Efficiency of SAR for disease control in Rhubarb: a preliminary study

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Know-how for Horticulture™

Applied Horticultural Research Pty Ltd

Project VG09031

Efficacy of SAR for disease control in Rhubarb:
A preliminary study.

Prepared by Dr Jenny Jobling

Horticulture Australia Ltd Final Report

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Efficacy of SAR for disease control in Rhubarb: a preliminary study

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Media Summary

Healthy plants have the capacity to protect themselves from fungal disease. When a fungal spore is detected the plant is triggered to switch on different reactions to build up its defences. These defences can include several barriers. For example they can be chemical (phenolic compounds that are toxic to the fungi), physical barriers (lignin deposits to prevent hyphal growth) and anti-fungal compounds (enzymes that attack fungal cells). Interestingly, the plant can also be tricked into this response and this is known as the induction of systemic acquired resistance (SAR). The plant is ready before a pathogen attacks and so the protection is much better. This is a novel method of disease control that has been shown in many research trials that it can be a valuable part of an integrated disease management program.

However, it has only been recently that elicitors of SAR have been marketed for use on agricultural crops. This project investigated the use of SAR in rhubarb. Two trials were done; one in a glasshouse and one field trial. Plants were treated with either BION® (BTH, Syngenta, Basel, Switzerland @ 50 ppm), Milsana® (KHH BioSci, Inc of NC, USA @ 1% v/v), Rezist® (Stoller enterprises, Houston, Texas, USA @ 44 ppm); Betaine® (Sigma Aldrich, USA @ 50 mM) or water as a control.   The induction of resistance in the plant was assessed by scoring the leaf spot disease caused by *Ascochyta rhei* (with a characteristics disease symptoms of more or less circular, light-tan spots) and by measuring the activity of the SAR marker enzymes, chitinase and β-glucanse.

The glasshouse results showed that plants treated with BION® 50 ppm and Betaine® 50 mM had a significantly lower level of leaf spot disease than the other treatments. However, in the field trial there were no significant differences between the levels of disease severity for any of the treatments and no difference in the levels of enzyme activity.

The results suggest that SAR may have been induced in the glasshouse but not in the field trial. One of the reasons for a reduced response was suggested by the grower. He observes that at the time of year when the trial was done the crop suffered from a transitional effect due to seasonal change and at this time the plants are more susceptible to disease. It would be good to repeat the experiments with a young, healthy crop to see if the variability in response is related to plant health at the time of application of the chemicals. The SAR response is an active plant response requiring energy and if the plant is under stress as a result of seasonal conditions then there may not be enough energy reserves available to establish SAR.

SAR has been shown to be a useful tool in many other crops. The variable results from this work warrant further investigation. The results show that more work is needed to adapt this technology to rhubarb. The establishment of SAR results in long-lasting resistance against a broad range of plant pathogens, including viruses, bacteria and fungi. The main benefits of SAR are that it exploits the natural defences of the plants against disease and pathogens will not develop resistance to this approach. It could therefore be a useful addition to an integrated disease control strategy for rhubarb growers.

Technical Summary

It has only been recently, that elicitors of systemic acquired resistance (SAR) have been marketed for use on agricultural crops. The most-studied resistance activator is acibenzolar-S-methyl (BTH, BION® (Syngenta, Basel Switzerland)). BION® is a synthetic analogue of salicylic acid that amplifies a wave of signals throughout the plant that results in a heightened defence level. With these increased defences, usually mediated by increased anti-fungal compounds or pathogenesis-related (PR) proteins, plants are significantly more resistant to disease.

However, it is likely the most effective disease control strategy is to use several chemicals in an integrated control program. One generally regarded as safe (GRAS) option is the product called Milsana® which is an extract from the Giant Knotweed (*Reynoutria sachalinensis*) (KHH BioSci, Inc of NC, USA). This is a bioprotectant and our laboratory has shown some very promising results using this product in combination with BION® against powdery mildew in cucurbits.

Rhubarb plants were grown in a glasshouse at the University of Sydney and were treated with either BION® (BTH, Syngenta, Basel, Switzerland @ 50 ppm), Milsana® (KHH BioSci, Inc of NC, USA @ 1% v/v), Rezist® (Stoller enterprises, Houston, Texas, USA @ 44 ppm); Betaine® (Sigma Aldrich, USA @ 50 mM) or water as a control. The induction of resistance in the plant was assessed by scoring the leaf spot disease caused by *Ascochyta rhei* and by measuring the activity of the SAR marker enzymes chitinase and β-glucanse.

The glasshouse results showed that plants treated with BION® 50 ppm and Betaine® 50 mM had a significantly lower level of leaf spot disease than the other treatments. Rezist® 44 ppm and Milsana® 1% (v/v) did not significantly reduce the disease incidence compared to the control plants. However, despite the positive result for BION® 50 ppm and Betaine® 50 mM there were no significant differences in the level of activity of chitinase and β-glucanse enzymes.

Further work was done using the same chemicals in a field trial. Rhubarb (cv. Sydney Crimson) was grown on a commercial property on the out skirts of Sydney were treated with BION® 50 ppm, BION® 100 ppm, Betaine® 50 mM, Betaine® 75 mM, Milsana® 1% and a water control.

In this field trial there was no significant difference between the levels of disease severity for any of the treatments. The results from the analysis of the enzyme activity of the leaf tissue for the field grown rhubarb also showed no significant differences between the treatments.

The results show an inconsistent response. It is not clear if SAR was induced in rhubarb or not. More work is needed to explain the difference in response between the glasshouse trial and the field trial. It would be good to repeat the experiments with a young, healthy crop as plant health may have been a contributing factor to the difference in results. It has been shown that the induction of SAR is greater in plants that are healthier in other crops.

SAR has been shown to be a useful tool in many other crops. The variable results from this work warrant further investigation. The results show that more work is needed to adapt this technology to rhubarb. The establishment of SAR results in long-lasting resistance against a broad range of plant pathogens, including viruses, bacteria and fungi. The main benefits of SAR are that it exploits the natural defences of the plants against disease and pathogens will not develop resistance to this approach. It could therefore be a useful addition to an integrated disease control strategy for rhubarb growers.

Introduction

This project investigated using systemic acquired resistance (SAR) and generally regarded as safe (GRAS) chemicals as part of an integrated programme to control the major diseases of rhubarb. SAR and GRAS chemicals have the advantage of having multiple modes of action and they are not pathogen specific. As a result several diseases can be controlled using the same chemicals and the risk of developing resistance is very low. The development of resistance by pathogens to chemical controls is a big problem for growers. Growers are finding that their armoury against plant diseases is dramatically shrinking. The use of SAR might provide growers with a new tool that won't develop resistance against plant disease.

It is important to point out that SAR is unlikely to control disease to the level the market requires when used alone. In situations where the disease severity is high it will also be necessary to use a fungicidal solution. The ideal control solution would be an integrated approach that uses SAR to reduce the plants susceptibility to disease with chemical controls as required.

Many diseases impact the yield and quality of rhubarb. In a rhubarb growers workshop held by HAL, October 17th 2008, several disease problems were identified. They included a range of viruses, root diseases such as *phythothera* sp, *Rhizoctonia sp* and *Pythium sp* and there are also foliar pathogens such as downy mildew, leaf spot and rust. The workshop also acknowledged that there are few fungicides registered for the control of these diseases *(Source: http://www2.dpi.qld.gov.au/horticulture/5196.html).* The use of systemic acquired resistance (SAR) therefore could offer another tool to growers in terms of integrated disease management.

1. Induced Resistance or Systemic Acquired resistance (SAR)

It has only been recently that elicitors of SAR have been marketed for use on agricultural crops. The most-studied resistance activator is acibenzolar-S-methyl (BTH, BION[®] (Syngenta, USA)).

