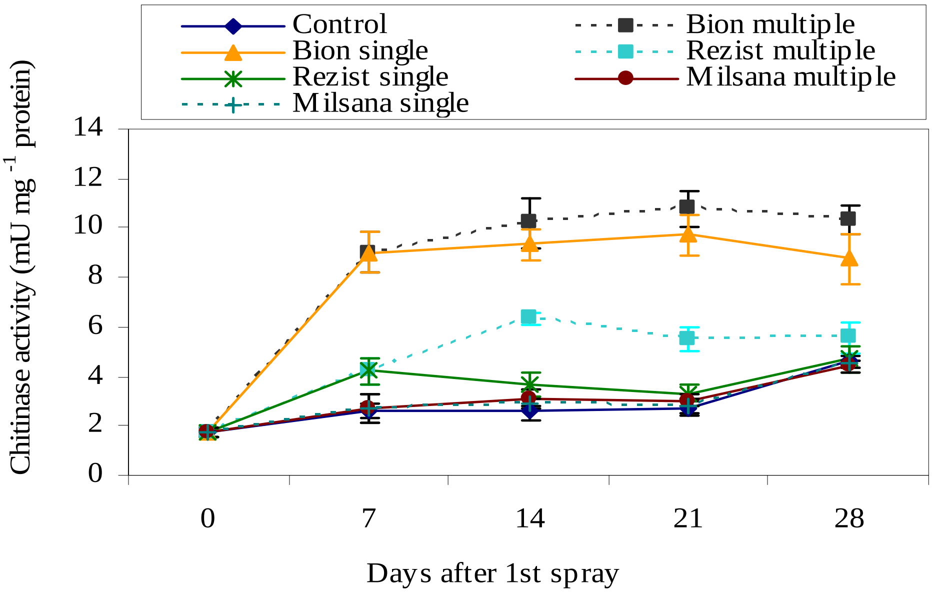

Figure 14 Effect of chemical elicitors on the chitinase activity of cucumber leaves sprayed with either Bion® or Rezist® or Milsana® once (single) or twice (multiple). The values in the graph are the average of three replicates assayed in duplicate. The bars in the lines represent the standard errors.

The effect of the application of the SAR chemicals on the growth of cucumber plants was also recorded. The application of Bion® in this experiment caused some leaf curling as a result of phytotoxicity of the chemical (Fig. 15C). A Milsana® spray before or after a Bion® spray minimized the amount of leaf curling (Fig. 15A and `5B). Treatment with Milsana®, Rezist® or the commercial fungicide did not show any effect on the growth of the cucumber plants (Fig. 15D, 15E and 15F).

