# Grower Summary

## FV 378

Assessment of plant elicitors to induce resistance against head-rot in broccoli

Final 2012

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| **Project Number:** | FV 378 |
| **Project Title:** | Assessment of plant elicitors to induce resistance against head-rot in broccoli |
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## Headline

Experimental trials investigating the effect of plant elicitors on head rot in broccoli did not show any significant levels of control due to low levels of disease while a commercial trial showed that all the elicitors except Justice gave a similar level of head rot control to the fungicide Cuprokytl.

## Background

Head-rot in broccoli is a major cause of crop loss in the UK and abroad. It is caused by bacteria in the soil that are able to infect the plants under ‘ideal’ conditions, in particular during periods of high humidity. The bacteria are common in soil and because they will only infect plants under the most conducive conditions, they are termed 'opportunistic' pathogens. Current treatments rely on copper oxychloride (e.g. ‘Cuprokylt’), which acts as a disinfectant on the plant. However, because the source of the bacteria is effectively limitless and the chemicals can be damaging to the plants and to the environment, finding suitable alternative treatments is a high priority. Plant defence elicitors do not have anti-microbial activity against fungi, bacteria or other pathogens, but instead they trigger a defensive reaction in the plant, making the plants more resistant to infection. Application of elicitors can be incorporated into a normal fungicide regime. Together these aspects make them an excellent candidate for treatment against head-rot in broccoli.

## Summary of the project and main conclusions

A two-year project (2010 – 2012) was undertaken to assess whether elicitors can be used to protect broccoli against bacterial head-rot. Some of the elicitors have been used successfully against bacterial pathogens on other crops, for example Probenazole (‘Oryzmate’) is a standard treatment against rice blast in Asia. Others have shown promise in experimental greenhouse trials, including BABA and Bion against head-rot in broccoli. Elicitors are potentially a good alternative to disinfectants for diseases such as head-rot where the bacteria that cause the disease are opportunistic pathogens, ubiquitous and present in high levels in the soil.

### *Methods*

Broccoli transplants (vars. Marathon, Parthenon) were planted in mid April and mid July 2010 and 2011, at replicated experimental sites (James Hutton Institute (JHI): Invergowrie and

Scottish Agricultural College (SAC): Pitlochie, Peacehill, Kirkton Barns) for trials 1 to 4. In addition, a commercial site (Peacehill) was used to test licensed fungicides, where the transplants were planted in July 2010, for trial 5. The trial sites were approximately 100 m x 80 m, made up of six beds of treated plants, each bordered by 'guard' beds containing un-treated plants. In each treatment plot 20 plants were assessed for trials 1 - 4, and 60 plants assessed for trial 5. Each treatment plot was replicated three times. Assessment was carried out approximately nine weeks after planting.

A group of five elicitors (BABA, Bion, *cis*-jasmone, Probenazole, Yea foliar) and three licensed fungicides with known or expected elicitor activity ('Amistar', 'Justice', 'Flyer'; a.i. azoxystrobin, proquinazid and pyraclostrobin, respectively) were selected for the trial (Table 1). 'Cuprokylt' (a.i. copper oxychloride) was included as a positive control in the commercial trial and all trials included a negative, untreated control. The elicitors were used either singly or in combination, with the aim of triggering multiple defensive pathways in the plant. Elicitors were applied three times in 10-day intervals; the first application was approximately one week before head initiation. A cocktail of head-rot bacteria was applied twice with a one-week interval and the first application was between the first and second elicitor application.

**Table 1:** Treatments used in the trials.

| **Treatments in trials 1--4** | **Concentrations used** |
|---|---|
| BABA [a] (Elicitor) | 1 mM |
| Bion (Elicitor) | 1 mM |
| *cis*-jasmone [a] (Elicitor) | 3.2 mM |
| Yea foliar (Elicitor) | 0.3 % (v/v) |
| Probenazole [a b] (Elicitor) | 0.2 mM (trial 1), 1 mM (trial 2-4) |
| Amistar (Fungicide with elicitor activity) | 1 L / ha |
| **Treatments in trial 5 (commericial trial)** | **Concentrations used** |
| Amistar (Fungicide with elicitor activity) | 1 L / ha |
| Probenazole [a b] (Elicitor) | 1.0 mM |
| Justice (Fungicide with elicitor activity) | 0.25 L / ha |
| Flyer (Fungicide with elicitor activity) | 1 L / ha |
| Cuprokylt (Fungicide) | 5 kg / ha |

All treatments were applied at a rate of 400 L / ha.