BION[®] is a synthetic analogue of salicylic acid that amplifies a wave of signals throughout the plant that results in a heightened defense level (Kunz et al., 1997). With these increased defenses, usually mediated by increased anti-fungal compounds or pathogenesis-related (PR) proteins, plants are significantly more resistant to disease (Bokshi et al 2005a, 2005b, 2006, 2007; McConchie et al., 2006).

A recent review of the scientific literature found that there is limited research using SAR on rhubarb. However BION[®] was found to reduce the crown rot disease in strawberry (Eikem et al, 2002) and tomato (Benhamou and Theriault 1992) by the induction of SAR against the pathogens. It is therefore very likely that the same response would be induced in rhubarb.

Other research has shown a significant reduction in the severity of downy mildew on cauliflower through the induction of SAR by the application of BION[®] (Sharma et al, 2004, Ziadi *et al*. 2001) or BABA (acid beta-aminobutyric acid）(Silue *et al*., 2002). There is also research to show that SAR can reduce the severity of rust and leaf spot however not specifically in rhubarb. The success of SAR in other vegetable crops suggests that the same results are likely in rhubarb. In the HAL funded project VG05034 "Managing mildews: prevention using systemic acquired resistance (SAR) in greenhouse and field grown cucurbits" it was found that the response of SAR was more consistent when plants were growing well and that stress as a result of inadequate water or nutrition reduced the level of disease control when SAR was used.

It is possible that the most effective disease control strategy using several chemicals is an integrated control program. One GRAS option is the product called Milsana® which is an extract from the Giant Knotweed (*Reynoutria sachalinensis*) (KHH BioSci, Inc of NC, USA). This is a bioprotectant and our laboratory has shown some very promising results using this product in combination with BION® against powdery mildew.

This project aimed to screen the elicitors of SAR and Milsana® for their effectiveness as part of a disease control strategy for rhubarb. The compounds identified from this study that show promise can then be included in a larger trial and in that trial, data can be collected to meet the requirements for a minor use permit.

Materials and Methods

The SAR inducing chemicals that were trialled include BION® (BTH, (Syngenta, USA)) Rezist® (Stoller) and Milsana® (KHH BioSci, Inc of NC, USA). Betaine® (Sigma Aldrich, USA) which is used in human health was also included in this study because it has recently been shown to induce SAR in other crops (Vechet et al, 2009). This chemical is not registered for plant use at this stage. In the original proposal we proposed to also trial silicate. Our work with cucumbers showed that SAR was not induced with potassium silicate and as a result we substituted Betaine® as a result of successful results recently reported in the scientific literature. These products need to be trialled for efficacy, application rate and application timing. This research involved a preliminary glasshouse trial and a field trial.

The experiments focused on the leaf spot disease caused by *Ascochyta rhei* (Figure 1).

The preliminary trials were done at the University of Sydney glasshouse and the field trials were done by Applied Horticultural Research Pty Ltd in collaboration with a commercial grower, J & G Camilleri, Horsley Park, NSW 2175.

Rhubarb plant in the commercial farm infected with leaf spot (caused by the fungus *Ascochyta rhei*).

Figure 1. Symptoms of leaf spot disease in field grown rhubarb.

1. Laboratory and Glasshouse Screening

A glasshouse trial was conducted on rhubarb (cv. Sydney Crimson) to determine the effectiveness of a range of SAR inducing chemicals. The method was revised to the original one proposed.

Our original plan was to challenge inoculated discs of detached leaf discs in enclosed Petri dishes to determine the optimum concentration of the SAR chemicals and how long after treatment the plant was protected. This technique was not used as the preliminary trials with plants in pots showed that the natural inoculum was sufficient to show treatment effects which were very low.

Extra time was spent adapting the enzyme assays we used on cucurbits to rhubarb. The low pH of the rhubarb leaves meant that the original protocols needed to be revised and several attempts were needed until an effective method was developed.

For the glasshouse screening trial the rhubarb plants (cv. Sydney Crimson) were sourced from a commercial grower. The plants were grown in 8 litre pots containing commercially prepared potting mix. There were three pots per treatment and there were three replicates of each treatment. The SAR inducing chemicals were applied twice to four months old plants at one week intervals at the following rates:

1.   BION® @ 50 ppm
2.   Milsana® @ 1% (v/v)
3.   Rezist® @ 44 ppm
4.   Betaine® @ 50 mM
5.   Water control

The induction of resistance in the plant was assessed by scoring the leaf spot disease caused by *Ascochyta rhei* (with characteristic disease symptoms). Also, leaf samples were collected to determine the activities of two enzymes that are involved in the generation of pathogenesis related proteins (PR proteins); β-glucanase and chitinase. The incidence of the disease was scored on the third fully expanded leaf of each plant, three and six weeks after the first application of the SAR inducing chemicals using a severity scale of 1-5 where: 1 = no spots or fungal colony: 2 = <10 spots per leaf; 3 = ≥10 to <25 spots; 4 = ≥25 to <50 spots; 5 = >50 spots per leaf.

Enzyme analysis

The leaf samples used for the enzyme analysis were collected from the third fully expanded leaves of each plant one and two weeks after the first application of the SAR inducing chemicals and stored at -80°C.

Analysis of chitinase and β-glucanse were performed following the methods of Bokshi *et al*. (2006) and Rivie`re *et al*. (2008) with some modifications. In brief, 0.5 g of frozen leaf tissue was ground in liquid nitrogen with 2% v/v PVP (polyvinylpyrrolidone) in a mortar and transferred to a 2 ml micro-tube. The tissue was homogenised in 1 ml 50 mM potassium acetate buffer, pH 3.5, containing 1 mM EDTA and 5 mM reduced glutathione. Reduced glutathione (5 mM) was added to the buffer on the day of extraction. The homogenates were allowed to stand for 30 min in ice, and were stirred intermittently with an inoculation loop. To these homogenates

40 µl of 1 M NaOH was added and mixed well by stirring with an inoculation loop to bring the pH level to approximately 5.0. The homogenates were centrifuged at 10,000 rpm for 10 min at room temperature and the supernatants were transferred to micro-tubes to assay enzymes and total soluble protein.

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°C for 5 min. The reaction was started by adding 0.1 ml aqueous CM-Chitin-RBV (2 mg/ml; Loewe Biochemica, Sauerlarch, Germany) for chitinase or 0.1 ml of laminarin azure (4 mg/ml; Sigma) for β-glucanse. After 20 min, the reaction was terminated by adding 0.1 ml 2N HCl which precipitated the undegraded substrate. The tubes were cooled in ice for approx. 10 min, and then centrifuged for 5 min at 9000 rpm. The absorbance of the supernatant was measured at 545 nm for chitinase or 600 nm for β-glucanase against their acetate buffer blank. Results were calculated using a linear equation derived from the standard curves of the absorbance of serial dilutions of standard pure chitinase from Sigma Chemical Co., St Louis, MO, USA (product number C6137) or Malt β-glucanase, from Megazyme (Deltagen Australia Pty. Ltd). The specific activity of chitinase and β-glucanase were expressed as mUnits mg^{-1} of total soluble protein.

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

2. Commercial Field Trial

The results from the glasshouse trial showed that BION$^{®}$, Betaine$^{®}$ and Milsana$^{®}$ should be tested further under field conditions. A field trial was run (start 4th March 2010) on a commercial farm (Horsley Park, NSW 2175) with BION$^{®}$, Betaine$^{®}$ and Milsana$^{®}$ (Figure 2). The crop was a red stalked rhubarb (Sydney Crimson) originally planted during May 2009 in 1.2 m raised beds in double rows that were watered regularly with a sprinkler irrigation system. The rhubarb crop was managed using best commercial practice (Figure 1).

