Know-how for Horticulture™

Developing cost effective UV protection of biological pesticides

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Know-how for Horticulture™

PUBLIC
FINAL REPORT

Horticulture Australia Project VX01006

Developing Cost Effective UV Protection for Biological Pesticides

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Media summary

The principal objective for Integrated Pest Management (IPM) systems is to reduce the amount of hard (broad-spectrum) chemicals needed to control insect pests in crops. Part of the strategy to achieve this objective is to use highly selective biological pesticides in place of hard chemicals wherever possible. Unfortunately, many good biological pesticides are very quickly rendered less effective by their susceptibility to degradation by the sun's rays.

This project looked at developing a sunscreen based on titanium dioxide that would protect biological pesticides from UV degradation, thus enabling existing biological pesticides to be used more effectively and make others that are non viable become viable.

A tank mix additive has been developed that can be used with both biological and hard chemical pesticides. Thus, it has the potential to render biological actives more effective as well as reduce the amount of any UV susceptible hard chemical pesticide required to achieve a given result.

The product tank mixes well over a pH range from 4 to 10, thus covering most conditions likely to be encountered in Australia. Moreover, the mixing qualities are unlikely to be adversely affected by formulation additives used in other tank mix components.

In laboratory trials by IPM Technologies Pty Ltd, with 2nd instar *Plutella xylostella* larvae on cabbage leaves, the formulation was shown to provide protection for *Bacillus thuringiensis* insecticide against UV degradation down to 0.1% on spray water. Also in laboratory trials, the formulation, when used at 1.0% on spray water, was shown not to deter the feeding of 2nd instar *P. xylostella* larvae. A successful field trial has been carried out on cabbages under pressure from *P xylostella*, where the product was shown to yield markedly superior performance against first and second instar larvae.

Experiments conducted on the Eureka AgResearch rainfall simulator showed that the product was also very effective as a sticker to enhance rainfastness.

Successful commercialisation of this product will allow growers to expect improved performance from soft pesticide options and reductions in the application rates for UV susceptible hard chemical pesticides.

The University of Sydney and HAL are now seeking an industry partner who is interested in taking this product to the market.

The project was funded by Horticulture Australia and the vegetable and potato industries.

Technical summary

Minimising the use of hard chemical pesticides and replacing them with biological pesticides is the subject of much agrochemical research. IPM strategists incorporate biological actives into their programs where these are available and sufficiently efficacious to allow reductions in hard chemical use. However, many potentially good biological actives are rendered inefficacious in the field by their susceptibility to the sun's rays. Providing effective protection for such actives will lead to more actives becoming available and those presently in use becoming more effective.

Inorganic pigments such as titanium dioxide and zinc oxide have been used as sunscreens for many years. However, this sort of technology has not previously been successfully exploited for the protection of sun sensitive biological pesticides.

The potential for titanium dioxide as a UV protection agent has been demonstrated in an earlier GWRDC-funded project. Although effective UV protection was demonstrated in this earlier project the protectant needed to be formulated with the active and the manufacturing procedure required made the approach non viable.

In this work an effective UV protection agent has been developed that has been demonstrated to improve the effectiveness of *Bacillus thuringiensis* (Bt) in situations where the active is under UV pressure. The formulation that has been developed is designed to be tank mixed with the active formulation and is expected to be compatible with both biological and chemical active formulations. Thus, the new UV protectant has the potential to reduce the use of chemical actives by making biological actives more efficacious and enabling reduced rates of sun sensitive chemical actives also.

During development the UV protection agent underwent extensive testing in the laboratories of IPM Technologies Pty Ltd and the efficacy of the optimised formulation has subsequently been confirmed in field trials. Field trials have so far been conducted at 0.2 % on spray water but in ongoing commercial trials the possibility of reducing the application rate below this level will be explored.

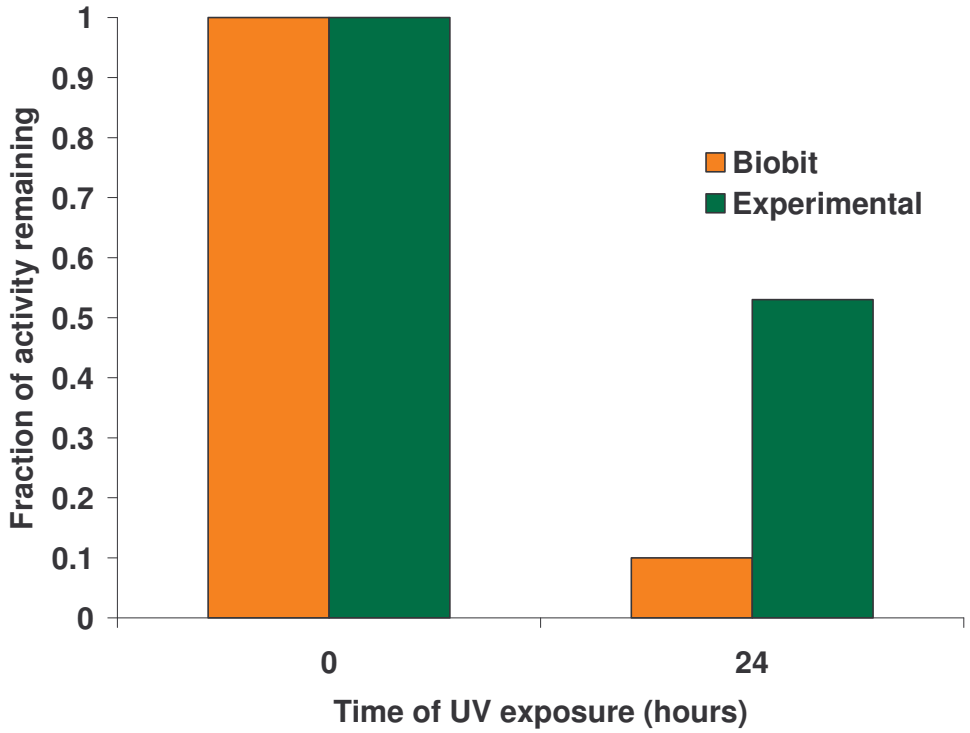
The UV protectant formulation is prepared by dispersing a commercial grade of titanium dioxide in water using an optimised blend of biocompatible surfactants. Dispersion is carried out using a high speed disperser, a cheap and common manufacturing device.