Activator-90 at 0.05 % (v/v) was added to all treatments.Key: a = addition of 0.01 % Tween 20

b = addition of 1 % (v/v) acetone

Laboratory experiments were carried out to determine the relative contribution of disease from three different head-rot bacteria species. The presence of head-rot bacteria on trial plants was detected using laboratory tests (Figure 1).

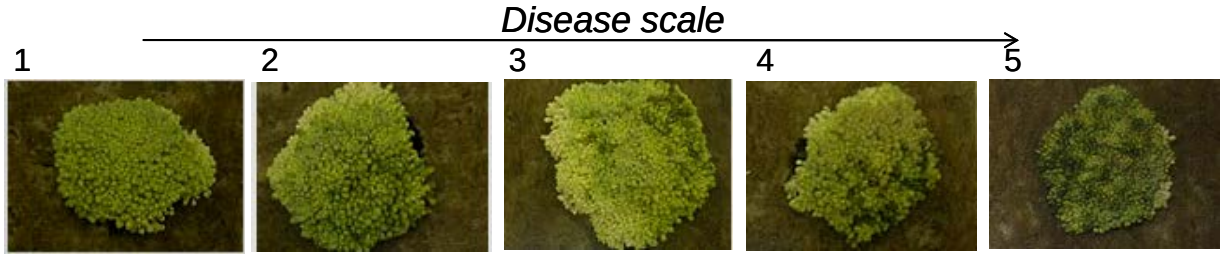


**Figure 1:** Disease assessment on detached broccoli florets, infected with a cocktail of head-rot bacteria. Disease scale showing degree of head-rot symptoms.

### *Main conclusions*

- Head-rot bacteria are common and can be isolated from healthy plants.
- Disease occurred in the absence of artificially added bacteria.
- Greater levels of disease are required to differentiate between the treatments.
  In the commercial trial greater levels of disease were observed and treatments, including Probenazole, gave similar results to ‘Cuprokylt’. whilst application of ‘Justice’ significantly increased the number of diseased plants.
- It appears that plants have a frequent exposure to the infecting bacteria during the season, so the effect of elicitor treatments could be short-term requiring repeated applications