The chemicals were applied at the concentrations used in the glasshouse trial, however two additional treatments were also included; BION$^{®}$ @ 100ppm and Betaine$^{®}$ 75 mM.

The experimental treatments include; BION$^{®}$ 50 ppm, BION$^{®}$ 100 ppm, Betaine 50 mM, Betaine 75 mM, Milsana 1% (v/v) and a water control.

Figure 2. *Field experiment on rhubarb with SAR inducing chemicals for the induction of systemic resistance in a commercial farm at Horsley Park, NSW.*

The SAR elicitors were trialled using a factorial design experiment. There were 3 replicates per treatment with 4 plants in each replication. At the time of the first spray application there were some visible signs of leaf spot disease. The fact that there was disease present may have contributed to the lack of treatment effects. Once a fungal disease is established in a plant then the SAR response can slow the rate of infection but it is unable to kill the disease and completely prevent the spread of infection. This will be discussed in more detail in the results section.

The SAR inducing chemicals were sprayed twice at one-week intervals and a third spray was applied two weeks after the second treatment (application 0, 1, 2 and 4 weeks). During the experimental period no other agrochemicals were used on the plants.

The plants were examined for disease at the time of the first spray application and they were scored again 3 and 6 weeks after the first application of the chemicals. The incidence of the leaf spot disease was scored on the third fully expanded leaf, using a severity scale described in the earlier section.

Leaf samples were also taken for the analysis of chitinase and β-glucanase activity from samples collected one week and 2 weeks after the first application of the SAR inducing chemicals using the methods described in the earlier section.

The chemicals were only tested on their own and not in combination with commercial fungicides as the plant numbers were low as a result of rain prior to the start of the trial.

1.   Glasshouse Screening Trial Results

Experiment Aims

What is the optimal concentration of the SAR elicitors to the disease and how long after an application will it protect the host from disease?

How many times do the SAR elicitors need to be applied?

Results and Discussion

The glasshouse trial showed that spraying the leaves of rhubarb plants with BION$^{®}$ 50 ppm and Betaine$^{®}$ 50 mM treatments significantly reduced the severity of leaf spot disease compared to the control plants (Fig 3). The disease reduction from the application of Rezist$^{®}$ 44 ppm or Milsana$^{®}$ 1% was not significant compare to the control plants. The results of the experiment also show an increasing severity of disease over 6 weeks.

The results of the experiment suggested that there was some disease resistance development as a result of the application of BION$^{®}$ 50 ppm or Betaine$^{®}$ 50 mM. The average severity score for the control plants was 3.5 compared to 1.8 for BION® 50 ppm and 1.5 for Betaine® 50 mM. This means that there were still leaf spots on the leaves (< 10 spots) compared to 10 to 25 spots on the control leaves.
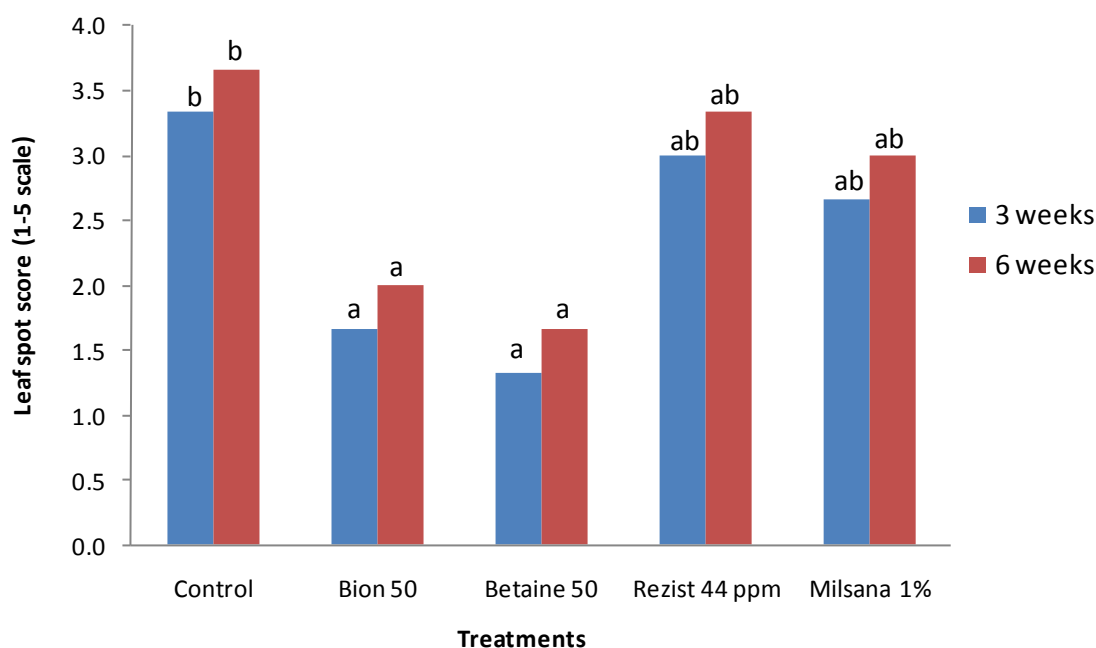
Figure 3. *Effect of SAR inducing chemicals on the severity of leaf spot disease of rhubarb plants. The disease severity was examined at three weeks (3 weeks) and six (6 weeks) after the first application of the treatments. The data is the mean of the severity scores. Columns with a different letter are significantly different (p = 0.05).*

It was also observed that the plants from the Betaine® and Milsana® treatments were greener than the control while the BION$^{®}$ treated plants showed signs of leaf senescence by being a paler green. A similar result was observed with Milsana® treated cucurbit plants (Bokshi et al, 2008).

Milsana® has been reported to make the plant foliage darker as it supplements nitrogenous nutrients (Copping and Duke 2007). As a result the rotation of Milsana® with SAR activators may control disease without compromising the yield. The reason for the darker foliage as a result of the application of Betaine® is unclear and more work is needed to understand this response.

The results of the enzyme analysis indicated no significant change in the activity of chitinase or β-glucanase from the application of SAR inducing chemicals (Table 1). The level of enzyme activity was unchanged in the samples collected one week after the spray with only a small increase (not significant) of both chitinase and β-glucanase activity in the BION® 50 ppm treated plants two weeks after the first application of the spray. The low level of enzyme activity suggests that there was no induction of resistance in the treated plants in this experiment. However more work is needed to confirm this result as the SAR response may induce other enzymes such as peroxidase or a response may be seen by screening for the induction of phytoalexins or pathogenesis related proteins and genes. This detailed analysis was beyond the scope of this project.

Table 1 *Activity of chitinase and β-glucanase in rhubarb leaves grown in the glasshouse treated with SAR inducing chemicals. The leaf samples were collected one week and two weeks after the first application of SAR inducing chemicals.*

Treatment	Chitinase activity (mUnit/mg protein)		β-glucanase activity (mUnit/mg protein)	
	One week	Two weeks	One week	Two weeks
Control	13.06 ($\pm$1.16)	15.96 ($\pm$1.99)	290.74 ($\pm$25.60)	355.41 ($\pm$16.06)
BION® 50 ppm	13.04 ($\pm$1.88)	17.531 ($\pm$1.79)	287.01 ($\pm$16.33)	364.24 ($\pm$15.76)
Betaine® 50 mM	12.78 ($\pm$2.12)	16.188 ($\pm$1.31)	294.20 ($\pm$14.06)	359.61 ($\pm$44.23)
Rezist® 44 ppm	12.95 ($\pm$0.98)	16.386 ($\pm$1.43)	292.09 ($\pm$23.37)	347.33 ($\pm$19.73)
Milsana® 1%	13.00 ($\pm$0.89)	15.556 ($\pm$1.74)	283.75 ($\pm$27.55)	358.88 ($\pm$20.56)
Level of significance (p=0.05)	NS	NS	NS	NS

Figures in the parenthesis indicate standard deviations (SD)

Due to the lack of significant treatment effects in this trial we were unable to clearly answer the questions originally posed; the ideal concentration and the duration of the SAR response. Extra treatments (BION® 100ppm and Betaine® 75 mM) were added to the field trial in an effort to determine a concentration effect. It was also hoped that the field trial results would determine the length of time of the SAR response.

