Evaluation of BOTRY-Zen and BZII on Process Tomatoes in Hawke's Bay: 2004-2005

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Confidential report to Botry-zen NZ. Ltd.

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This report has been prepared by The Horticulture and Food Research Institute of NZ Ltd (HortResearch), which has its Head Office at Mt Albert Research Centre, Private Bag 92 169 Auckland. The report has been approved by:

Research Scientist

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Date:

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EXECUTIVE SUMMARY

Background

Field tomatoes are grown in many countries and used in many food products. Unlike glasshouse tomatoes, field tomatoes have been specifically bred for mechanical harvesting and processing. They have a growth habit is quite different to the glasshouse varieties, forming a low, compact, dense canopy. The "close to ground" nature of the crop creates an ideal environment for several fungal pathogens; the most important including, *Botrytis cinerea*, three species of *Sclerotinia* (*Sclerotinia sclerotiorum*, *Sclerotinia minor*, and *S. rolfsii*) and *Alternaria alternata*. Large crop losses due to one or more of these pathogens have been reported when conditions are favourable for infection, especially close to harvest.

BOTRY-Zen NZ Ltd, (BZL) approached HortResearch in 2004, with a view to field evaluating two of their products for suppression of *Botrytis* and *Sclerotinia* in field grown tomatoes for processing. This request was based upon their market research that identified a significant global opportunity for their biological control product, BOTRY-Zen (BZ) and new product (BZII). If field trials in NZ were successful, BZL would proceed with further evaluation in Italy, the second largest global producer of tomatoes for processing after the USA.

The aim of this part of the BZL programme for 2004-05 was to evaluate BOTRY-Zen® based programmes for control of *Botrytis*, *Sclerotinia* and harvest rots (e.g. in process tomatoes in Hawke's Bay.

Key Findings

- Despite the relatively low levels of disease, the BZ applications over flowering showed a clear reduction of *Botrytis* incidence compared with the unsprayed and fungicide (Shirlan®) control treatments, as measured by fruit affected by *Botrytis* at harvest.
- Neither the addition of extra applications of BZ pre-harvest nor the addition of BZII (chitosan) showed any incremental improvement in *Botrytis* control over the flower applications alone.
- Neither crop maturity nor fruit soluble solids levels (Brix) were affected by any treatment.
- No clear differences amongst treatments were found for *Sclerotinia*.

Recommendations

These conclusions should be treated as preliminary, and as such, be reconfirmed preferably under higher *Sclerotinia* disease pressure, and in a more commercial setting.

INTRODUCTION

Field tomatoes are grown in many countries and used in many food products. Unlike glasshouse tomatoes, field tomatoes have been specifically bred for mechanical harvesting and processing. Their growth habit is quite different to the glasshouse varieties, forming a low, compact, dense canopy. The "close to ground" nature of the crop creates an ideal environment for several fungal pathogens; the most important including, *Botrytis cinerea*, three species of *Sclerotinia* (*Sclerotinia sclerotiorum*, *Sclerotinia minor*, and *S. rolfsii*) and *Alternaria alternata*. Large crop losses due to one or more of these pathogens have been reported when conditions are favourable for infection, especially close to harvest.

Disease control with current registered agrichemicals is an option, but these are not always effective based upon the experiences of Heinz-Wattie field technical staff in Hawke's Bay (Kale *pers comm.*). Consequently, this industry is actively seeking alternative methods of disease control, which are not based upon registered agrichemicals.

BOTRY-Zen NZ Ltd, (BZL) approached HortResearch in 2004, with a view to field evaluating two of their products for suppression of *Botrytis* and *Sclerotinia* in field grown tomatoes for processing. This request was based upon their market research that identified a significant global opportunity for their biological control product, BOTRY-Zen (BZ) and their new chitosan based product (BZII). If field trials in NZ were successful, BZL would proceed with further evaluation in Italy, the second largest global producer of tomatoes for processing after the USA (Anon, 2003).

The aim of this part of the BZL programme for 2004-05 was to evaluate BOTRY-Zen® based programmes for control of *Botrytis*, *Sclerotinia* and harvest rots in process tomatoes grown in the Hawke's Bay.

METHODS

Site and crop management

A collaborative approach was adopted for this field trial. Crop & Food Research staff at the Lawn Road Research Centre (Clive, Hawke's Bay) maintained the site and provided crop production inputs including a general pest and disease spray programme, weed control, and irrigation. HortResearch staff applied all treatment applications and carried out mid season disease assessments. Elak Consultants Ltd. provided crop management advice as well as flower and harvest assessments.