Introduction

The principal objective for Integrated Pest Management (IPM) systems is to reduce the amount of hard (broad-spectrum) chemicals needed to control insect pests in crops. Part of the strategy to achieve this objective is to use highly selective biological pesticides in place of hard chemicals wherever possible. Unfortunately, many good biological pesticides are very quickly rendered less effective by their susceptibility to degradation by the sun's rays.

An earlier GWRDC funded project completed in the early 1990s demonstrated the effectiveness of the approach of using titanium dioxide as a UV protection agent for *Bacillus thuringiensis* (Bt) insecticides. However, the formulation developed at that time was a wettable-powder that was too difficult to manufacture and therefore not commercially viable. Moreover, the early formulation was such that it had to be formulated with the active and could not be used as a tank mix. Some results obtained for that original formulation are shown in Figure 1.

Figure 1: The effectiveness of the original Bt wettable powder formulation compared to a standard commercial Bt formulation



The formulating objective for this project was to develop a tank mix additive, based on titanium dioxide, which would be compatible with a wide range of active formulation types and can thus be used to protect both biological and hard chemical actives from UV degradation. Such a formulation would thus have a maximum IPM benefit as it would have the potential to reduce the rate at which some hard chemicals are used as well as making biological actives more viable. Moreover, a tank mix additive is not constrained

to be used with a particular product but can be used with all formulations irrespective of source.

Materials and Methods (Formulating)

In general, a number of important factors need to be recognised in pesticide formulating. For example, the formulation must be cost effective, be able to be readily manufactured on available equipment, properties need to be stable to storage and handling, the formulation must be readily dispersed in a spray tank under the minimal shear conditions available in such systems and should have minimal impact on non target species. If the pesticide is one that requires the pest to eat the active (as opposed to simply contact it) formulation ingredients must not deter the target pest from feeding on the plants being protected.

When formulating for biological actives there is the added requirement that formulation components must not have a deleterious effect on the active itself. Experience suggested that the surfactants used to stabilise the dispersions of formulation components were the most likely materials present in a formulation that would cause harm to biological actives. Thus, a dispersion of commercial titanium dioxide was generated using a blend of surfactants that were known not to have a deleterious effect on the survival of biological organisms. While these are not surfactants that would generally be used for the dispersion of titanium dioxide it was felt that the need to used biologically compatible surfactants was the overriding consideration in their choice. Surfactants can be characterised by a quantity known as their hydrophilic lipophilic balance (HLB) and surfactants of different HLB can be blended to obtain a surfactant blend of intermediate HLB. When using a blend of surfactants to disperse a solid particulate material in water there will generally be an optimum HLB that provides a dispersion of minimum viscosity. The optimum blend of the surfactants used in this work was determined by plotting dispersion viscosity as a function of surfactant blend HLB. The final formulation established contained titanium dioxide, water and the surfactant blend.

Materials and Methods (Agronomic Testing)

The agronomic testing of the formulations generated in this project covered a number of aspects: i) the need for the UV protectant to be palatable to pest species and thus not deter their feeding; ii) the efficacy of the UV protectant in protecting the biological active from UV degradation and iii) the rainfastness of the UV protectant and its capacity to enhance the rainfastness of the biological active formulation.

A standardised bioassay procedure was used for assessment of UV protectiveness of the experimental formulations.

A single batch of the biological insecticide, *Bacillus thuringiensis*, (Dipel®) was used for all laboratory-based experiments. Suspensions of Dipel were prepared at the desired rates (between 0.25 – 1.0 g/L), and the experimental UV formulations were mixed with the Bt at desired rates (between 0.1 – 1.0 %). The protectant work was started at 1 % on spray water because we believed this to be the viable concentration from a cost point of view. Effective results at this high concentration led us to explore lower concentrations. Leaves from organically grown cabbages were used by cutting leaf discs of 40mm diameter. The leaf discs were dipped in the suspensions for 10 seconds and then air-dried

by hanging from pegs in a fume cupboard. There were four repetitions of each treatment. Control discs were dipped into water.

The fume cupboard was fitted with four 300mm UV_A fluorescent lamps arranged as two lamps on each side of the hanging leaf discs at a distance of 200mm from the discs. This apparatus allowed equivalent exposure of both sides of all leaf discs to ultraviolet irradiation. The discs were exposed while drying for up to 150 minutes; longer times were found to degrade the leaf disks.

After UV exposure, the cabbage leaf discs were placed in ventilated plastic 70mL containers and approximately ten second-instar *P. xylostella* larvae were introduced. Treatments were then incubated at 25°C (photoperiod 13L:11D; set at 14 hours light and 10 hours darkness) for 72 hours, after which mortality of *P. xylostella* larvae was assessed. Mortality was defined as inability to walk when prodded with a fine paint brush. Data was analysed to determine differences between treatment means.

A field trial was conducted on cabbages under very high pressure from *P. xylostella* and is described in detail in Appendix 4.

Rainfastness assessments were carried out using the Eureka AgResearch rainfall simulator at Werribee, Victoria and are described in detail in Appendix 5.

Results (Formulation Physical Testing)

Any dispersion that is to be incorporated into a spray tank needs to be readily dispersed under the minimal shear that is characteristic of such systems over the range of pH conditions likely to be encountered in the field. To test the formulation with regard to its ability to be dispersed and remain dispersed under spray tank conditions the formulation was dispersed in water at various pH values under conditions of shear that did not create a vortex. The pH of the water was adjusted with sodium hydroxide or hydrochloric acid, as required. The formulation was mixed into the water, under minimal shear conditions, to yield a dispersion at 0.63% solids. The results obtained are shown in Figure 2. Relative to the number average that most people are familiar with, the Z average particle size obtained from light scattering is very sensitive to the presence of a small number of large particles. The primary particle size for the titanium dioxide used in the formulation was about 240 nm and, although the formulation performs slightly worse under acid conditions relative to basic conditions, the results confirm that the UV protectant works adequately under all pH conditions likely to be experienced in the field.

The effect of spray tank pH on the particle size achieved when the titanium dioxide formulation was dispersed under simulated low shear spray tank conditions was further investigated by measuring the initial particle size of a 1.0% dispersion after being added to a gently stirred vessel (Figure 3). The results obtained confirm that better results are obtained at higher pH. Nevertheless, the UV protectant works adequately under every pH condition likely to be experienced in the field.