2. Field Trial Results

The results from the assessment of the disease severity from the field trial showed that there were no significant differences between the treatments at any scoring time (Fig 4). However, there was a trend for the reduction in the level of disease severity over the 6 week period for plants treated with BION$^®$ 50 ppm and BION$^®$ 100 ppm. Treatment with Betaine$^®$ 50 mM and Betaine$^®$ 75 mM also showed a reduction in disease severity but this was not statistically significant.
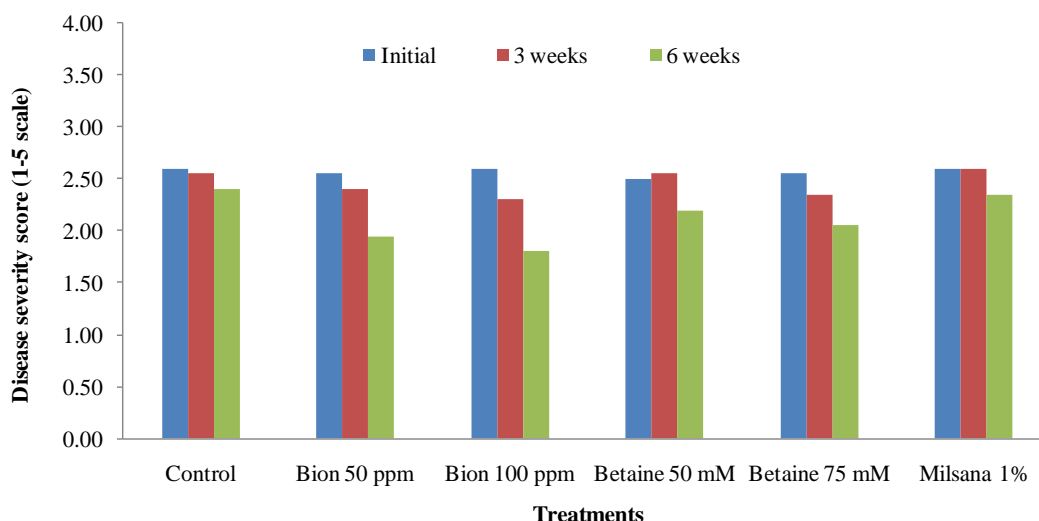

Figure 4. Disease severity of rhubarb plants treated with SAR inducing chemicals. Initial disease severity was scored at the time of the first application of the SAR inducing chemicals and then again 3 and 6 weeks after the first application of the chemicals. There were no significant differences between the levels of disease severity for any treatment at any time.

The leaf spot severity scores suggest that SAR was not induced in this trial. One of the reasons for a reduced response could have been that at the time of year when the trial was done the crop suffers from a transitional effect due to seasonal change and at this time the plants are more susceptible to disease. More work is needed to confirm this result. The overall disease incidence was also low with the disease score being between 10 to 25 spots per leaf. It might be better to have a more accurate scoring system in future experiments with smaller increments between categories.

The results from the analysis of the enzyme activity on the leaf tissue of the field grown rhubarb was similar to the results of the glasshouse experiment. There were no significant differences between the treatments (Table 2).

Table 2. *Activity of chitinase and β-glucanase in rhubarb leaves from the field trial treated with SAR inducing chemicals. The leaf samples were collected one week and two weeks after the first application of SAR inducing chemicals.*

Treatment	Chitinase activity (mUnit/mg protein)		β-glucanase activity (mUnit/mg protein)	
	One week	Two weeks	One week	Two weeks
Control	10.41 ($\pm$2.06)	10.69 ($\pm$1.28)	299.05 ($\pm$39.55)	303.99 ($\pm$28.10)
BION® 50 ppm	10.82 ($\pm$2.63)	11.03 ($\pm$1.09)	302.52 ($\pm$61.71)	311.02 ($\pm$68.08)
BION® 100 ppm	11.11 ($\pm$2.56)	11.07 ($\pm$1.23)	307.21 ($\pm$22.77)	309.85 ($\pm$32.31)
Betaine® 50 mM	10.58 ($\pm$0.75)	10.55 ($\pm$1.48)	303.02 ($\pm$16.08)	318.54 ($\pm$30.03)
Betaine® 75 mM	11.93 ($\pm$0.97)	10.76 ($\pm$0.98)	305.97 ($\pm$71.12)	315.74 ($\pm$67.20)
Milsana® %	10.26 ($\pm$0.88)	10.59 ($\pm$0.69)	298.44 ($\pm$19.58)	300.73 ($\pm$48.65)
Level of significance ($p = 0.05$)	NS	NS	NS	NS

Figures in the parenthesis indicate the standard deviation of the mean.

Control Bion 50 Bion 100 ppm
Betaine 50 mM Betaine 75 mM Milsana 1%

Figure 5. *Examples of the appearance of plants from each treatment showing the disease condition and growth status 6 weeks after the application of the chemicals. The control plant was the least healthy. It is interesting to note that Betaine® 75 mM made the plant greener than the other treatments. The application of Milsana® also made the plant greener but less than with Betaine® 75 mM. Also, unlike other crops BION® 100 ppm did not cause any phytotoxicity.*

General Discussion

There was a significant reduction in the incidence of leaf spot disease in the SAR treated rhubarb plants in the glasshouse trial but not in the field trial. The level of disease was reduced by one score category (down from 10 to 25 spots per leaf to less than 10 spots per leaf) in the glasshouse trial.

The reason for the variability in response warrants further investigation. Previous work in cucurbits has shown that healthy plants have a greater SAR response than unhealthy or stressed plants (Bokshi *et al*. 2008). The reason for this is that the induction of SAR requires energy for the production of new proteins, genes and enzymes. If the plant is under stress already then there are few reserves of energy left to be used for the induction of SAR. It may be that the plants in the field were under some environmental stress at the time of the trial. The grower suggested that at the time of year when the trial was done the crop suffers from a transitional effect due to seasonal change and at this time he notices that the plants are more susceptible to disease.

It is important to note that in both trials there was no induction of the chitinase and β-glucanase enzyme activity in the leaves of rhubarb treated with the SAR elicitors and this result suggests that SAR was not induced. The induction of SAR results from a cascade of signals through the plant and many enzymes and genes are activated. In other work chitinase and β-glucanase have been reliable marker enzymes for the induction of SAR (Bokshi et al, 2008, 2007). It is possible that this is not the case in rhubarb however and so this result needs to be confirmed by testing other enzymes such as peroxidase or by screening for phytoalexins or the induction of pathogenesis related proteins and genes that could also be induced and be better markers of a systemic response in rhubarb.

Systemic acquired resistance is a fundamental plant process that has been shown to be able to be beneficially exploited in other crops. This work with rhubarb shows that there is still a lot for us to learn. The response is not the same in all crops and more basic research is needed to optimise the response in this crop. The benefits for further work are that the induction of SAR can protect the plant from a range of plant diseases and the defences are diverse which means that there is little chance that the pathogens will develop resistance to this approach.

Technology Transfer

1.   Draft article for Vegetables Australia

This article will be submitted to Vegetables Australia on acceptance of the final report by HAL.

With a little help from you: Rhubarb can protect itself from leaf spot disease.

