Desktop evaluation on commercially available microbial-based products for control or suppression of *Pseudomonas syringae* pv. *actinidiae*

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October 2011
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1. Introduction

The following report presents the findings of a desktop evaluation of biocontrol agents with potential to control or suppress bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* (Psa). The review identifies the most likely candidates of locally available and overseas products based on a ranking approach and discusses their suitability.

2. Background

Biological control of plant pests and diseases relies on the use of one living organism to control another through antagonistic interactions. Generally, biocontrol agents are predators, pathogens and parasites of the organism to be controlled. In the specific case of the bacterium Psa, biological control might be achieved through three different mechanisms, which include (i) amensalism, i.e. antibiotic and lysis; (ii) competition; and (iii) parasitism and predation. In addition, for the purpose of this report, we have identified the following modes of action as having potential biocontrol properties against Psa, either by themselves or in combination with other products as part of an integrated pest management strategy: (iv) induced resistance and (v) plant growth promotion or regulation.

(i) Amensalism describes an interaction in which the biocontrol organism produces metabolic substances that inhibit growth, certain metabolic pathways or multiplication (antibiosis) or even lead to the complete breakdown of the cells of the target organism (lysis, the mechanism used by bacteriophages).

(ii) Competition refers to the contest between biocontrol agent and pathogen for space and resources. A quickly growing and multiplying biocontrol organism, for example, can colonise flowers, leaves or roots to the extent that it replaces or prevents any subsequent colonisation by the pathogenic organism. However, there is evidence in some cases that if the antagonist is applied when the pathogen is already present it is not effective. This suggests that agents with competition as the sole mode of action are best used on a regular basis as a preventative measure before disease outbreak.

(iii) The third mechanism of action is hyperparasitism (i.e. the biocontrol organism is parasitic to plant parasites/ pathogens), in which case the biocontrol organism lives off the pathogen, killing it in the process.

(iv) Like elicitors or chemical / biochemical compounds, some biological organisms help promote plant resistance by exuding compounds that stimulate the defence responses.

(v) Similarly, certain metabolic compounds produced by microbial organism might promote plant growth and crop production by improving seed germination and fruit ripening, enhancing nutrient uptake and boosting protein synthesis. This will increase disease resistance and help the plant to withstand stress conditions, which might not lead to disease control by itself but could be a mechanism contributing to a successful pest management strategy.
The control of plant diseases using biological agents is an attractive prospect not just within integrated crop management. Especially in view of the ever growing number of plant pathogen strains resistant or tolerant to widely used chemical compounds, alternatives such as biocontrol have become almost an imperative to ensure the continued viability of crop production. While biological control can be very effective, it is necessary to understand that its nature makes biological control variable. Its efficacy hugely depends on environmental conditions. Just like the pathogen, the biocontrol organism requires optimum temperature and moisture conditions to proliferate and spread. Biocontrol seldom means complete eradication of the unwanted organism but rather reducing its population to levels below the economic threshold.

3. Product Evaluation

3.1. Overview

As part of this study, a range of biocontrol products (commercially available locally and / or overseas) and organisms (where no commercial product is available\(^1\)) suitable for the control of agricultural and horticultural plant pathogens (including fungi and bacteria) were identified and ranked based on the following criteria: Target pathogen/disease; target crop; mode of action; availability in NZ/ ease of import:

**Target pathogen/ disease:**
- 5 – *Pseudomonas*;
- 4 – Other bacterial diseases on fruit (e.g. *Erwinia amylovora*, fire blight);
- 3 – All other bacteria;
- 2 – Foliar diseases caused by fungi;
- 1 – Soil-borne diseases.