All efforts were made to manage the crop using standard commercial practices. The tomato plants were propagated commercially and plug transplants planted in a single row bed system with 1.5-meter centers. Plants were spaced 30 centimeters apart as per normal commercial practice and fertiliser applied just prior to raising the beds. The transplanter applied starter fertiliser and sidedress fertiliser at the time of planting.

Initial trial establishment was poor (uneven plant take), including foliar symptoms that resembled herbicide contamination. The trial site was then completely re-worked and replanted on December 4th. Although the re-planting was carried out relatively late in the growing season, it had the advantage of providing more favorable conditions for *Botrytis* and *Sclerotinia* development.

However, in the subsequent planting there were still some residual herbicide-like symptoms. These symptoms are now believed to relate to an application of Tordon® to the pasture in the previous year.

Careful selection of the plot rows was undertaken to minimize any impact of this residue on the results, and it was agreed to continue with the trial. As the crop grew, the symptoms disappeared, and no obvious effect on crop growth or production from the herbicide residue was observed.

Details of weed, pest and disease management can be found in Appendix 2. Copper and Mancozeb were used during the early part of the growing season but Mancozeb was not applied again after the 16 January (start of flowering) due to incompatibility with BZ. Insecticides were used for insect control as required. Routine weed management was carried out using a combination of herbicides and manual weeding. Overhead irrigation was applied to the whole site at regular intervals throughout the growing season. Ethrel®, a fruit-ripening hormone that induces ethylene production in the plant and is used commercially to enhance tomato ripening, was applied over the entire trial area prior to harvest.

Each of the six treatments (listed in Table 1) was randomly assigned to six replicate blocks (details of the trial layout can be found in Appendix 1). All products were mixed directly with clean tap water and the adjuvant Nu-Film™ 17 was added at the recommended field rate (30 ml 100 ℓ^{-1}) to the spray tank whilst filling. Treatments were applied to run-off using a motorised hydraulic pump sprayer, delivering a water rate of 600 ℓ ha^{-1}, 800 ℓ ha^{-1} and 950ℓ ha^{-1} for the flowering sprays. All preharvest sprays were applied at 950ℓ ha^{-1}.

Experimental design and spray treatments

A replicated block design with six replicates was used in this study. Extra rows were placed as buffer rows around the trial. Each plot comprised three rows with harvest measurements being taken from the center three meters of the center row. Total plot size was 18m^2.

Table 1. Treatments applied to replicate tomato plots in Hawke's Bay during 2004-05.

Treatment	Product	Rate	Growth stage	Spray dates
Nil (control)[1]				
Fungicide (Flowering only)	Shirlan®	12.5ml/10L	Early flowering	22 Jan 2005
	Shirlan®	9.5ml/10L	Mid flowering	2 Feb 2005
	Shirlan®	3.8ml/10L	Half rate at late flowering	11 Feb 2005
BOTRY-Zen® (Flowering only)	BOTRY-Zen®	2 x 10^6 spores ml^{-1}	Early flowering	22 Jan 2005
			Mid flowering	2 Feb 2005
			Late flowering	11 Feb 2005
BOTRY-Zen® (Full season)	BOTRY-Zen®	2 x 10^6 spores ml^{-1}	Early flowering	22 Jan 2005
			Mid flowering	2 Feb 2005
			Late flowering	11 Feb 2005
			Fruit ripening	3 March 2005
			Fruit ripening	15 March 2005
			Late fruit ripening	21 March 2005
BOTRY-Zen® with BZII (Flowering only)	BOTRY-Zen®	2 x 10^6 spores ml^{-1}	Early flowering	22 Jan 2005
			Mid flowering	2 Feb 2005
	Chitosan	0.3g/L	Late flowering	11 Feb 2005
BOTRY-Zen® at flowering	BOTRY-Zen®	2 x 10^6 spores ml^{-1}	Early flowering	22 Jan 2005
			Mid flowering	2 Feb 2005
			Late flowering	11 Feb 2005
Then BZII pre-harvest	Chitosan	0.3g/L	Fruit ripening	3 March 2005
			Fruit ripening	15 March 2005
			Late fruit ripening	21 March 2005

[1] Nil *Botrytis* and *Sclerotinia* fungicides during flowering.

Note that chlorothalonil (Chlorotek®) was used for control of black mold (*A. alternaria*) across the whole trial area at the time of Ethrel® application (2 weeks prior to harvest on 31 March).