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Healthy plants have the capacity to protect themselves from fungal disease. When a fungal spore is detected the plant is triggered to switch on different reactions to build up its defences. These defences can include several barriers. For example they can be chemical (phenolic compounds that are toxic to the fungi), physical barriers (lignin deposits to prevent hyphal growth) and anti-fungal compounds (enzymes that attack fungal cells). Interestingly, the plant can also be tricked into this response and this is known as the induction of systemic acquired resistance (SAR). The plant is ready before a pathogen attacks and so the protection is much better. This is a novel method of disease control that has been shown in many research trials to be a valuable part of an integrated disease management program.

However, it has only been recently that elicitors of SAR have been marketed for use on agricultural crops. The most-studied resistance activator is acibenzolar-S-methyl (BTH, BION[®] (Syngenta, Basel Switzerland). BION[®] is a synthetic analogue of salicylic acid that amplifies a wave of signals throughout the plant that results in a heightened defence level.

Recently this approach was trialled in rhubarb. Rhubarb plants grown in a glasshouse at the University of Sydney were treated with either BION[®] (BTH, Syngenta, Basel, Switzerland @ 50 ppm), Milsana[®] (KHH BioSci, Inc of NC, USA @ 1%), Rezist[®] (Stoller enterprises, Houston, Texas, USA @ 44 ppm); Betaine[®] (Sigma Aldrich, USA @ 50 mM) or water as a control. The induction of resistance in the plant was assessed by scoring the leaf spot disease caused by *Ascochyta rhei* (with a characteristics disease symptoms of more or less circular, light-tan spots). The leaves were scored 3 and 6 weeks after the first application of the chemicals. The third full leaf was scored for all plants and a severity scale of 1-5 was used where: 1 = no spots: 2 = <10 spots per leaf; 3 = ≥10 to <25 spots; 4 = ≥25 to <50 spots; 5 = >50 spots per leaf.

Figure 1 shows that plants treated with BION[®] 50 ppm and Betaine[®] 50 mM had a significantly lower level of leaf spot disease than the other treatments. Rezist[®] 44 ppm and Milsana[®] 1% (v/v) did not significantly reduce the disease incidence compared to the control plants.

Further work was done using the same chemicals in a field trial. Rhubarb (cv. Sydney Crimson) was grown on a commercial property on the out skirts of Sydney. The plants were treated with BION[®] 50 ppm, BION[®] 100 ppm, Betaine[®] 50 mM, Betaine[®] 75 mM, Milsana[®] 1% or a water Control.

In this field trial there were no significant differences between the levels of disease severity for any of the treatments. The results from the analysis of the enzyme activity for the leaf tissue from the field grown rhubarb also showed no significant differences between the treatments.

The results suggest that there was no induction of SAR in the field grown rhubarb plants as a result of the application of these SAR inducing chemicals. One of the reasons for a reduced response was suggested by the grower. He observed that at the time of year when the trial was done, the crop suffered from a transitional effect due to seasonal change and at this time the plants are more susceptible to disease. Therefore, more work is needed to clarify these results. It would be good to repeat the experiments with a young, healthy crop to see if the variability in response is related to plant health. The SAR response is an active plant response requiring energy and if the plant is under stress as a result of seasonal conditions then there may not be enough energy reserves available to establish SAR.
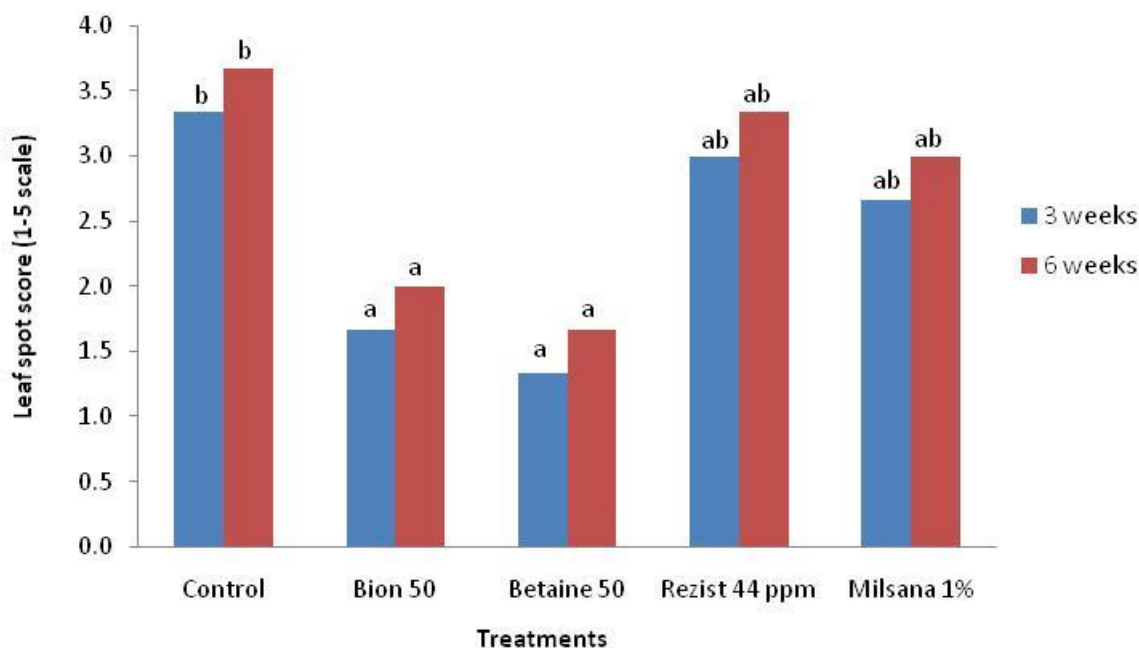
Figure 1. *The effect of SAR inducing chemicals on the severity of leaf spot disease on rhubarb plants. The disease severity was examined at three weeks and six 6 weeks after the first application of the chemicals. The data in the graph are the means of the disease severity scores. Treatments with a different letter are significantly different at 5% level of significance (data analysed from leaf spot counts).*

2. *Draft research paper for VG09031*

This research paper could be submitted to the Journal of Horticultural Science and Biotechnology. However more work is needed before it can be submitted to clarify the variable results found. The field trial needs to be repeated and further enzyme work done.

Managing rhubarb diseases through induction of systemic acquired resistance

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Abstract

In this experiment Rhubarb plants were grown in 8 litre pots using a commercial potting mix and were watered and fertilised in line with commercial practice. Four month old plants were treated with the following SAR (systemic acquired resistance) inducing chemicals, BION® (50 ppm), Milsana® (1%), Rezist® (44 ppm), Betaine® (50 mM) and a water only control.

The chemicals were applied twice at one week intervals. Leaf samples were collected one and two weeks after the first application of the chemicals. The plants were assessed for the appearance of leaf spot disease (most likely caused by *Ascochyta rhei* leaf spot diseases can be caused by several pathogens and the exact one was not identified in these experiments) two weeks after the first spray. From the disease score it was found that plants treated with Betaine® and BION® had reduced disease.

Further work was done using the same chemicals in a field trial. Rhubarb (cv. Sydney Crimson) was grown on a commercial property on the out skirts of Sydney. The plants were treated with BION® 50 ppm, BION® 100 ppm, Betaine® 50 mM, Betaine® 75 mM, Milsana® 1% and a water control.

In this field trial there was no significant difference between the levels of disease severity for any of the treatments. The results from the analysis of the enzyme activity of the leaf tissue of the field grown rhubarb also showed no significant differences between the treatments.

The results suggest that there was no induction of systemic acquired resistance in the field grown rhubarb plants as a result of the application of these SAR inducing chemicals. More work is needed to clarify these results. It would be good to repeat the experiments with a young, healthy crop to see if the variability in response is related to plant health. The SAR response is an active plant response requiring energy and if the plant is under stress as a result of seasonal conditions then there may not be enough energy reserves available to establish SAR.