Figure 2: The impact of spray tank pH and time on the TiO_2 particle size

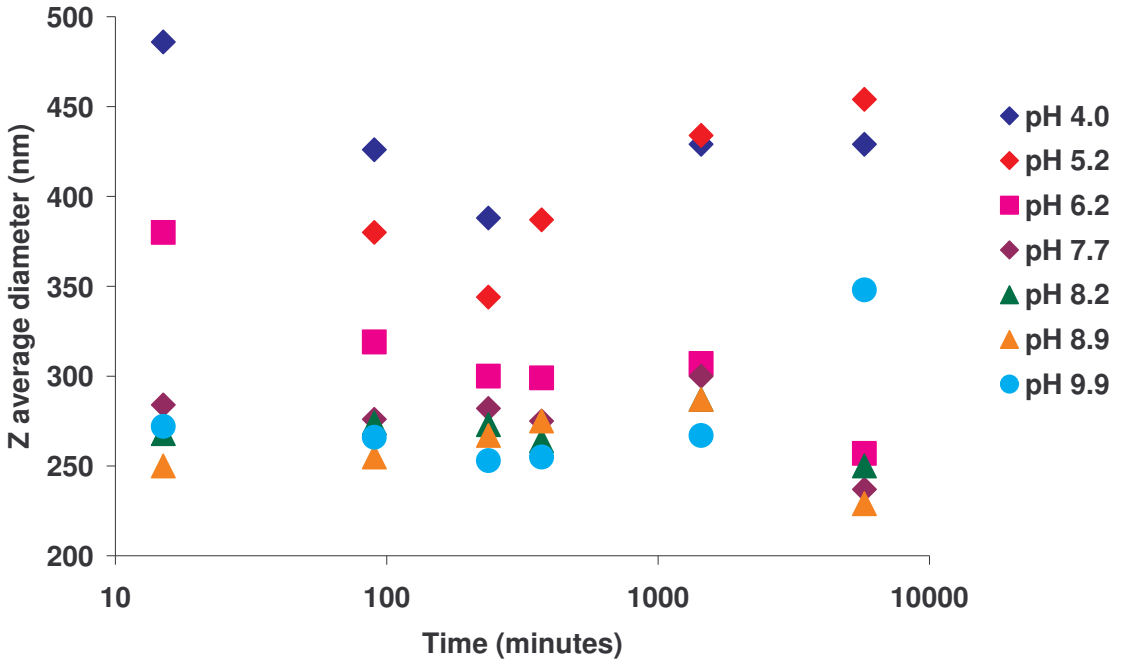
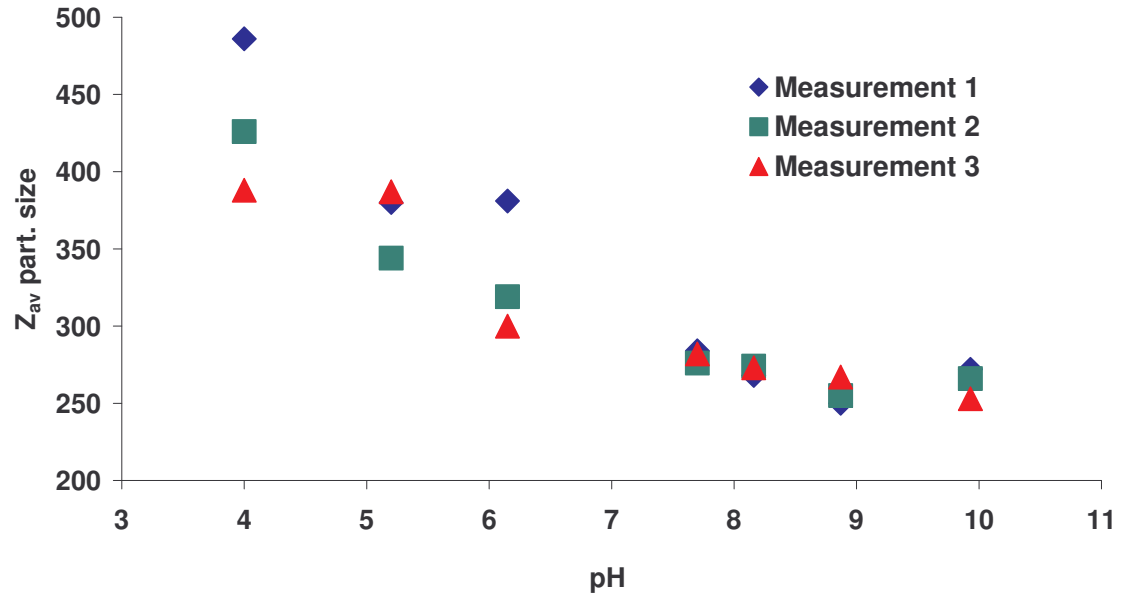


Figure 3: The impact of time and pH on the particle size in a simulated spray tank



Results (Agronomic testing)

Feeding tests involving measurement of leaf areas consumed on both treated and untreated cabbage leave discs showed that the UV protectant did not deter the feeding of *P. xylostella* larvae. There was no difference between the control (water) and with the addition of the UV protectant at 0.5% and 1.0% on spray water. There was also no

difference in feeding between Bt treatments with and without the addition of the UV protectant.

The UV protectant formulation was tested for its effectiveness in protecting Bt from the effect of UV exposure. Figure 4 shows that when the UV protectant was used at 1.0% on spray water and Bt (Dipel®) was used at 1.0g/L 100 minutes of UV exposure had no significant effect on the efficacy of the Bt. There was, however, a significant reduction in the efficacy of the Bt when no protectant was used. In this assay, 2nd instar *P. xylostella* larvae were exposed to residues of Bt on cabbage leaf disks. This first experiment was carried out at a level of protectant that was regarded as being at the highest level that could be regarded a viable, in order to establish proof of concept of the approach being used (Bioassay details in Appendix 1).

In subsequent experiments both lower levels of protectant and higher levels of UV exposure were evaluated. Figures 5 and 6 show results that are representative of a considerable body of laboratory testing of the UV protectant. Figure 5 shows the results obtained when the UV protectant was used at 0.2% on spray water to protect Bt (Dipel; used at 0.5g/L). This assay also used 2nd instar *P. xylostella* larvae on cabbage leaves and the UV exposure was extended to 150 minutes. Again there is no significant difference between the mortality of the *P. xylostella*, with and without UV exposure, when the Bt was protected, whereas the unprotected Bt showed a significant reduction in efficacy after UV exposure (Bioassay details in Appendix 2).