Disease Assessments

Visual score of Botrytis and Sclerotinia per plot

After the last flowering spray, a visual assessment of both *Sclerotinia* and *Botrytis* was carried out by Elak Consultants Ltd on 25 Feb 2005. Only one plant had *Sclerotinia* infection at this time. There was very little *Botrytis* fruit infection but active *Botrytis* was seen on the later flower trusses (decaying flower parts around very small fruitlets (Figure 1 below). *Botrytis* "strikes" were assessed by visually inspecting all plants in a plot and recording the number of immature fruit with typical *Botrytis*-like symptoms (Figure 2).

Figure 1. Photograph showing actively sporulating *Botrytis* on the old petal and calyx.

Figure 2. Photograph of a typical *Botrytis* strike post flowering (25 February 2005). A water soaked pale tan rot has progressed down the fruit towards the stylar end. The infection has also moved up the pedicel and a canker- like lesion has formed on the main truss.

Sources of Botrytis and Sclerotinia inoculum in field tomato canopies

A field inspection identified several potential sources of *Botrytis/Sclerotinia* infection/inoculum sites in the tomato canopy (Figure 3 below). These were termed "floral tissues" and consisted of; green calyx with a senescing petal still attached, calyx minus any senescing floral tissues and necrotic petals adhering to the truss. Other canopy tissues identified were necrotic leaves from the bottom of the canopy and green leaves in the canopy above ground with necrosis. *Botrytis* infection of immature green fruit in many other crops has been reported and was not known for process tomatoes therefore, these fruit were also sampled for latent fruit infection.

Figure 3. Field inspection and sampling of floral, and vegetative tissues and green fruit immediately post-flowering on 23 February

Infection of floral tissues
Twelve days after the late flowering spray (11 Feb), a sample of ten flower trusses minus developing fruit were taken at random from each plot and returned to HortResearch Ruakura. From this sample, three sub-samples were cut from the main trusses with flame-sterilised dissection scissors then placed into high humidity chambers consisting of sterile plastic petri dishes with two layers of sterile Whatman filter paper, to which one millilitre of sterile water was added. The sub samples were;
Calyx plus petals (min/plot=7; max per plot=11)
Calyx minus petals (min/plot=3; max per plot=10)
Petals adhering to the truss "Petals only" (min/plot=4; max per plot=11)

The high humidity chambers were placed in plastic Plix lunch boxes to prevent drying and incubated for 5 days at room temperature to simulate disease-conducive conditions. The number of tissue types in each petri dish with *Botrytis* sporulation /*Sclerotinia* mycelium was recorded and the results expressed as a percentage. Each tissue type with *Botrytis*/plot was also visually rated for the extent of *Botrytis* inoculum production (0-5 scale) based upon the number of conidiophores on the tissue after incubation where, (1 = 0–5, 2 = 6–10, 3 = 1-20, 4 = 21–40, 5 = 40–80). A *Botrytis* severity index per plot for each tissue type was then calculated.

Botrytis infection of necrotic leaf tissues: incubation tests
In addition to the flowering trusses sampled on 11 Feb, a sample of up to 10 completely necrotic old leaves from the bottom of the canopy ("dead leaves", DL) and up to 5 green leaves with necrosis (GLWN) was collected. All tissue types were taken at random from each plot and returned to HortResearch Ruakura for processing. Tissues were evenly spread out over a pre moistened paper towel in a plastic tray, then placed in *Botrytis* conducive conditions by sealing several trays in large plastic bags to maintain high humidity at room temperature. The incidence, area (cm^2) and severity of *Botrytis* sporulation on dead leaflets, dead petioles and GLWN were recorded after six days. For each tissue type with *Botrytis* sporulation, the intensity of sporulation was allocated a score from 1-5. Where 1 = sparse sporulation, 2 = sparse to moderate intensity, 3 = moderate intensity, 4 = moderate to very dense sporulation, 5 = very dense sporulation. An estimate of *Botrytis* inoculum potential from each tissue type was calculated from the product of sporulation area (cm^2) and sporulation intensity (1-5).

Botrytis infection of immature green fruit: The ONFIT test
In addition to the samples above a sample of 10 immature green fruit from fruiting trusses in the canopy was also collected at random from each plot, and these were sent HortResearch Ruakura for processing. Fruit were triple surface sterilised by soaking in 70% alcohol, then 0.5% sodium hypochlorite, then 70% alcohol again. After this process fruit were rinsed thoroughly in running tap water, placed in plastic letter trays and frozen overnight at minus 20C to break down any host barriers to pathogen expression. After freezing, trays were placed in *Botrytis*-conducive conditions by sealing in plastic bags to maintain high humidity at room temperature. The number of fruit with *Botrytis* was recorded after six days.