`

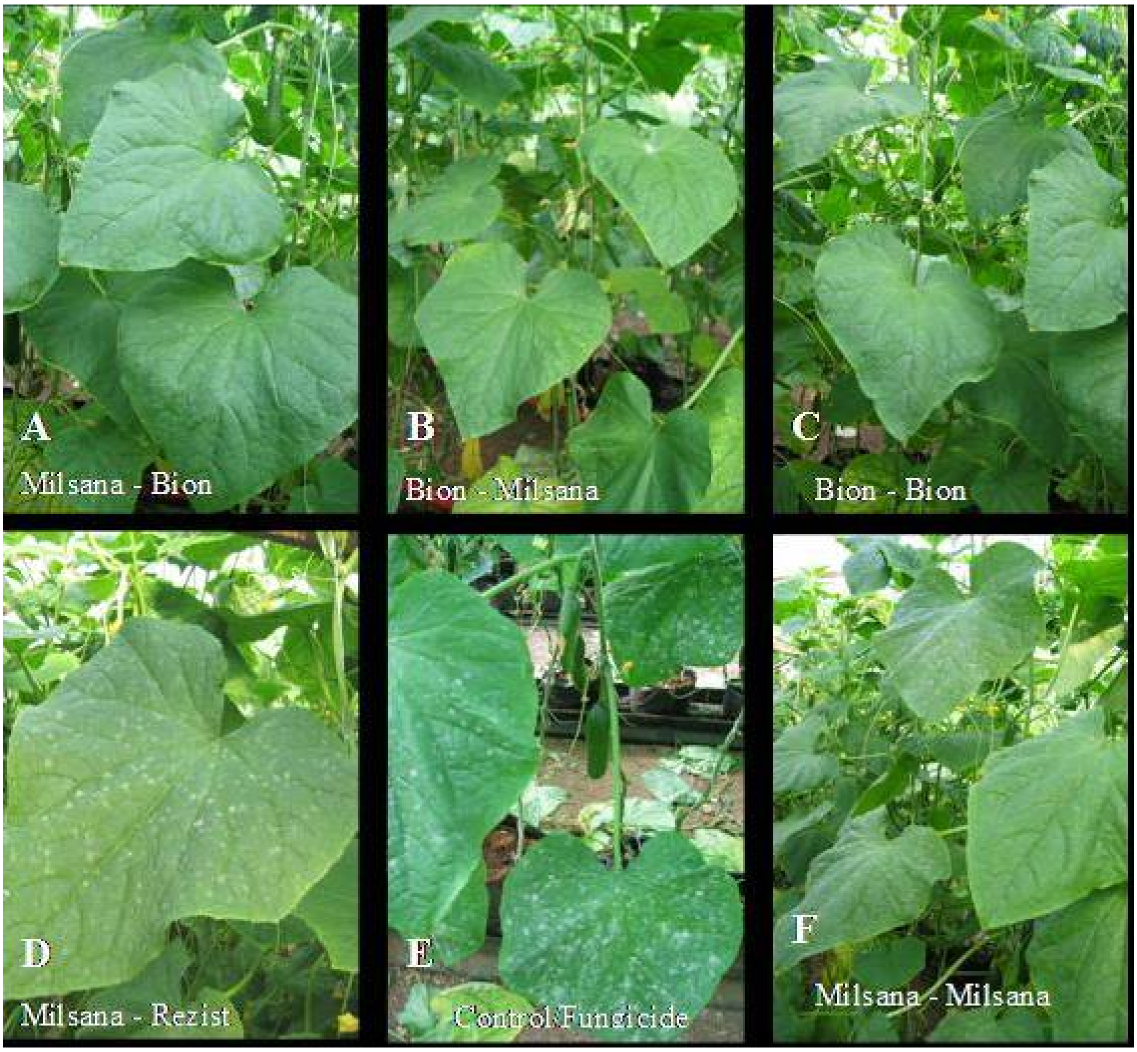

Figure 15 The level of powdery mildew that developed on cucumber plants treated with different chemicals. Pictures were taken four weeks after the 1st spray of the chemicals. The control plants were treated with the fungicide Triad 125® @ 0.075% plus silica magic at 200 ppm at the time of the 1st spray and a second spray was applied four weeks later.

Plants not treated with Bion® or Milsana® had very severe powdery mildew infections which resulted in some defoliation of the plants (Fig. 16B).

`

Figure 16 Cucumber plants treated with chemical elicitors with healthy basal leaves (A) vs control plants (fungicide treated) with premature defoliation due to infection with powdery mildew (B). The plants treated with Bion® alone or in combination with Milsana® were protected from powdery mildew and had healthy basal leaves compared to the control plants.

`

The effect of the chemical elicitors on the yield of cucumber was assessed by measuring the number and weight of fruit harvested. The results showed a significant reduction in the number of fruit from the two applications of Bion® (Fig.17). There was however, an increase in the number of fruit from the Milsana® treated plants (single or multiple sprays). Treatments that contained Milsana® in rotation also showed an increase in the total fruit number compared to a single spray of Bion® or Rezist®. Similar results were obtained for the total weight of fruit, where the Milsana® treatments showed an increase in fruit weight compared to the other treatments (Figure 18).