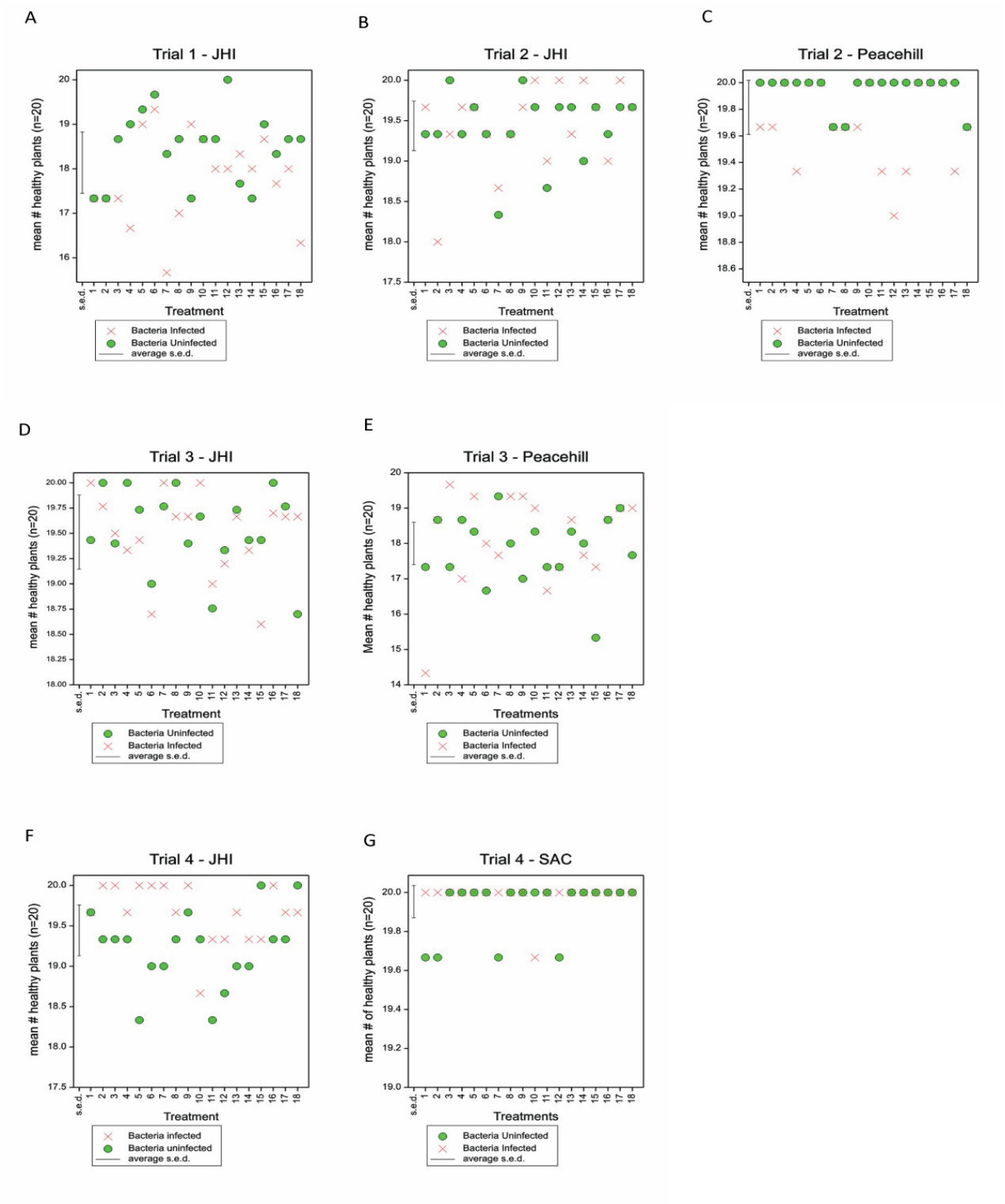


**Figure 2:** Graphs showing the mean numbers of healthy plants from each trial. Trials 1 - 4 from JH1 (A, B, D, F) and SAC sites (C, E, G). N.B. trial 1 at the SAC site yielded 100 % healthy plants (not shown).

- Trial 1 (April 2010): Application of bacteria to the trial run at JHI resulted in a significant increase in the number of diseased plants although no significant effects were seen for the treatments (Fig. 2A). Probenazole (#5) and Amistar (#6) and their combinations appeared to provide some protection, in contrast to the combination of BABA and Bion (#7) which gave the highest level of disease. No disease symptoms were observed on any plants at the SAC site (Pitlochie). Possible reasons for the lack of disease were a particularly dry June and low levels of nitrogen.
- Trial 2 (July 2010): A lack of disease symptoms meant that it was not possible to statistically distinguish any differences between the treatments (both experimental sites). This occurred despite increasing the concentration of added bacteria 100-fold (to $10^6$ cfu/ml). There was a significant increase in the number of diseased plants at the SAC site (Peacehill), but not at the JHI site (Fig. 2B, 3C). As with trial 1, the combination of BABA and Bion (#7) resulted in the highest number of diseased plants.
- Trial 3 (May 2011): An increased level of disease was observed at the SAC site (Peacehill) and there was a marginal effect with treatment type, which arose from an increased number of symptomatic plants treated with BABA (#1) and *cis* jasmone + Amistar (#15). On this occasion there was no effect with addition of bacteria. In addition, there were no significant effects with either treatment or application of bacteria at the JHI site.
- Trial 4: (July 2011): Only a low level of disease was observed for both trials with no significant differences between the treatment types or addition of bacteria.

The effect of licensed fungicides, 'Amistar', 'Flyer' and 'Justice', and Probenazole were compared to Cuprokylt on a commercial site (Peacehill). The plants were not artificially infected with head-rot bacteria, so that the trial relied on 'natural' infection of the plants from soil-borne bacteria.