Harvest measurements
Elak Consultants Ltd carried out all harvest assessments on 13 April 2005. During the harvesting operation, fruit rots were recorded and separated into three categories: *Botrytis*, *Sclerotinia*, and Other Rots. The main components of the Other Rot category were Black Mould, Anthracnose and ground rots. For this category, any fruit with symptoms only just apparent or considered minor (i.e. less than 5 % of the fruit surface affected), was classified as sound and not included in the rot category. This is in line with standard grading practice for late season process crops.
Field assessment of *Sclerotinia* and *Botrytis* can at times be difficult to differentiate even by an experienced field manager, therefore a combined category was also included in the analysis of component fruit rots.

To determine treatment effects on soluble solids (an important factor in paste production) Brix measurements were taken from three separate samples from each plot. The standard method developed by Heinz-Waittie's for determining Brix in tomatoes was used in this study and an Atago digital refractometer was used to measure the Brix.

Statistical analysis

All data were subject to analysis of variance (ANOVA) using a Generalised Linear Model in the Genstat™ statistical package. Least significant differences (LSD) were calculated in order to separate treatment means for significance at P=0.05. Count data for the ONFIT of green immature fruit was analysed using a generalised linear model with binomial distribution fitted.

RESULTS

Seasonal weather conditions

December was an unusually cool wet month, with regular rainfall through until the first of the flowering sprays in mid January (For details please see Appendix 1). The weather pattern then changed to provide warm dry conditions until mid March. During this period regular irrigation was applied to the trial site, as per a normal commercial crop. The possible need for additional irrigation was discussed but not requested. This period of warm dry weather extended from just prior to flowering, until mid way between the end of flowering and harvest. From mid March through to harvest, on the 14th April, the conditions were cloudy and wet with long periods of leaf and fruit wetness. Rainfall was measured on at least 15 of the last 18 days in March. These conditions would be considered by growers to be especially favorable for *Botrytis* and *Sclerotinia* problems to develop in a tomato crop. In summary, while the weather conditions were not considered ideal for disease development in 2004-05 season, they were regarded as "normal" for this region.

Disease measurements

Post flowering assessment

After the flowering sprays, Elak Consultants Ltd carried out a visual assessment of both *Sclerotinia* and *Botrytis* on 25 Feb 2005. Only one plant had *Sclerotinia* infection at this assessment time. There was very little *Botrytis* fruit infection but active *Botrytis* was seen on the later flower trusses (decaying flower parts around very small fruitlets). Only the *Botrytis* flower strike category is presented, due to the very low incidence for the other disease categories (Table 2).

Table 2: Post-flower *Botrytis* infections

Treatment	Post flowering *Botrytis* strike count	
Nil	7.7	a
Shirlan	6.2	a
BZ at flowering only	6.7	a
BZ flowering then BZ pre harvest	7.2	a
BZ plus chitosan (flowering only)	8.0	a
BZ flowering then chitosan pre harvest	5.5	a
LSD (P=0.05)	3.19	

Means followed by the same letter are not significantly different (P>0.05; LSD test)

Sources of Botrytis and Sclerotinia inoculum in field tomato canopies

1. Infection of floral tissues

Only one floral tissue (calyx with a petal attached) had typical *Sclerotinia* symptoms, indicating that very little *Sclerotinia* infection in the canopy had occurred. The incidence of *Botrytis*, in the three different floral tissues is presented in Table 3. None of the flowering treatments significantly (P>0.05) reduced the incidence of *Botrytis* on floral tissues or *Botrytis* severity. There was considerable plot-to-plot variation (relatively high LSD values) and this has contributed to a lack of significant differences being detected.

Table 3: Incidence of *Botrytis* and *Botrytis* severity on tomato floral tissues in Hawke's bay in 2005.

Treatment	Calyx + Petal (%)	Calyx minus petal (%)	Adhering petals (%)	All floral tissues (%)	Average *Botrytis* severity on all floral tissues
Nil	7.1	5.2	17.5	8.58	3.8
Shirlan®	1.7	2.1	4.2	3.07	0.7
BZ at flowering only	13.5	0.0	3.8	7.02	2.7
BZ flowering then BZ pre harvest	3.3	4.2	3.9	4.08	0.7
BZ plus chitosan (flowering only)	13.3	2.4	0.0	6.1	3.8
BZ flowering then chitosan pre harvest	10.6	2.8	1.7	6.8	1.3
ANOVA significant at P<0.05	ns	ns	ns	ns	ns
LSD (P=0.05)	**9.9**	**7.3**	**15.5**	**6.1**	**3.6**

ns= not significant (P value >0.05)

Interestingly, the average *Botrytis* severity was low (nil=av. of 3.8 conidiophores/floral tissue), in the nil flowering treatment, suggesting that conditions had not been favorable for floral tissue infections. Low severity also indicates that these tissues may not be an important source of inoculum relative to other sources (e.g. leaf petioles as discussed in the next section).