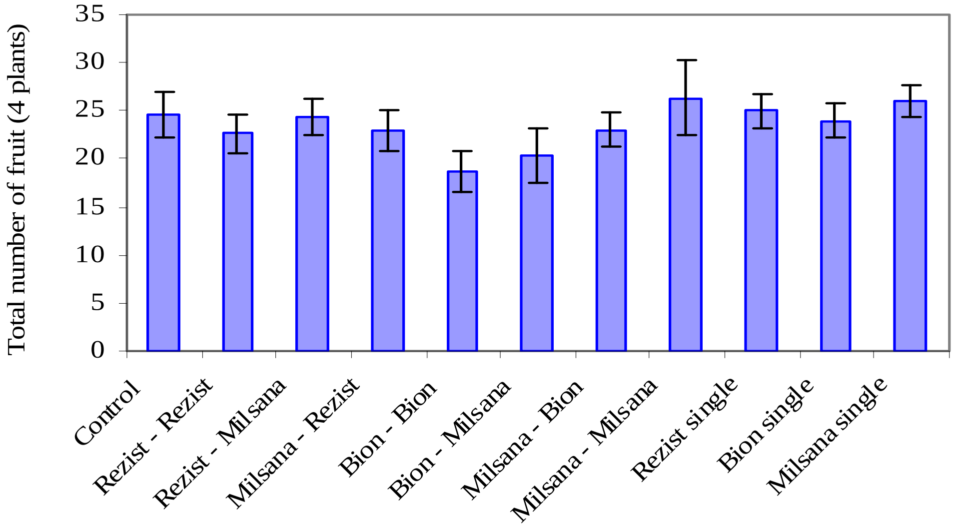

Figure 17 The effect of chemical elicitors on the number of fruit harvested per treatment. Harvest data was collected by continuously harvesting commercial sized fruit for two weeks on alternate days. The bars on the columns represent the standard errors of the treatments.

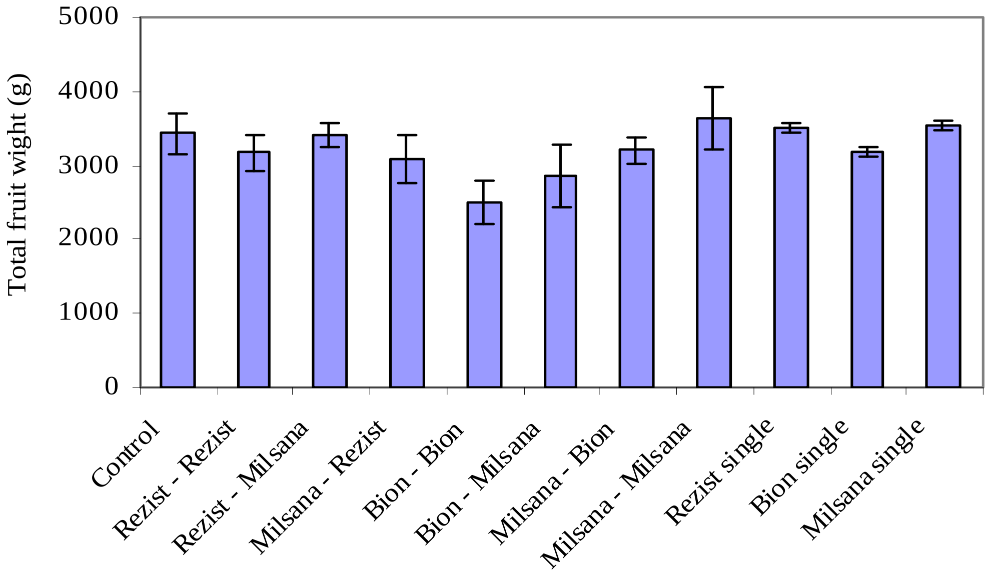

Figure 18 The effect of the chemical elicitors on the total fruit weight harvested over two weeks. The values are averages of three replicates. The bars on the columns represent the standard errors of the treatments.

`

Discussion – Second Field Trial of Cucumbers

The study on cucumber plants showed a reduction in the severity of powdery mildew on plants treated with Milsana® and Bion®. The disease reduction was most significant for the plants treated with Bion® and Milsana® in rotation. In this study a single or multiple application of Milsana® did not yield significant control of powdery mildew of cucumber plants. The analysis of chitinase activity in the leaves showed a positive correlation with the reduction in the severity of powdery mildew on the cucumber plants. The occurrence of a higher chitinase activity and a lower severity of disease indicated a high level of SAR as a result of the Bion® treatments. Although there was a moderate increase in the activity of chitinase in the Rezist® treated leaves, the enzyme activity may not have been high enough to protect the plant from powdery mildew in a high disease pressure situation as occurred in this experiment.