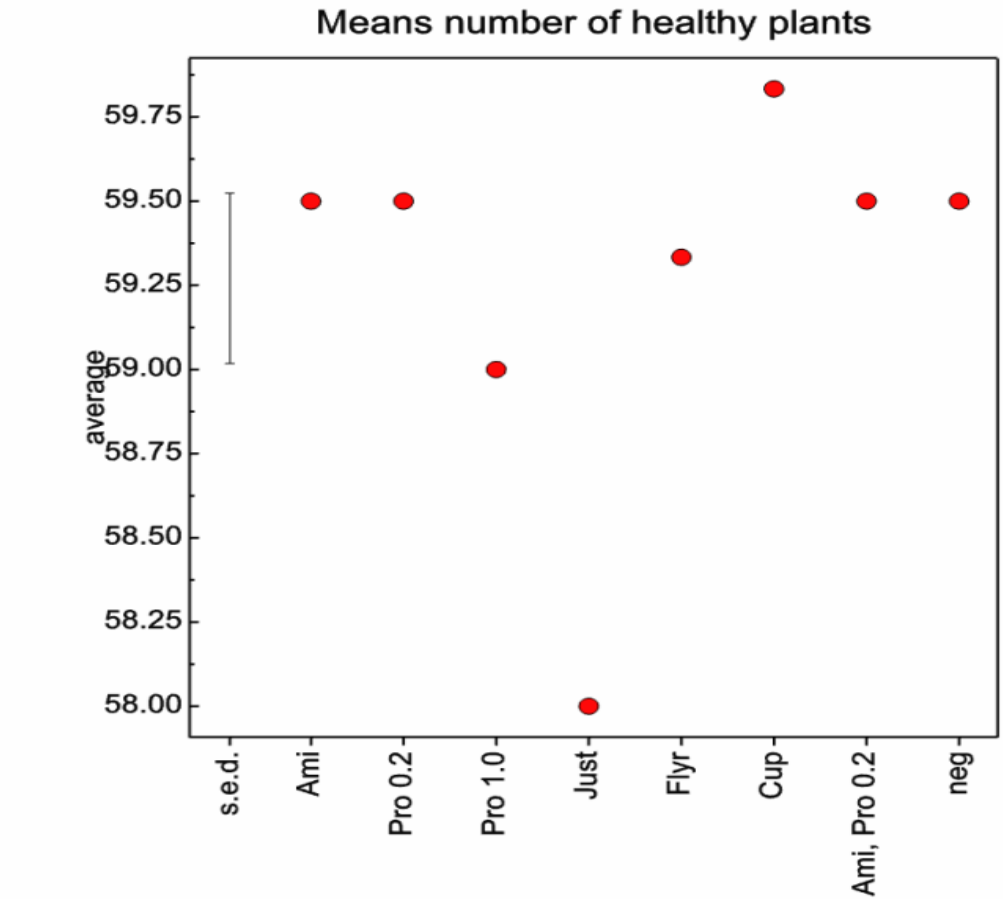


**Figure 3:** Average number of plants without head-rot symptoms for each of the eight treatments used on the commercial trial (Peacehill, 2010).

- Trial 5 (July 2010): The majority of treatments gave similar protection to Cuprokylt, with no significant differences (Fig. 3). However, plots treated with 'Justice' had significantly greater numbers of diseased plants; in addition, the extent (spread) of head-rot was greater across the diseased plants (not shown).

Laboratory analysis showed that one bacteria species, *Pseudomonas fluorescens* (Psf), was responsible for the greatest extent of disease in comparison to *Ps. marginalis* (Psm) and *Pectobacterium carotovorum* subsp*. carotovorum* (Pcc), although all three species were capable of causing typical head-rot symptoms on broccoli heads. It is of note that it was possible to detect head-rot bacteria from diseased and healthy plants in trial 3, the commercial site, where bacteria were not artificially added.

Further work is required to definitively show a role for elicitors in control of head-rot bacterial in broccoli. While there is some evidence for a positive effect, some elicitor or fungicide

treatments can increase the level of disease. One possibility is through the use of smaller-scale, protected trials.

## Financial benefits

At this point it is not possible to accurately cost the elicitors since they are still experimental.

## Action points for growers

No action points from this work at present