Key words: Rhubarb, systemic acquired resistance, leaf spot disease**,** *Ascochyta rhei*

Introduction

It has only been recently that elicitors of SAR have been marketed for use on agricultural crops. The most-studied resistance activator is acibenzolar-S-methyl (BTH, BION® (Syngenta, Basel Switzerland).

BION® is a synthetic analogue of salicylic acid that amplifies a wave of signals throughout the plant that results in a heightened defence level (Kunz et al., 1997). With these increased defences, usually mediated by increased anti-fungal compounds or pathogenesis-related (PR) proteins, plants are significantly more resistant to disease (Bokshi et al 2006, 2007, 2008).

A recent review of the scientific literature found that there is limited research using SAR on rhubarb. However BION® was found to reduce the crown rot disease in strawberry (Eikemo et al, 2002) and tomato (Benhamou and Theriault 1992) by the induction of systemic acquired resistance against the pathogens. It is therefore very likely that the same response would be induced in rhubarb.

Other research has shown a significant reduction in the severity of downy mildew on cauliflower through the induction of SAR by the application of BION® (Sharma et al, 2004, Ziadi *et al.* 2001) or BABA (acid beta-aminobutyric acid)(Silue *et al.*, 2002). There is also research to show that SAR can reduce the severity of rust and leaf spot however not specifically in rhubarb (Sharma et al., 2004). The success of SAR in other vegetable crops suggests that the same results are likely in rhubarb.

It is possible that the most effective disease control strategy using several chemicals is an integrated control program. One generally regarded as safe GRAS option is the product called Milsana® which is an extract from the Giant Knotweed (*Reynoutria sachalinensis*) (KHH BioSci, Inc of NC, USA). This is a bioprotectant and our laboratory has shown some very promising results using this product against powdery mildew in cucurbits (Bokshi et al, 2008).

This project aimed to screen the elicitors of SAR and Milsana for their effectiveness as part of a disease control strategy for rhubarb. The main disease the research focused on was the leaf spot disease caused by *Ascochyta rhei* (with characteristic disease symptoms of more or less circular, light-tan spots) (Zhao. et al. 2006).

Materials and methods

Glasshouse Trials

A glasshouse trial was conducted on rhubarb where systemic acquired resistance (SAR) inducing chemicals were tested for their capacity to control leaf disease. The plants were grown in 8 litre pots containing commercially prepared potting mix and there were 4 replicate plants per treatment. Two applications of the SAR inducing chemicals were applied to four month old plants one week apart. The following chemicals and rates were applied; BION® (BTH, Syngenta, Basel, Switzerland @ 50 ppm), Milsana® (KHH BioSci, Inc of NC, USA @ 1%(v/v)), Rezist® (Stoller enterprises, Houston, Texas, USA @ 44 ppm); Betaine® (Sigma Aldrich, USA @ 50 mM) and a water control.

The induction of resistance in the plant was assessed by scoring the leaf spot disease caused by *Ascochyta rhei* (with characteristic disease symptoms of more or less circular, light-tan spots). Also, the leaf samples were collected to analyse the activities of the enzymes β-glucanase and, chitinase. These enzymes induce reactions in the plant that help protect it from fungal infection. The incidence of the disease was scored on the third fully expanded leaf, three and six weeks after the first application of the SAR inducing chemicals using a severity scale of The incidence of the disease was scored on the third fully expanded leaf of each plant, three and six weeks after the first application of the SAR inducing chemicals using a severity scale of 1-5 where: 1 = no

spots or fungal colony: 2 = <10 spots per leaf; 3 = ≥10 to <25 spots; 4 = ≥25 to <50 spots; 5 = >50 spots per leaf.

Enzyme analysis

The leaf samples used for the enzyme analysis were collected from the third fully expanded leaves of each plant one and two weeks after the first application of the SAR inducing chemicals and stored at -80°C.

Analysis of chitinase and β-glucanse were performed following the methods of Bokshi *et al*. (2006) and Rivie`re *et al*. (2008) with some modifications. In brief, 0.5 g of frozen leaf tissue was ground in liquid nitrogen with 2% v/v PVP (polyvinylpyrrolidone) in a mortar and transferred to a 2 ml micro-tube. The tissue was homogenised in 1 ml 50 mM potassium acetate buffer, pH 3.5, containing 1 mM EDTA and 5 mM reduced glutathione. Reduced glutathione (5 mM) was added to the buffer on the day of extraction. The homogenates were allowed to stand for 30 min in ice, and were stirred intermittently with an inoculation loop. To these homogenates 40 µl of 1 M NaOH was added and mixed well by stirring with an inoculation loop to bring the pH level to approximately 5.0. The homogenates were centrifuged at 10,000 rpm for 10 min at room temperature and the supernatants were transferred to micro-tubes to assay enzymes and total soluble protein.

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°C for 5 min. The reaction was started by adding 0.1 ml aqueous CM-Chitin-RBV (2 mg/ml; Loewe Biochemica, Sauerlarch, Germany) for chitinase or 0.1 ml of laminarin azure (4 mg/ml; Sigma) for β-glucanse. After 20 min, the reaction was terminated by adding 0.1 ml 2N HCl which precipitated the undegraded substrate. The tubes were cooled in ice for approx. 10 min, and then centrifuged for 5 min at 9000 rpm. The absorbance of the supernatant was measured at 545 nm for chitinase or 600 nm for β-glucanase against their acetate buffer blank. Results were calculated using a linear equation derived from the standard curves of the absorbance of serial dilutions of standard pure chitinase from Sigma Chemical Co., St Louis, MO, USA (product number C6137) or Malt β-glucanase, from Megazyme (Deltagen Australia Pty. Ltd). The specific activity of chitinase and β-glucanase were expressed as mUnits mg^{-1} of total soluble protein.

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

Field Trial

The results from the glasshouse trial show that BION®, Betaine® and Milsana® should be tested under field conditions. A field trial was carried out (start 4th March 2010) on a commercial farm (Horsley Park, NSW, 2175) with BION®, Betaine® and Milsana® (Figure 1). The crop was a red stalked rhubarb (Sydney Crimson) planted during May 2009 in 1.2 m raised beds in double rows that were watered regularly with a sprinkler irrigation system. The rhubarb crop was managed using best commercial practice.

The chemicals were applied at the concentrations used in the glasshouse trial. The experimental treatments include; BION® 50 ppm, BION® 100 ppm, Betaine® 50 mM, Betaine® 75 mM,

Milsana® 1% (v/v) and a water control.

The SAR elicitors were trialled using a factorial design experiment. There were 3 replicates per treatment with 4 plants in each replication. At the time of the first spray application there were some visible signs of leaf spot disease.

The SAR inducing chemicals were sprayed twice at one-week intervals and a third spray was applied two weeks after the second treatment (application 0, 1, 2 and 4 weeks). During the experimental period no other agrochemical were used on the plants.

The plants were examined for disease at the time of the first spray application and they were scored again 3 and 6 weeks after the first application of the chemicals. The incidence of the leaf spot disease was scored on the third fully expanded leaf, using a severity scale described in the earlier section.

Leaf samples were also taken for the analysis of chitinase and β-glucanase activity from samples collected one and two weeks after the first application of the SAR inducing chemicals using the methods described in the earlier section.

Statistical analysis

Data from all experiments were subjected to one or two-way ANOVA in a linear model (GLM) using Simstat (Provalis Research, Montreal, Canada). When appropriate, means were separated by Fisher's protected least significant difference test (LSD, $P=0.05$ or 0.01).