The greatest reductions in *Botrytis* incidence were seen on the adhering petals and BZ was similar to Shirlan® (Figure 4). The combination of BZ with chitosan resulted in no *Botrytis* being detected at all on the petals adhering to the truss.

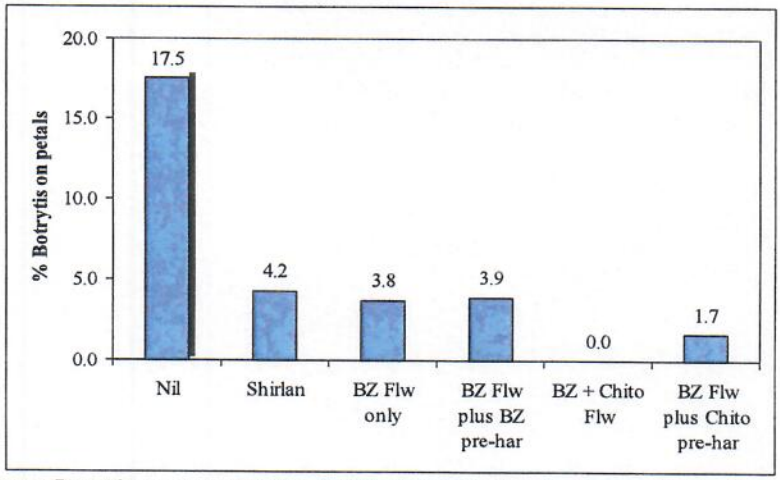

Figure 4: Percent Botrytis on petals (11 Feb, 2005) adhering to tomato trusses. Vertical bar = LSD (15.5) at P=0.05

Because of the nature of flowering in field tomatoes (developing fruit and flowers on the same truss) it is possible that the floral tissues sampled actually emerged after our flowering applications and as a consequence would have had little BZ establishment on them. Visual observations of BZ colonization of the floral tissues tended to support this hypothesis since BZ colonization was much lower than we would have expected (e.g. compared to kiwifruit with >90% of each petal colonized by BZ).

2. *Botrytis* infection of necrotic leaf tissues

Two necrotic leaf types were sampled for incidence and extent of *Botrytis* colonization after the flowering sprays in 2005. An estimate of *Botrytis* inoculum potential from each tissue type was calculated from the product of sporulation area (cm^2) and sporulation intensity (1-5). The incidence of *Botrytis* and *Botrytis* inoculum potential on necrotic leaflets at the bottom of the canopy (Figure 5) was not significantly different between BZ and Shirlan treatments (Table 4).

Table 4: Incidence of *Botrytis* and *Botrytis* inoculum potential on necrotic tomato leaves sampled from the bottom of the tomato canopy and on green leaves with necrosis (GLWN) in the canopy assessed post flowering in 2005.

Treatment	Necrotic leaflets with *Botrytis* (%)	*Botrytis* inoculum potential on necrotic leaflets[1]	Necrotic leaf petioles with *Botrytis* (%)	*Botrytis* inoculum potential on necrotic petioles[1]	GLWN with *Botrytis* (%)	*Botrytis* inoculum potential on GLWN[1]
Nil	12.8	4.8	10.0	25.4	1.5	1.0
Shirlan®	11.8	4.2	23.3	34.5	6.1	0.5
BZ at flowering only	7.9	3.5	33.3	44.0	9.5	1.7
BZ flowering then BZ pre harvest	13.6	3.9	26.7	25.8	4.3	0.8
BZ plus chitosan (flowering only)	4.6	1.1	13.3	18.0	1.2	1.0
BZ flowering then chitosan pre harvest	9.2	6.0	40.0	58.0	1.5	1.5
ANOVA at P<0.05	ns	ns	*	ns	*	ns
LSD (P=0.05)	**10.6**	**6.0**	**25.0**	**56.8**	**7.4**	**2.7**

[1] is the product of the area in Botrytis sporulation x the severity of sporulation (1-5 scale) and is an estimate of potential Botrytis inoculum production from this inoculum source relative to other leaf sources.