Figure 4: The impact of ultraviolet radiation on Bt (Dipel) + 1.0% UV protectant as measured by the mortality 2nd instar P. xylostella

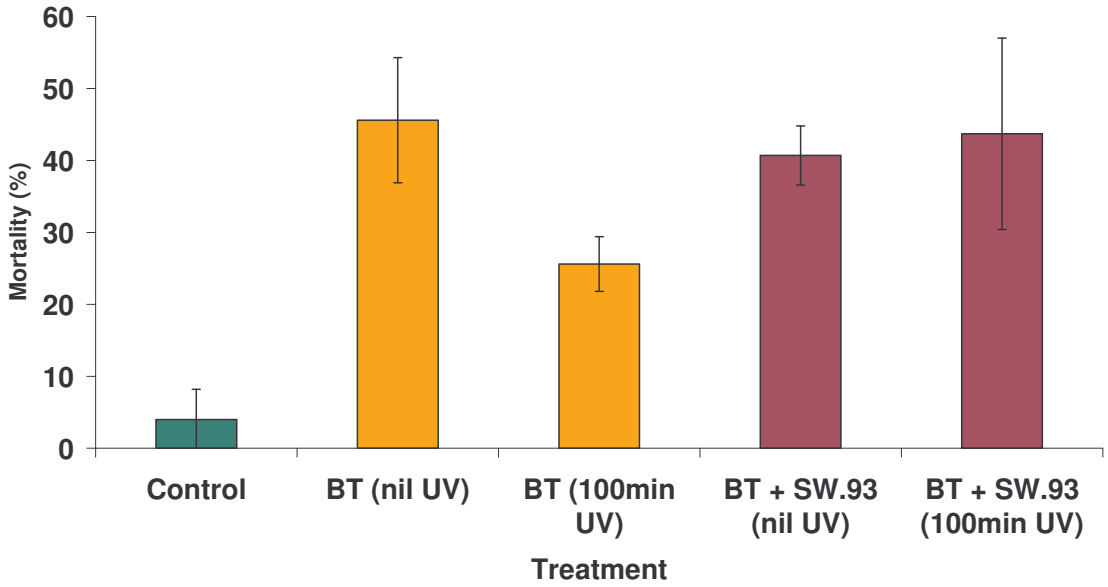


Figure 5: The impact of ultraviolet radiation on Bt (Dipel) + 0.2% UV protectant as measured by the mortality 2nd instar P. xylostella

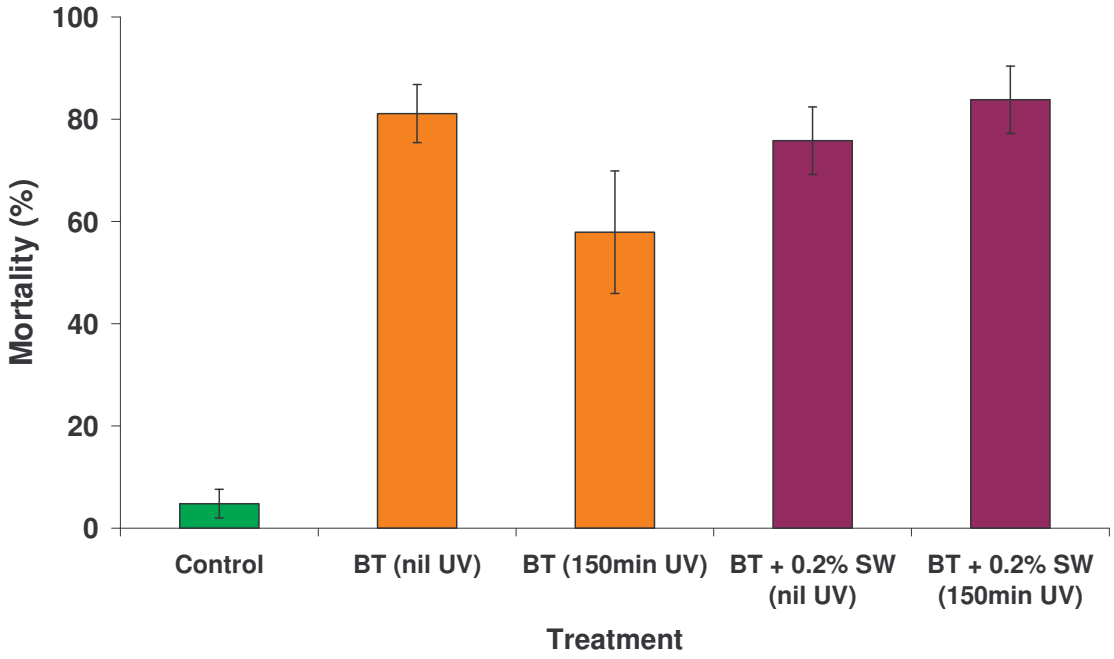
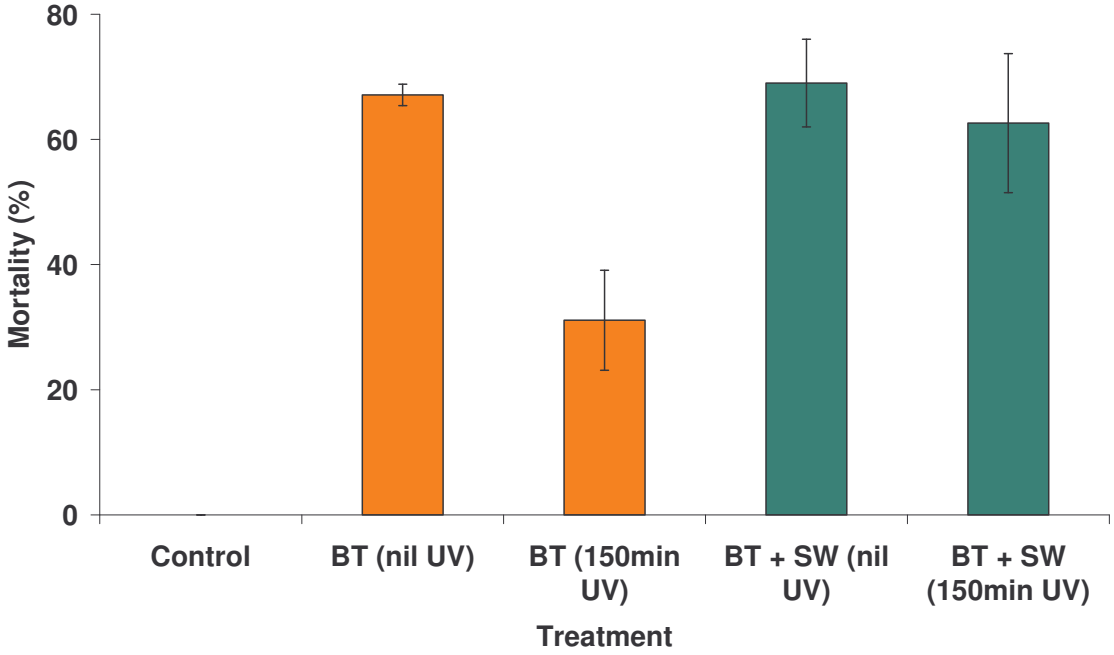