Results and discussion

Glasshouse Trial

The results from the glasshouse disease data indicated a significant reduction in leaf spot disease obtained from the plants treated with BION® 50 ppm and Betaine® 50 mM between the three and six week assessments (Fig 1). Rezist® 44 ppm and Milsana® 1% did not significantly reduce the disease incidence compared to the control plants. The disease severity for plants treated with all the chemicals increased from week 3 to week 6. It was also observed that the plants treated with Betaine® and Milsana® were greener than the control plants and the BION® treated plants showed signs of leaf senescence and were a paler green than the other treatments.

The results of the enzyme analyses indicated no significant change in the activity of chitinase or β-glucanase from the application of SAR inducing chemicals (Table 1). The level of enzyme activity was unchanged in the samples collected one week after the spray with only a small increase (not significant) of both chitinase and β-glucanase activity in the BION® 50 ppm treated plants two weeks after the first application of the spray. The low level of enzyme activity suggests that there was no induction of resistance in the treated plants in this experiment. However more work is needed to confirm this result as the SAR response may be confirmed present or not by testing other enzymes such as peroxidase or by screening for the induction of pathogenesis related proteins and genes.
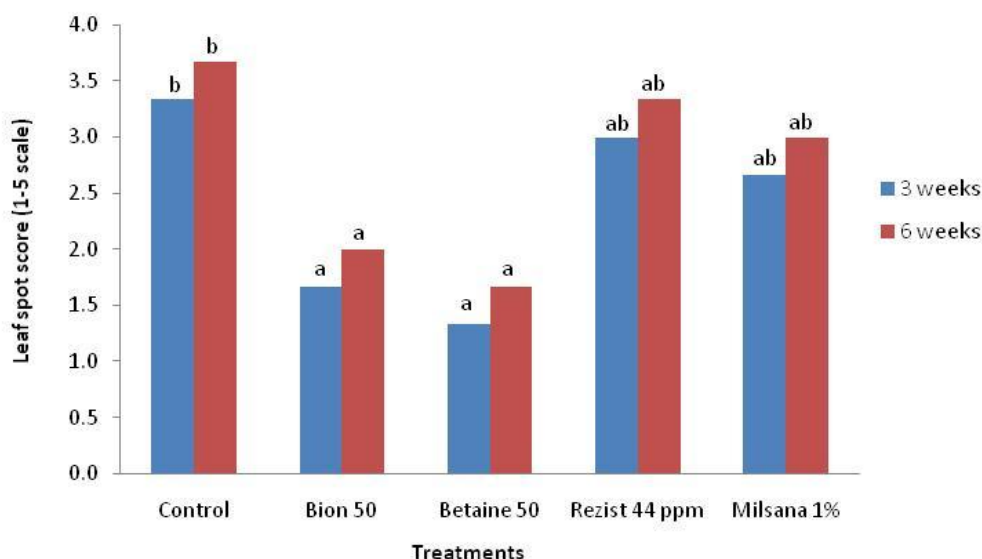
Figure 1. *The effect of SAR inducing chemicals on the severity of leaf spot disease on rhubarb plants. The disease severity was examined at 3 and 6 weeks after the first application of the chemicals. The data in the figure are the means of the disease severity scores. Treatments with a different letter are significantly different at 5% level of significance (data analysed from leaf spot counts).*

Table 1. *Activity of chitinase and β-glucanase in rhubarb leaves grown in the glasshouse treated with SAR inducing chemicals. The leaf samples were collected one week and two weeks after the first application of SAR inducing chemicals.*

Treatment	Chitinase activity (mUnit/mg protein)		β-glucanase activity (mUnit/mg protein)	
	One week	Two weeks	One week	Two weeks
Control	13.06 ($\pm$1.16)	15.96 ($\pm$1.99)	290.74 ($\pm$25.60)	355.41 ($\pm$16.06)
BION® 50 ppm	13.04 ($\pm$1.88)	17.531 ($\pm$1.79)	287.01 ($\pm$16.33)	364.24 ($\pm$15.76)
Betaine® 50 mM	12.78 ($\pm$2.12)	16.188 ($\pm$1.31)	294.20 ($\pm$14.06)	359.61 ($\pm$44.23)
Rezist® 44 ppm	12.95 ($\pm$0.98)	16.386 ($\pm$1.43)	292.09 ($\pm$23.37)	347.33 ($\pm$19.73)
Milsana® 1%	13.00 ($\pm$0.89)	15.556 ($\pm$1.74)	283.75 ($\pm$27.55)	358.88 ($\pm$20.56)
Level of significance (p=0.05)	NS	NS	NS	NS

Figures in the parenthesis indicate standard deviations (SD)

There was however some reduction in the level of disease severity in the treated glasshouse plants and SAR may still have occurred but via different chemical pathways. More research is needed to determine the nature and mechanism of disease control in rhubarb as a result of the application of these chemicals.

Field Trial

The results from the assessment of the disease severity from the field trial showed that there were no significant differences between the treatments at any scoring time (Fig 2). However, there was a trend for the reduction in the level of disease severity over the 6 week period for plants treated with BION$^®$ 50 ppm and BION$^®$ 100 ppm. Treatment with Betaine$^®$ 50 mM and Betaine$^®$ 75 mM also showed a reduction in disease severity but it was also not significant.

The leaf spot severity scores suggest that SAR was not induced in this trial. One of the reasons for a reduced response could have been that at the time of year when the trial was done the crop suffers from a transitional effect due to seasonal change and at this time the plants are more susceptible to disease. More work is needed to confirm this result. The overall disease incidence was also low with the leaves having between 10 to 25 spots per leaf. It might be better to have a more accurate scoring system in future experiments with smaller increments between categories.

The results from the analysis of the enzyme activity on the leaf tissue of the field grown rhubarb was similar to the results of the glasshouse experiment. There were no significant differences between the treatments (Table 2).
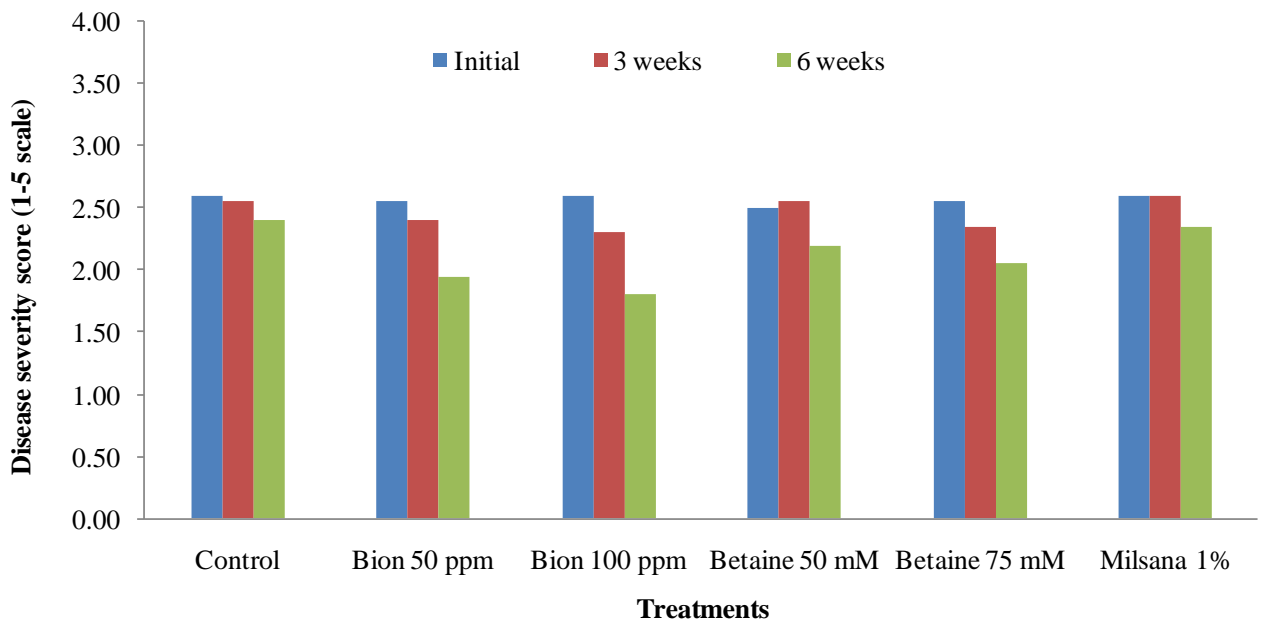

Figure 2. *Disease severity of rhubarb plants treated with SAR inducing chemicals. Initial disease severity was scored at the time of first application of the SAR inducing chemicals and then again 3 and 6 weeks after the first application of the chemicals. There were no significant differences between the levels of disease severity for any treatment at any time.*

Table 2. Activity of chitinase and β-glucanase in rhubarb leaves from the field trial treated with SAR inducing chemicals. The leaf samples were collected one and two weeks after the first application of SAR inducing chemicals.