In the previous field experiment on cucumber during the spring, multiple sprays with Milsana® had shown greater control of powdery mildew. The lower effectiveness of Milsana® in this study may be because of seasonal variation which affected the virulence of the pathogen and/or the disease pressure which was too high to be overcome by weekly sprays with Milsana®. This study has shown that treatment with Milsana® rotated with Bion® at weekly intervals did effectively control powdery mildew.

Although two sprays with Bion® significantly increased resistance in the plants against powdery mildew, this treatment did affect plant development and as a result, compromised yield. The study however, showed that treatment with Bion® in rotation with Milsana® can minimize the adverse phytotoxic affects of Bion® or Rezist® applied on there own.

Results - Zucchini

There was no significant difference in the severity of the powdery mildew incidence on the leaves of the different treatments for zucchini (Figure 19). However there was an indication that treatment with Rezist® and Milsana® did reduce the disease severity but the variation between replicates was high and so the treatment effect was not significant.

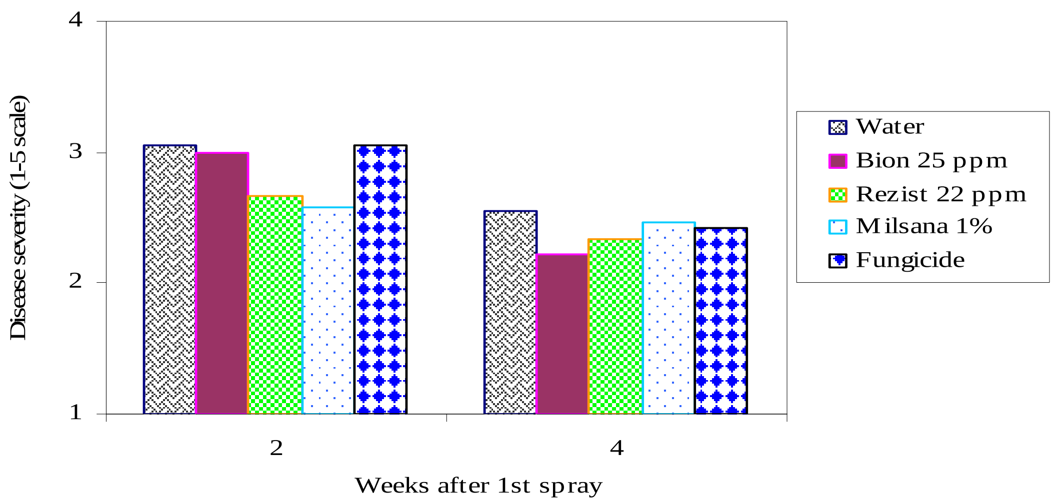

Figure 19 The effect of chemical elicitors on the severity of powdery mildew of zucchini grown in the field. Two week old plants first sprayed with Bion® or Rezist® or Milsana® or fungicide (Amistar®) or water. The second and third applications of the chemicals were applied at fortnightly intervals. The severity of powdery mildew was scored two and four weeks after the first spray application.

`

Figure 20 shows that there was a significant increase in the chitinase activity of zucchini plants 14 days after the first spray application of Bion® ond Rezist® (Fig 20). The increase in chitinase activity dropped by day 28 in most of the treatments except for the multiple application of Bion® at 50 ppm. Multiple application of Bion® at 50 ppm had significantly increased chitinase activity compared to Bion® at 25 ppm or a single spray at 50 ppm. No significant increases were observed in chitinase activity for single or multiple sprays of Rezist®.