* significant at P<0.05

ns= not significant (P value >0.05)

Overall, *Botrytis* incidence and *Botrytis* inoculum potential on the necrotic petioles of leaves from the bottom of the canopy (gray shaded columns, Table 4) was higher compared to the leaflets at the bottom of the canopy (Figure 5). None of the flowering treatments reduced incidence or inoculum potential on this tissue type and there were no significant differences between Shirlan® or BZ treatment at flowering (Figure 4, Table 3). No additional benefit of adding BZII (chitosan) was measured. Shirlan®, BZ, and BZII have been reported as having good botryicidal activity and this finding indicates there was insufficient active compound or BCA inoculum present to protect the tissue at the time of necrosis. The dense nature of the tomato canopy suggests that it is difficult to penetrate with conventional spray equipment and

improvements to application technology and/or increased water rates at flowering are required if these products are to be effectively evaluated in the future. *Botrytis* incidence in one of the BZ flowering treatments (BZ at flowering then BZ chitosan preharvest) was significantly ($P<0.05$) higher compared to the nil but the overall average of BZ treatments over flowering was not significantly different from the nil treatment.

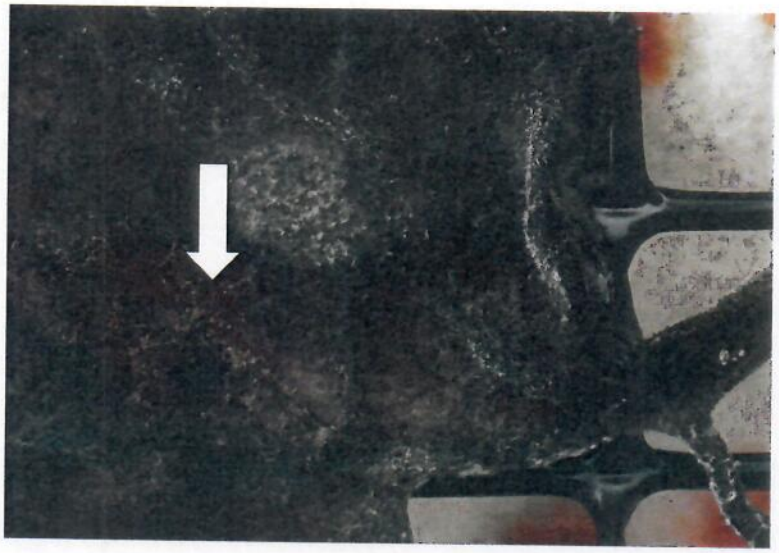

Figure 5. Sparse *Botrytis* sporulation (arrow) typically found on the leaflet of tomato leaves sampled from the bottom of the canopy.

Figure 6. Compared to fruitlets, profuse *Botrytis* sporulation (white arrow) occurred on the petioles of tomato leaves (from the dead leaf sample from the bottom of the canopy).

Botrytis incidence and *Botrytis* inoculum potential on green leaves with necrosis (GLWN) was low in the nil treated plots (1.5% and 1 respectively, Table 4). This finding indicates that this tissue type may not be as favourable for *Botrytis* infection and colonization, compared to the other tissue types identified.

Botrytis latent infection of immature green fruit

Latent infection of green immature tomatoes was detected and the highest incidence occurred in the nil treated plots (Figure 7). All treatments reduced latent infections and analysis of treatments for significant difference compared to the nil are presented in Table 5.