Treatment	Chitinase activity (mUnit/mg protein)		β-glucanase activity (mUnit/mg protein)	
	One week	Two weeks	One week	Two weeks
Control	10.41 (+2.06)	10.69 (+1.28)	299.05 (+39.55)	303.99 (+28.10)
BION® 50 ppm	10.82 (+2.63)	11.03 (+1.09)	302.52 (+61.71)	311.02 (+68.08)
BION® 100 ppm	11.11 (+2.56)	11.07 (+1.23)	307.21 (+22.77)	309.85 (+32.31)
Betaine® 50 mM	10.58 (+0.75)	10.55 (+1.48)	303.02 (+16.08)	318.54 (+30.03)
Betaine® 75 mM	11.93 (+0.97)	10.76 (+0.98)	305.97 (+71.12)	315.74 (+67.20)
Milsana® %	10.26 (+0.88)	10.59 (+0.69)	298.44 (+19.58)	300.73 (+48.65)
Level of significance ($p = 0.05$)	NS	NS	NS	NS

Figures in the parenthesis indicate the standard deviation of the mean.

Overall Conclusion

The results show an inconsistent response. It is not clear if SAR was induced or not. More work is needed to explain the difference in response between the glasshouse trial and the field trial. It would be good to repeat the experiments with a young, healthy crop as plant health may have been a contributing factor to the difference in results. It has been shown that the induction of SAR is greater in plants that are healthier in other crops (Bokshi *et al*, 2008). The SAR response may also be confirmed present or not by testing other enzymes such as peroxidase or by screening for the induction of pathogenesis related proteins and genes

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Recommendations

1. There was a significant effect of the SAR treatments in the glasshouse rhubarb trial (disease score was <10 spots per leaf for the BION$^{®}$ 50 ppm and Betaine$^{®}$ 50 mM treatments compared to 10 to 25 spots per leaf for the other treatments and the control). There was no treatment effect in the field trial.

    It is important to note that in both trials there was no induction of chitinase and β-glucanase enzyme activity in the leaves of rhubarb treated with the SAR elicitors and this suggests that SAR was not induced. The induction of SAR results from a cascade of signals through the plant and many enzymes and genes are activated. In other work chitinase and β-glucanase are reliable marker enzymes for the induction of SAR.   The results described here need to be confirmed by testing other enzymes such as peroxidase or by screening for phytoalexins or the induction of pathogenesis related proteins and genes that could also be induced and be markers of a systemic response in rhubarb.

    One of the reasons for a reduced response in the field trial could have been that at the time of year when the trial was done the crop suffered from a transitional effect due to seasonal change and at this time the grower observes that the plants are more susceptible to disease. The SAR response is an active plant response requiring energy and if the plant is under stress as a result of seasonal conditions then there may not be enough energy reserves available to establish SAR.

2. More work is needed to confirm this result and to investigate the activity of other pathogenesis related enzymes, such as peroxidase that might be better markers of SAR in rhubarb. There is no evidence at this stage to support the application of a minor use permit for these chemicals for use in rhubarb.

3. The use of systemic acquired resistance (SAR) for disease control in horticultural crops warrants more work. SAR provides protection against a range of diseases and the risk of disease resistance developing is minimal. The difficulty with this approach is that more basic research is needed before it can be effectively adopted into commercial horticulture. Most of the research focuses on grains (monocots) and as seen by this work in rhubarb the response to the SAR elicitors is not necessarily the same in all plants. More basic research is required to understand the difference and range of responses if SAR is to be exploited in horticultural crops.

    The other limitation is the cost for chemical companies to register a new product for small industries such as the rhubarb industry. The application of minor use permits goes someway to addressing this problem but the high cost of registration does not encourage chemical companies to pursue basic research unless the cost benefit for them is obvious.

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Appendix 1: Letters of Support for Project

STOLLER AUSTRALIA PTY LTD
ABN 35 065 320 747

Dr Jenny Jobling   May 21 2009
Adjunct Associate Professor
Applied Horticultural Research
c/- Woolley Building, A20
University of Sydney NSW, 2006, Australia

Dear Jenny,

Ref: Project: VG09031 - Preliminary study to look at the efficacy of SAR for disease control in Rhubarb

As you know, Stoller has a trial product called ReZist that has shown some SAR qualities in helping plants deal with various stresses and diseases by improving their resistance.

We note with interest your proposed project looking at SAR for disease control in Rhubarb and the inclusion of Stoller's ReZist in the project.

We support this project and will view the results with interest and hope that there may be opportunities in the future to investigate the option to commercialise ReZist for use on Rhubarb and other crops.

Regards

Richard Emery

General Manager

Stoller Australia

Marrone
Bio Innovations

Dr Jenny Jobling
Applied Horticultural Research Pty Ltd
C/- Woolley Building, A20
University of Sydney   NSW   2006

Dear Dr Jobling,

Re: VG09031 - Efficacy of SAR for disease control in Rhubarb: a preliminary study

This letter is in response to your question as to whether Marrone Bio Innovations intends to register Regalia SC (formerly Milsana) in Australia.

You are aware that Regalia SC is a product with specific activity useful in protecting crops against certain diseases. The product is not curative. Nor is the product of broad-spectrum.

The specificity of Regalia SC and its mode of action make it ideal for use in IPM and other sustainable horticultural systems. While useful in specific situations, the specificity of the product limits market opportunities for the product. Consequently, it is necessary to ensure that registration of the product will result in a reasonable return on the investment made in obtaining registration.

Marrone Bio Innovations is committed to developing and registering Regalia SC, including in Australia, provided suitable regulatory pathways are available.

I draw your attention to the substantial increase in fees proposed by APVMA which have not yet been finalised. As such matters as future fees payable to the APVMA are not known at this time or whether APVMA will require studies to be conducted that have not been requested in other countries, we cannot guarantee that registration will be pursued in Australia. However, I can assure you that it is our intention to obtain registration for Regalia SC if the as yet unidentified obstacles do not adversely affect viability of the project.

Pam Marrone
CEA & Founder, Marrone Bio Innovations

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Ken McKee,
Field Development
Manager

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Dr Jenny Jobling
University of Sydney NSW, 2006,

5/28/2009
Bion Interest

Hello Jenny,

As you're aware Syngenta registered Bion (acibenzolar-s-methyl) for the first time in Australia around 2 years ago for use as a seed treatment in cotton.

We are continuing to evaluate the product for both seed applied and foliar uses however at present we have not identified opportunities of sufficient commercial interest to justify investment in label extensions.

While I am unable to guarantee any future registration plans with Bion I can say that our medium term screening activities will most likely centre on the products performance in mixtures with conventional "fungicides" to enhance existing disease management programs. I think future label extension with Bion are more likely to reach the market in this form as opposed to the solo product.

I hope this reply is of some value. Please let me know if I can be of any further service in this or related matters.

Sincerely,

Ken McKee

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