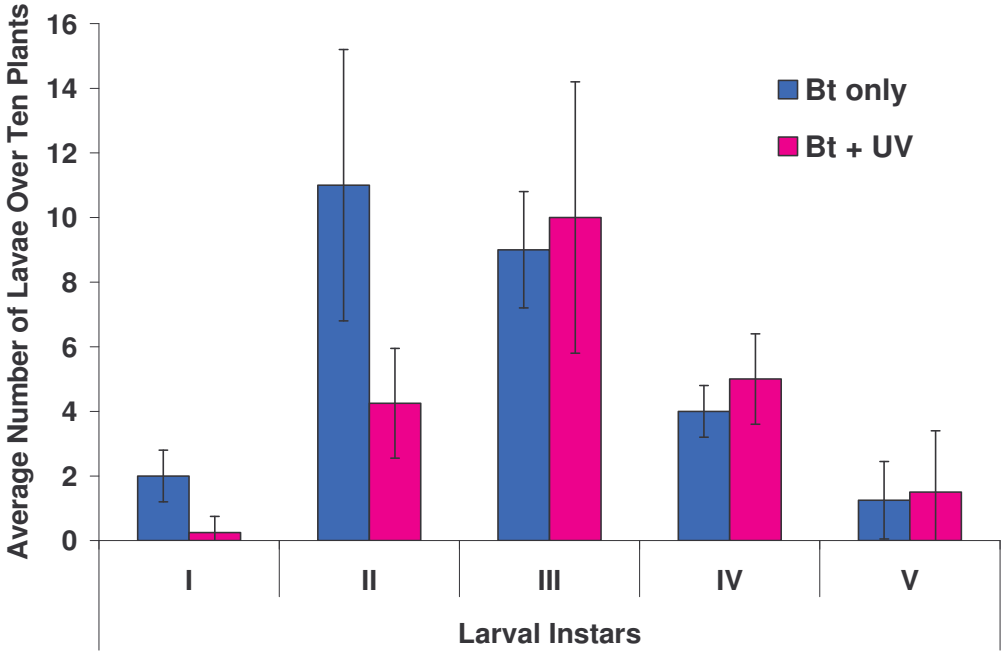
Figure 6: The impact of ultraviolet radiation on Bt (Dipel) + 0.1% UV protectant as measured by the mortality 2nd instar P. xylostella



The effectiveness of the UV protectant in protecting Bt (Dipel; used at 0.5g/L) from 150 minutes of UV exposure was also tested at 0.1% on spray water. The results obtained are shown in Figure 6. Again, the effectiveness of the UV protectant is demonstrated as significant reductions in mortality are encountered for the unprotected Bt whereas no change in efficacy is found where the protectant is used (Bioassay details in Appendix 3). On the basis of the results obtained in the laboratory bioassays field trial work was undertaken at a UV protectant usage rate of 0.2% on spray water. The authors recognise that the results obtained suggest that even lower levels of protectant may well be effective but believe that these need to be established by extensive field experience.

A field trial was performed during December 2003 and January 2004 on cabbage plants grown at the property of Mr. P. Schreurs, Devon Meadows, in southern Victoria (Detailed report in Appendix 4). The trial was performed under conditions of very high insect pressure. Application was by boom sprayer at an application rate of 1000L/Ha. The UV protectant was used at a rate of 0.2 % on spray water the commercial Bt products at 700g/Ha. Milk powder was applied at a rate of 1.5 kg/Ha as a feeding stimulant. The result (Figure 7) showed very significant reductions in the number of small caterpillars in the UV protectant treatment but no significant reductions in the larger caterpillars. We believe that there was no difference in numbers of the larger caterpillars because they were protected from the insecticide applications by being inside the developing cabbage.

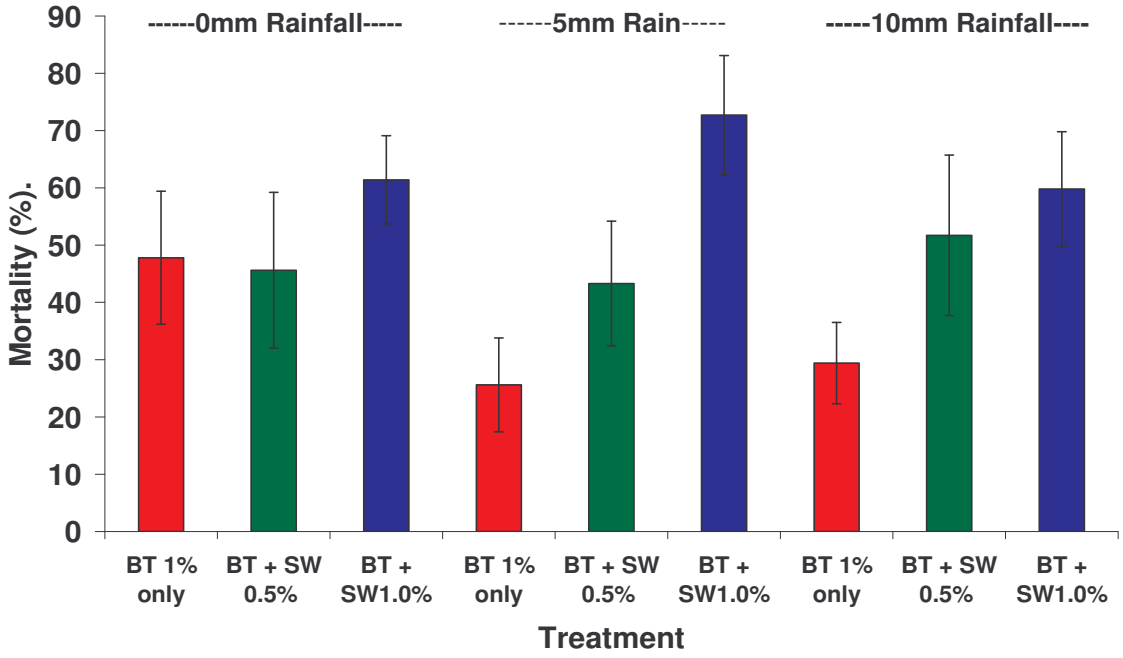
Figure7: The effect of UV protectant on the toxicity of Bt towards P. xylostella larvae (cabbage field trial)



The rainfastness of a Bt formulation protected by the UV protectant was also tested (Appendix 5). In these tests the UV protectant was used at 0.5 % and 1 % on spray water

and cabbage seedlings were sprayed to run-off with a hand-held atomizer. Plants were air-dried and stored at 20^{O}C. Rainfall simulation was applied using the Eureka AgreSearch rainfall simulator 19 hours post spray. *P. xylostella* larvae (2nd instar) were applied to excised leaves 4-6 hours after rainfall. Leaves were incubated at 20^{O}C for 72 hours before mortality was assessed. Six replicates per treatment were carried out. The results (Figure 8) clearly demonstrate the superior rainfastness of the applications that included the UV protectant.