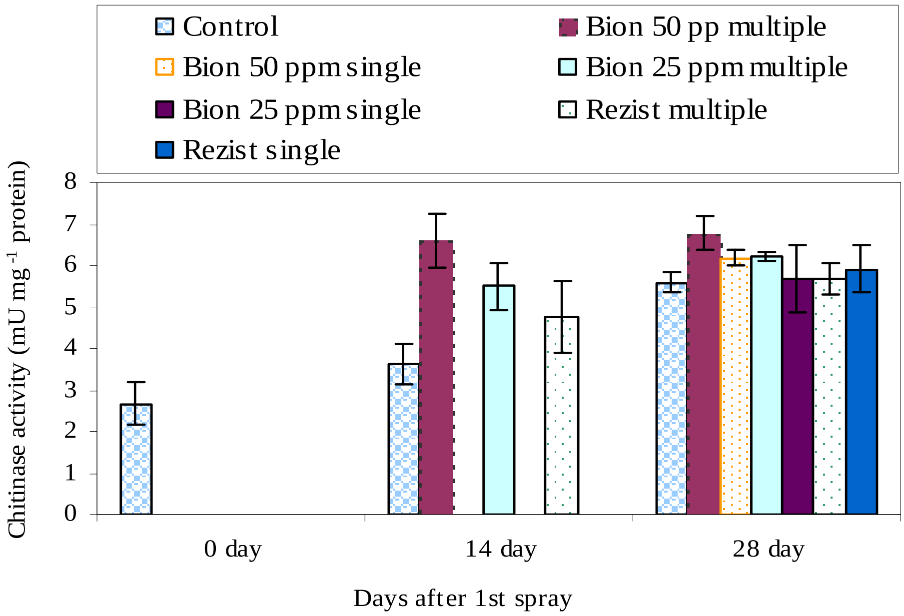

Figure 20 The effect of chemical elicitors on the level of chitinase activity of zucchini plants. Leaf samples were collected on day 0, 14 and 28 after the first spray of the chemicals by cutting off a quarter of the 4th fully expanded leaf from four plants of each replicate. The values in the graph are the average of three replicates assayed in duplicate. The bars on the column represent the standard errors of the treatments.

Discussion – Second Field Trial of Zucchini.

In both trials the zucchini cultivar "Columbia" was grown. This was a very vigorous cultivar. It seemed that this cultivar had some inherent disease resistance as the response to the treatments was very variable between replicates in both trials. However during the first 4 weeks of the trials there was an indication that the Rezist® and Milsana® treatments did reduce the severity of the powdery mildew infection. However the variability between the replicates meant that the results were not statistically significant. Further work is needed to determine if in very vigorous cultivars the treatments need to be applied over shorter intervals or at higher concentrations to induce a sustained response that can control powdery mildew in zucchini.

It is interesting to note the difference in the level of response to the SAR elicitors seen in cucumbers and zucchini's.

`

Technology Transfer

1. Article for Good Fruit and Vegetable Magazine July 2005 "Plants Can Protect Themselves Against Disease"

2. Article for Good fruit and Vegetable Magazine January 2007 "Systemic Acquired Resistance (SAR) controls powdery mildew in cucumbers"

3. Conference presentation Australasian Plant Pathology Conference Adelaide Sept 2007 "Exploitation of natural plant resistance to control powdery mildew in cucurbits"

4. Presentation to ACIAR SAR workshop in Brisbane October 2007

5. Article in Vegetables Australia September 2007 "Self defence Unlocks the Door"

6. A research paper that will be submitted to an appropriate refereed journal is in being prepared.

`

Conclusions

The research showed that powdery mildew could be controlled in cucumbers grown in poly tunnels using elicitors of SAR.

- Bion® provided consistent and long lasting control of powdery mildew
- At high disease pressure Milsana® and Rezist® were less effective
- Bion® or Rezist® applied 3 times on their own affected plant growth
- Rotational application of elicitors improved outcomes
- Milsana® followed by Bion® provided better disease control than Bion® followed by a Milsana®
- A rotational spray of Milsana® minimised the phytotoxicity of Bion® or Rezist®
- A rotational spray program using Bion® and Milsana® performed much better than the conventional fungicide program
- In zucchini the application of Bion® and Rezist® induced a systemic response as indicated by an increase in chitinase activity.
- In zucchini the application of Bion® and Rezist® did not significantly reduce the severity of the powdery mildew infection. Part of the reason for this was the large variability in response between treatment replicates. More work is required for zucchini.
- This work shows that the tested chemical elicitors do have the potential to exploit the plants' natural disease resistance and as a result their use could minimise the use of synthetic fungicides in cucurbit crops.