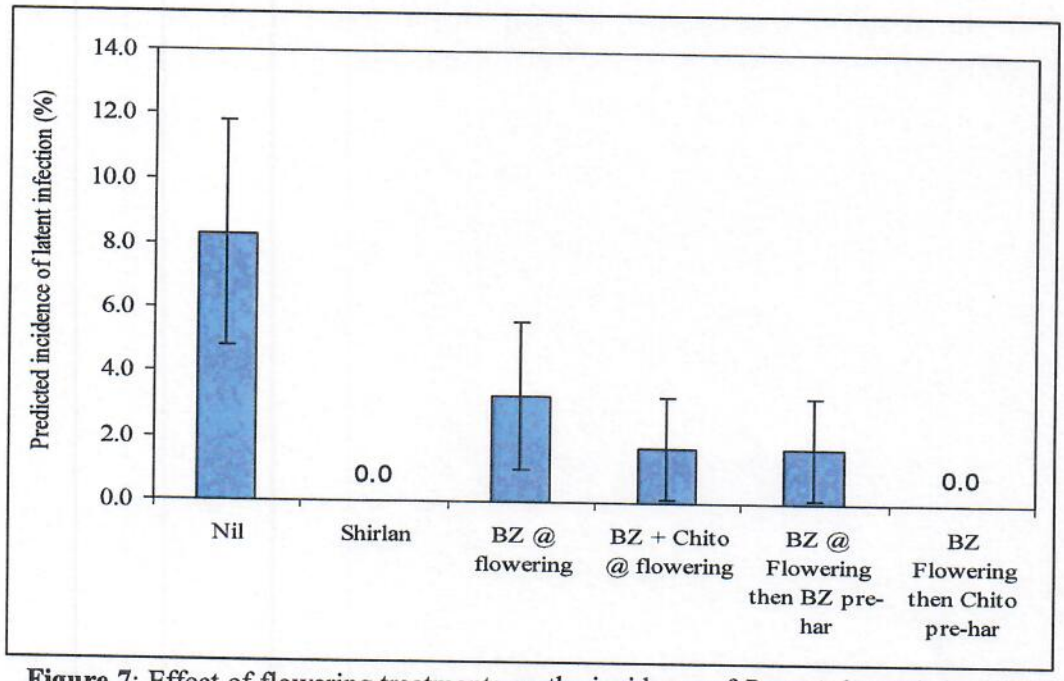

Figure 7: Effect of flowering treatments on the incidence of *Botrytis* latent infections in green immature tomatoes sampled after flowering in 2005. Bars are plus and minus the standard error of the mean.

Treatment with Shirlan® and one of the flowering BZ treatments ("BZ flowering the chitosan) completely prevented the formation of latent infections by *Botrytis*. However, since all the BZ treatments had BZ applied over flowering and the latents were sampled after the last flowering spray it would be more appropriate to conclude that BZ flowering treatment reduced latent infections from 8.3% (the level on the nil) to 1.7% (the average of the BZ treatments), an 80% reduction.

Table 5. Analysis of actual and predicted incidence of *Botrytis* latent infections in green immature tomato fruit sampled at the end of flowering in 2005.

	Treatment	Difference	SED	P value	Significance
Nil vs	Shirlan®	8.3%	3.5%	0.026	<0.05
Nil vs	3 x BZ @ flowering only	5.0%	4.2%	0.247	NS
Nil vs	BZ @ flowering + BZ preharvest	6.7%	3.9%	0.099	<0.10
Nil vs	BZ + BZ chitosan @ flowering only	6.7%	3.9%	0.099	<0.10
Nil vs	BZ @ flowering then BZ chitosan pre harvest	8.3%	3.5%	0.026	<0.05

Analysis of actual and predicted incidence of Botrytis latent infections in green immature tomato fruit sampled at the end of flowering in 2005.

Shirlan® and one of the four BZ flowering treatments, significantly (P<0.05) reduced *Botrytis* latent infections compared to the nil (Table 5). Two of the other BZ flowering treatments rduced Botrytis latent latent infections compared to the nil but this only significant at P<0.10 and not P<0.05. These findings indicate that *Botrytis* latent infections occur in field tomatoes and this is the first report that we are aware of that has reported these findings.

Epidemiologically, we are not certain if this relates to crop loss but our finding that BZ significantly reduced *Botrytis* crop loss at harvest suggests that there may be a link. The data indicates (Figure 8) that there may be a stronger association between latents and crop loss compared to other inoculum sources measured (e.g. petioles and floral tissues). The pathway from flowering to latent infection is not clear in this crop since there have been so few reports of epidemiological studies in process tomatoes.