Figure 8: The rainfastness of the UV protectant as measured by the mortality 2nd instar P. xylostella exposed to treated plants



Discussion

This project set out to develop a UV protectant for biological actives based on titanium dioxide. Feeding tests involving measurement of leaf areas consumed on both treated and untreated cabbage leave discs showed that the UV protectant did not deter the feeding of *P. xylostella* larvae. There was no difference between the control (water) and with the addition of the UV protectant at 0.5% and 1.0% on spray water. There was also no difference in feeding between Bt treatments with and without the addition of the UV protectant.

We have established that the UV protectant formulation developed in this project provides a definite agronomic benefit by providing significant protection for Bt against UV degradation. Effective use of the protectant at 0.2 % on spray water has been confirmed by field trials conducted by IPM technologies and by a commercial company interested in marketing the product. The commercial company is also planning field trials

that will explore the effectiveness of the UV protectant at lower protectant application rates.

An added, unplanned, advantage of the protectant formulation is that it is also effective as a sticker, enhancing the rainfastness of the Bt formulation.

There were some interesting efficacy anomalies between bioassays conducted at different times over the years of this project. For example, in separate experiments, Dipel used at 1% on spray water (Figure 4) appeared less efficacious than when used at 0.5% on spray water (Figure 5). However, this trend appears reversed within the single rainfastness experiment (Figure 8), where 1% treatments are more efficacious than 0.5% treatments. Increased efficacy with increases in Bt concentration would be expected, and is indeed observed within a single controlled experiment. Although examination of the reasons for efficacy differences between experiments done at different times was not undertaken as part of this project this may prove a worthy subject for further study.

Technology Transfer

Samples of the UV protectant for evaluation have been given under secrecy agreement to a number of companies with the potential to manufacture and market the product. Promising field trial results have been returned by one of these companies and discussions with potential companies are ongoing.

Recommendations

We do not believe that the UV protectant formulation that has been developed can be improved by further work and therefore do not recommend further work with titanium dioxide.

Further rigorous field trials are necessary to establish the optimum usage rate for cost effective performance and these are being undertaken by interested commercial organizations.

Investigations into the impact of leaf type on the effectiveness of the UV protectant may also prove interesting. We would not expect the leaf type to impact on the UV protectant but the protectant may ameliorate the impact of leaf type on the effectiveness of the Bt formulation.

APPENDIX 1

Bioassay data at 1.0% UV Protectant

Bt bioassay: UV degradation

Bt (Dipel) at 1.0g/L ± 1.0% UV protectant (SW.93)
2nd instar P. xylostella larvae on cabbage leaves

Treatment	dead	alive	total	% mort.	Mean mort.	SD	SE
Control	1	5	6	16.7			
Control	0	6	6	0.0			
Control	0	8	8	0.0			
Control	0	7	7	0.0	4.2	8.3	4.2
Bt (nil UV)	2	3	5	40.0			
Bt (nil UV)	3	5	8	37.5			
Bt (nil UV)	2	4	6	33.3			
Bt (nil UV)	5	2	7	71.4	45.6	17.5	8.7
Bt (100min UV)	2	11	13	15.4			
Bt (100min UV)	2	4	6	33.3			
Bt (100min UV)	2	5	7	28.6			
Bt (100min UV)	2	6	8	25.0	25.6	7.6	3.8
Bt + SW 1.0% (nil UV)	3	4	7	42.9			
Bt + SW 1.0% (nil UV)	2	3	5	40.0			
Bt + SW 1.0% (nil UV)	3	7	10	30.0			
Bt + SW 1.0% (nil UV)	3	3	6	50.0	40.7	8.3	4.1
Bt + SW 1.0% (100min UV)	4	2	6	66.7			
Bt + SW 1.0% (100min UV)	3	11	14	21.4			
Bt + SW 1.0% (100min UV)	4	2	6	66.7			
Bt + SW 1.0% (100min UV)	1	4	5	20.0	43.7	26.5	13.3

Graph

Treatment	Mortality (%)
Control	4
Bt (nil UV)	45.6
Bt (100min UV)	25.6
Bt + SW.93 (nil UV)	40.7
Bt + SW.93 (100min UV)	43.7

APPENDIX 2

Bioassay Data at 0.2% UV Protectant

Bt bioassay: UV degradation
Bt (Dipel) at 0.5g/L + 0.2% UV protectant (SW.98.13.3)
2nd instar *P. xylostella* larvae on cabbage leaves
72 hr assessment

Treatment	dead	alive	total	% mort.	Mean mort.	SD	SE
Control	0	9	9	0.0			
Control	1	10	11	9.1			
Control	1	9	10	10.0			
Control	0	12	12	0.0	4.8	5.5	2.8
Bt (nil UV)	8	3	11	72.7			
Bt (nil UV)	11	1	12	91.7			
Bt (nil UV)	7	3	10	70.0			
Bt (nil UV)	9	1	10	90.0	81.1	11.3	5.7
Bt (150min UV)	4	5	9	44.4			
Bt (150min UV)	6	4	10	60.0			
Bt (150min UV)	4	7	11	36.4			
Bt (150min UV)	10	1	11	90.9	57.9	24.1	12.0
Bt + 0.2% SW (nil UV)	9	3	12	75.0			
Bt + 0.2% SW (nil UV)	7	5	12	58.3			
Bt + 0.2% SW (nil UV)	8	2	10	80.0			
Bt + 0.2% SW (nil UV)	9	1	10	90.0	75.8	13.2	6.6
Bt + 0.2% SW (150min UV)	5	2	7	71.4			
Bt + 0.2% SW (150min UV)	8	1	9	88.9			
Bt + 0.2% SW (150min UV)	9	3	12	75.0			
Bt + 0.2% SW (150min UV)	11	0	11	100.0	83.8	13.2	6.6

Graph

Treatment	Mortality (%)	SE
Control	4.8	2.8
Bt (nil UV)	81.1	5.7
Bt (150min UV)	57.9	12
Bt + 0.2% SW (nil UV)	75.8	6.6
Bt + 0.2% SW (150min UV)	83.8	6.6

APPENDIX 3

Bioassay Data at 0.1% UV Protectant

Bt (Dipel) was used at a rate of 0.5g/L +/- 0.1% UV protectant (SW.98.13.3). Bioassay was carried out using 2nd instar *P. xylostella* larvae on cabbage leaves.
72 hr assessment.