`

Recommendations

1. It is recommended that more work is done to investigate the interaction between plant health, plant nutrition and the induction of SAR. This work could help to improve the consistency of the SAR response and subsequent disease control in vegetable crops.

2. It is recommended that Bion® and Milsana® are investigated further for use on vegetables by first applying for minor use permits. AgAware Consulting Pty Ltd have been asked by AusVeg to evaluate minor-use proposals, design data generation processes in support of permit applications, coordinate permit applications and liaise with the APVMA and growers. It is recommended that Bion® and Milsana® are included in this program.

`

Literature cited

Agostini, J.P., Bushong, P.M. and Timmer, L.W. 2003. Greenhouse evaluation of products that induce host resistance for control of Scab, Melanose and Alternaria Brown Spot of citrus. Plant Disease, 87: 69- 74.

Bokshi, A.I., Morris, S.C., McConchie, R. and Deverall, B.J. 2006. Pre-harvest application of 2,6-dichloroisonicotinic acid, β-aminobutyric acid benzothiadiazole to control post-harvest storage diseases of melons by inducing systemic acquired resistance (SAR). The Journal of Horticultural Science & Biotechnology, 81: 700-706.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities utilising the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.

Hammerschmidt, R. and Yang-Cashman, P. 1995. Induced resistance in cucurbits, p 63-85. In: Hammerschmidt R. and Kuć J. (eds.), Induced resistance to disease in plants. Kluwer Academic, Amsterdam.

Heil, M., Hilpert, A., Kaiser, W. and Linsenmair, K.E. 2000. Reduced growth and seed set following chemical induction of pathogen defence: does systemic acquired resistance (SAR) incur allocation cost? Journal of Ecology, 88: 645-654.

Kelly. G. 2007. Cucurbit production in Australia. Acta Horticulturae, 731: 479-484. Proceedings of the III international Symposium on Cucurbits.

Kirrane, E.F., Hoppin, J.A., Kamel, F., Umbach, D.M., Boyes, W.K., DeRoos, A.J., Alavanja, M and Sandler, D.P. 2005. Retinal degeneration and other eye disorders in wives of farmer pesticide applicators enrolled in the agricultural study. American Journal of Epidemiology, 161: 1020-1029.

Konstantinidou-Doltsinis, S., Markellou, E., Kasselaki, A.K., Fanouraki, M.N., Koumaki,, C.M., Schmitt, A., Liopa-Tsakalidis, A. and Malathrakis, N.E. 2006. Efficacy of Milsana®, a formulated plant extract from Reynoutria sachalinsis, against powdery mildew of tomato. BioControl, 51: 375-392.

Letham, D.B. and Priest, M.J. 1989. Occurance of cleistothecia of Sphaerotheca fuliginea on cucurbits in South Australia and New South Wales. Australian Plant Pathology, 18: 35-37.

McGrath, M.T. 2001. Fungicide resistance in cucurbits powdery mildew: Experiences and challenges. Plant Disease, 85: 236-245.

O'Brien, R.G. 1994. Fungicide resistance in populations of cucurbit powdery mildew (Sphaerotheca fuliginea). New Zealand Journal of Crop and Horticultural Science, 22: 145-149.

Raskin, I. 1998. Biological role of precursors and metabolites of salicylic acid. ICPP'98. Abstract (Web site).

Schirra, M., D'hallewin, G., Ben-Yehoshua, S. and Fallik, E. 2000. Host-pathogen interactions modulated by heat treatment. Postharvest Biology and Technology, 21: 71-85.

ahr applied horticultural research

PO Box 3114 Bundeena NSW 2230 Australia

phone +61 2 9527 0826 **fax** +61 2 9544 3782