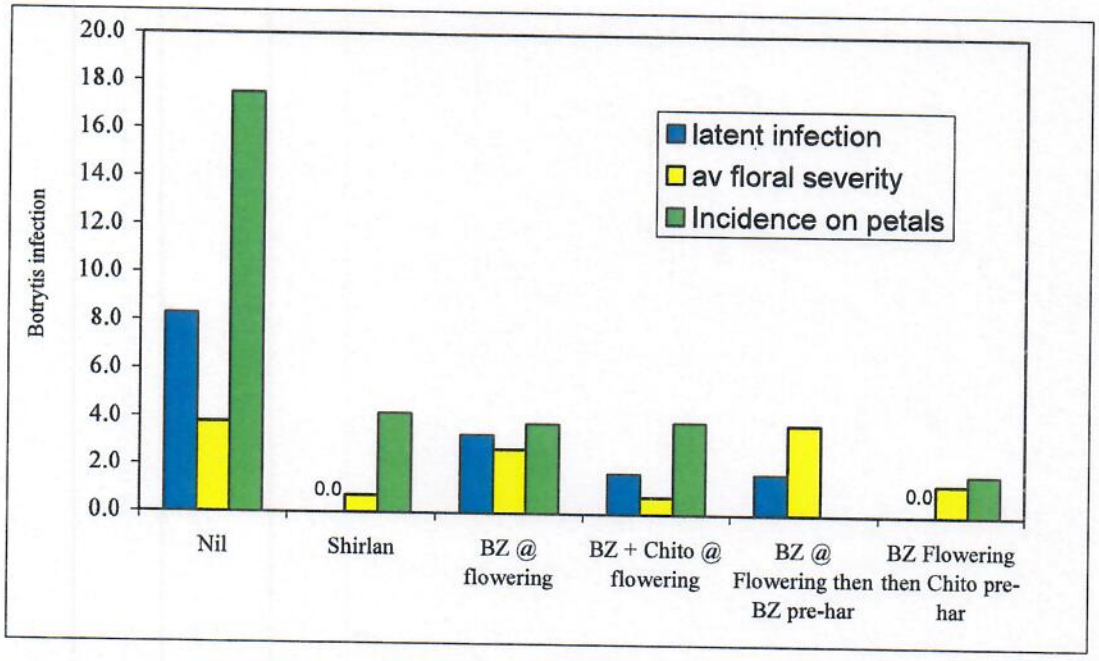

Figure 8: A proposed link between petal infection and latent green fruit infection. The correlation coefficient [r] between petal incidence and latent infection was 0.89. This is significant at P<0.05 (df=4) but does not necessarily mean there is a cause and effect.

Harvest Assessments

Treatment had no significant effect on total yield per plot, or weight of red fruit (Table 6) indicating that none of the treatments had any adverse effect on fruit set, subsequent fruit development or fruit ripening. These findings were supported by the Brix measurements, which found no differential treatment effects on fruit maturity (data not shown).

Table 6. Total yield, weight of the red fruit component per plot and yield per hectare in 2005

Treatment	Total fruit weight (kgs)	Weight of red fruit (kgs)	Red fruit yield (tons/ hectare)
Nil	31.3 a	27.9 a	61.9
Shirlan®	32.5 a	29.7 a	66.0
BZ @ flowering only	32.9 a	30.1 a	66.8
BZ @ flowering + BZ preharvest	31.4 a	28.3 a	62.9
BZ + BZ chitosan @ flowering only	33.0 a	30.5 a	67.7
BZ @ flowering then BZ chitosan pre harvest	31.9 a	28.3 a	63.0
LSD (P=0.05)	3.28	3.29	

Means followed by the same letter are not significantly different (P>0.05; LSD test)

Table 7. Average weight (kg) of tomatoes with fruit rots at harvest in 2005.

Treatment	Botrytis	Sclerotinia	Other Rots	Sclerotinia + Botrytis	All Rots
Nil	1.29 a	0.27 a	1.64 a	1.55 a	3.19 a
Shirlan®	0.91 b	0.09 a	1.53 a	1.00 b	2.54 ab
BZ @ flowering only	0.53 c	0.18 a	1.83 a	0.71 bc	2.54 ab
BZ @ flowering + BZ preharvest	0.60 c	0.20 a	1.92 a	0.80 bc	2.72 ab
BZ + BZ chitosan @ flowering only	0.54 c	0.14 a	1.50 a	0.68 c	2.18 b
BZ @ flowering then BZ chitosan pre harvest	0.63 bc	0.15 a	1.95 a	0.78 bc	2.72 ab
LSD (P=0.05)	0.30	0.19	0.73	0.30	0.88

Means followed by the same letter are not significantly different (P>0.05; LSD test).

Botrytis crop loss was highest for the nil treatment (1.3 kg) and all other treatments significantly (P<0.05) reduced *Botrytis* crop loss (Table 7). The BZ based programmes reduced *Botrytis* crop loss by 51-59% and three of these were significantly (P<0.05) better than the Shirlan® treatment. *Sclerotinia* crop loss was small (<300gm) none of the treatment s had any significant impact on *Sclerotinia* crop loss. Combining *Botrytis* and *Sclerotinia* rots gave a similar pattern of crop loss as that described for the Botrytis crop loss above.

These results indicate that the BZ treatments gave superior protection against *Botrytis* crop loss compared to the Shirlan® treatment. The results also indicate that there was no extra advantage gained from the addition of either the chitosan or the pre harvest applications, suggesting that application over flowering was more important in this growing season.