Treatment	dead	alive	total	% mort.	Mean mort.	SD	SE
Control	0	13	13	0.0			
Control	0	14	14	0.0			
Control	0	11	11	0.0			
Control	0	9	9	0.0	0.0	0.0	0.0
Bt (nil UV)	14	7	21	66.7			
Bt (nil UV)	10	6	16	62.5			
Bt (nil UV)	14	6	20	70.0			
Bt (nil UV)	9	4	13	69.2	67.1	3.4	1.7
Bt (150min UV)	1	8	9	11.1			
Bt (150min UV)	3	6	9	33.3			
Bt (150min UV)	3	7	10	30.0			
Bt (150min UV)	5	5	10	50.0	31.1	15.9	8.0
Bt + 0.1% SW (nil UV)	9	6	15	60.0			
Bt + 0.1% SW (nil UV)	7	4	11	63.6			
Bt + 0.1% SW (nil UV)	5	3	8	62.5			
Bt + 0.1% SW (nil UV)	9	1	10	90.0	69.0	14.1	7.0
Bt + 0.1% SW (150min UV)	12	1	13	92.3			
Bt + 0.1% SW (150min UV)	4	5	9	44.4			
Bt + 0.1% SW (150min UV)	10	5	15	66.7			
Bt + 0.1% SW (150min UV)	8	9	17	47.1	62.6	22.1	11.1

Graph

Treatment	Mortality (%)	SE
Control	0	0
Bt (nil UV)	67.1	1.7
Bt (150min UV)	31.1	8
Bt + SW (nil UV)	69	7
Bt + SW (150min UV)	62.6	11.1

APPENDIX 4

Field trial of UV protectant with Bt insecticide on cabbages

Aim

To assess the effect of experimental UV protectant on the toxicity of Bt-based insecticides on *P. xylostella* larvae.

Method

A field trial was performed during December 2003 and January 2004 on cabbage plants grown at the property of P. Schreurs, Devon Meadows, in southern Victoria.

Plants: Young cabbage plants

Test insect: Natural infestations of *P. xylostella* larvae

Insect pressure: Very high

Spray application: Boom sprayer, at application rate of 1000 L/Ha using Hardy turquoise nozzles (the colour of the nozzle is specific for the nozzle size) at 58 psi pressure (4 bar).

Spray application date	**Bt product***
16th December 2003	Delfin
19th December 2003	Delfin
23rd December 2003	Delfin
27th December 2003	Xentari
30 December 2003	Xentari
3rd January 2004	Xentari

*Bt product applied at 700 g/Ha

Milk powder (1.2 kg/Ha) included as feeding stimulant

Agral (60 ml/100L) included as wetting agent

Bt applied with and without experimental UV protectant at rate of 200 ml/100L.

The trial consisted of four replicates of 10 cabbage plants for each treatment. Plants were sprayed between 4:00am and 6:00am on each occasion, and assessments of the number of larvae were made on 24 December 2003 and 5 January 2004. The final assessment is reported here.

Assessments were performed by examining the cabbage plants and recording the number of live *P. xylostella* larvae within the plants.

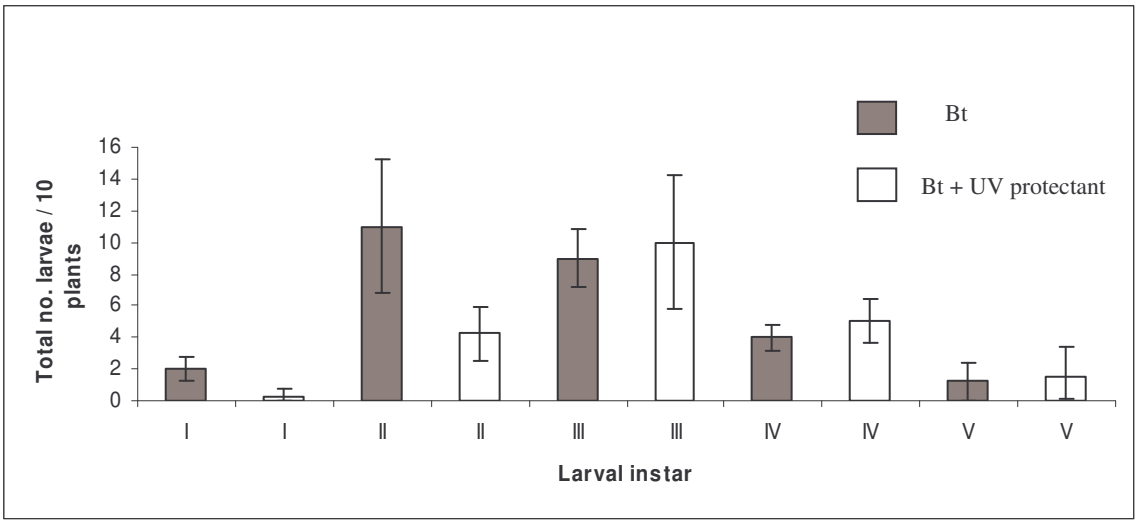
Results and Discussion

A range of *P. xylostella* larvae were found in the cabbages, from small first instars (approx. 2mm length) to the final fifth instars (approx. 10mm length). There were significantly fewer ($P<0.05$) first and second instar larvae in the cabbages treated with UV protectant than in the 'Bt only' treatments (Fig. A5.1). This suggests that the Bt with UV protectant is more effective than straight Bt at killing young *P. xylostella* larvae.

This may be due to enhanced longevity of the Bt toxin in the 'UV protectant' treatment by protection from ultraviolet degradation.

First and second instar larvae on cabbages are generally exposed to spray applications because they tend to be present on small plants and on the outer leaves of larger plants.

Fig. A5.1. Number of *P. xylostella* larvae in cabbages following applications of Bt insecticides with and without UV protectant.



Error bars indicate 95% confidence intervals.

There was no significant difference between treatments in the number of larger instar larvae (III - V instar). These instars were generally found inside the head of the developing cabbage where they are protected from insecticide sprays by several layers of cabbage leaves. This protection from spray applications is likely to be the reason that no difference was observed in the number of larvae between treatments.

Conclusion

The experimental UV protectant appeared to enhance the activity of the Bt insecticides on young larvae, which are exposed to spray applications.

Raw Data

Bt only

	No. of P. xylostella larval instars per 10 plants				
Rep	**I**	**II**	**III**	**IV**	**V**
1	2	17	7	3	1
2	1	10	10	5	0
3	3	10	11	4	1
4	2	7	8	4	3
Total	**8**	**44**	**36**	**16**	**5**

Mean/10 Plants	**2**	**11**	**9**	**4**	**1.25**
SD	0.8	4.2	1.8	0.8	1.3
SE	0.4	2.1	0.9	0.4	0.6
95% CI	0.8	4.2	1.8	0.8	1.2

Bt + UV protectant

	No. of P. xylostella larval instars per 10 plants				
Rep	**I**	**II**	**III**	**IV**	**V**
1	0	6	4	5	4
2	1	4	11	3	2
3	0	2	11	6	0
4	0	5	14	6	0
Total	**1**	**17**	**40**	**20**	**6**
Mean/10 Plants	**0.25**	**4.25**	**10**	**5**	**1.5**
SD	0.5	1.7	4.2	1.4	1.9
SE	0.3	0.9	2.1	0.7	1.0
95% CI	0.5	1.7	4.2	1.4	1.9

APPENDIX 5

Rainfastness Bioassay Data for formulation SW.98.13.3

Cabbage seedlings were sprayed to run-off with a hand-held atomizer. Plants were air-dried and stored at 20ºC. Rainfall simulation was applied 19 hours post spray. *P. xylostella* larvae (2nd instar) were applied to excised leaves 4-6 hours after rainfall. Leaves were incubated at 20ºC for 72 hours before mortality was assessed. Six replicates per treatment were carried out.

TABLE A6.1
Summary of Results

Treatment	Simulated Rainfall (mm)	Mort. (%)	SE	Fraction of activity remaining
Bt 1.0% only	0	47.8	11.6	1
Bt + SW 0.5%	0	45.6	13.6	1
Bt + SW1.0%	0	61.4	7.7	1
Bt 1.0% only	5	25.6	8.2	0.54
Bt + SW 0.5%	5	43.3	10.9	0.95
Bt + SW1.0%	5	72.7	10.4	1.18
Bt 1.0% only	10	29.4	7.1	0.62
Bt + SW 0.5%	10	51.7	14	1.13
Bt + SW1.0%	10	59.8	10	0.97

TABLE A6.2
Raw Data

Treatment	Rainfall (mm)	Dead	Alive	Total	Mortaltiy (%)	Mean mort. (%)	SD	SE
Control	0	0	5	5	0.0			
Control	0	0	5	5	0.0			
Control	0	0	5	5	0.0			
Control	0	0	5	5	0.0			
Control	0	0	5	5	0.0			
Control	0	0	5	5	0.0	0.0	0.0	0.0
Bt 1.0%	0	3	2	5	60.0			
Bt 1.0%	0	2	3	5	40.0			
Bt 1.0%	0	0	5	5	0.0			
Bt 1.0%	0	4	2	6	66.7			
Bt 1.0%	0	4	1	5	80.0			
Bt 1.0%	0	2	3	5	40.0	47.8	28.1	11.6
SW 0.5%	0	4	1	5	80.0			
SW 0.5%	0	4	1	5	80.0			
SW 0.5%	0	3	2	5	60.0			
SW 0.5%	0	2	4	6	33.3			
SW 0.5%	0	0	6	6	0.0			
SW 0.5%	0	1	4	5	20.0	45.6	33.0	13.6
SW 1.0%	0	3	2	5	60.0			
SW 1.0%	0	2	3	5	40.0			
SW 1.0%	0	6	1	7	85.7			
SW 1.0%	0	4	1	5	80.0			
SW 1.0%	0	3	2	5	60.0			
SW 1.0%	0	3	4	7	42.9	61.4	18.7	7.7
Bt 1.0%	5	1	4	5	20.0			
Bt 1.0%	5	1	4	5	20.0			
Bt 1.0%	5	3	2	5	60.0			
Bt 1.0%	5	2	4	6	33.3			
Bt 1.0%	5	1	4	5	20.0			
Bt 1.0%	5	0	5	5	0.0	25.6	20.0	8.2
SW 0.5%	5	0	5	5	0.0			
SW 0.5%	5	3	2	5	60.0			
SW 0.5%	5	2	3	5	40.0			
SW 0.5%	5	2	3	5	40.0			
SW 0.5%	5	4	1	5	80.0			
SW 0.5%	5	2	3	5	40.0	43.3	26.6	10.9
SW 1.0%	5	5	2	7	71.4			
SW 1.0%	5	3	2	5	60.0			
SW 1.0%	5	5	2	7	71.4			
SW 1.0%	5	5	0	5	100.0			
SW 1.0%	5	5	0	5	100.0			
SW 1.0%	5	2	4	6	33.3	72.7	25.3	10.4
Bt 1.0%	10	2	3	5	40.0			
Bt 1.0%	10	3	2	5	60.0			
Bt 1.0%	10	1	4	5	20.0			

Bt 1.0%	10	1	4	5	20.0			
Bt 1.0%	10	1	4	5	20.0			
Bt 1.0%	10	1	5	6	16.7	29.4	17.2	7.1
SW 0.5%	10	1	5	6	16.7			
SW 0.5%	10	4	1	5	80.0			
SW 0.5%	10	2	4	6	33.3			
SW 0.5%	10	5	0	5	100.0			
SW 0.5%	10	3	2	5	60.0			
SW 0.5%	10	1	4	5	20.0	51.7	34.0	14.0
SW 1.0%	10	2	4	6	33.3			
SW 1.0%	10	2	3	5	40.0			
SW 1.0%	10	6	1	7	85.7			
SW 1.0%	10	4	1	5	80.0			
SW 1.0%	10	4	1	5	80.0			
SW 1.0%	10	2	3	5	40.0	59.8	24.4	10.0