**Mode of action:**
- 5 – Several modes of action against bacterial pathogens (antibiosis, induced resistance, competition, parasitism);
- 4 – One mode of action against bacterial pathogens;
- 3 – Several modes of action against fungal pathogens;
- 2 – One mode of action against fungal pathogens;
- 1 – Plant growth promotion, if only mode of action

**Target crop:**
- 5 – Perennial vine crops (including grape and kiwifruit);
- 4 – Fruit trees;

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\(^1\) This might be due to a lapse of registration, where a company went out of business or because a new organism was identified to have biocontrol potential.
3 – Other perennial tree crops;
2 – Perennial vegetables / arable crops (incl. tomato);
1 – Annual vegetables / arable crops.

**Availability / ease of import:**
5 – Available in NZ;
3 – Most other products and organisms where species might be present in NZ and organism is not on MAF unwanted organism list;
1 – No product available, i.e. organism would need to be isolated or imported from culture collection;
0 – Organism on MAF list of unwanted organisms / regulated pest.

Table 1: Highest scoring microbial candidates for control and / or suppression of Psa.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Total Score</th>
<th>Product Name</th>
<th>Biocontrol agent/ organism*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>Serenade Max</td>
<td><em>Bacillus subtilis</em> strain QST 713</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>BacStar™</td>
<td><em>Bacillus subtilis</em> var. <em>amyloliquefaciens</em> D747</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>BlightBan® A506</td>
<td><em>Pseudomonas fluorescens</em> A506</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>Blossom Bless™</td>
<td><em>Pantoea agglomerans</em> P10c</td>
</tr>
<tr>
<td>5</td>
<td>17.5</td>
<td>Companion Gold</td>
<td><em>Bacillus subtilis</em> GB03</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>Sonata®</td>
<td><em>Bacillus pumilus</em> QST 2808</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>Bloomtime ®</td>
<td><em>Pantoea agglomerans</em> strain E325</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>Cease® / Rhapsody®</td>
<td><em>Bacillus subtilis</em> strain QST 713</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>Clarity/ Subtilex® NG</td>
<td><em>Bacillus subtilis</em> MBI600</td>
</tr>
<tr>
<td>10</td>
<td>15.5</td>
<td>Blossom Protect™</td>
<td><em>Aureobasidium pullulans</em> DSM 14941, <em>Aureobasidium pullulans</em> DSM 14940</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>Ballad® Plus</td>
<td><em>Bacillus pumilus</em> QST2808</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>Companion®</td>
<td><em>Bacillus subtilis</em> GB03, other <em>B. subtilis</em>, <em>B. licheniformis</em>, <em>B. megaterium</em></td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>Tri-D25™</td>
<td><em>Trichoderma koningi</em>, <em>Trichoderma hazianum</em></td>
</tr>
<tr>
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<td>14.5</td>
<td>Botry-Zen®</td>
<td><em>Ulocladium oudemansii</em></td>
</tr>
<tr>
<td>14</td>
<td>14.5</td>
<td>Vinevax™</td>
<td><em>Trichoderma harzianum</em></td>
</tr>
<tr>
<td>14</td>
<td>14.5</td>
<td>TripleX</td>
<td><em>Bacillus amyloliquefaciens</em> BS 1b</td>
</tr>
<tr>
<td>14</td>
<td>14.5</td>
<td>Bio Sol B Sub Plus</td>
<td><em>Bacillus subtilis</em> strain ATCC6051 + seaweed extract</td>
</tr>
<tr>
<td>18</td>
<td>14</td>
<td>AgriPhage™</td>
<td>bacteriophages</td>
</tr>
<tr>
<td>18</td>
<td>14</td>
<td>Sentinel®</td>
<td><em>Trichoderma</em> LC52</td>
</tr>
<tr>
<td>18</td>
<td>14</td>
<td>Superzyme®</td>
<td><em>Bacillus subtilis</em>, <em>Pseudomonas putida</em>, <em>Trichoderma koningii</em> &amp; <em>T. harzianum</em></td>
</tr>
<tr>
<td>18</td>
<td>14</td>
<td>Fultzyme Plus™</td>
<td><em>Bacillus subtilis</em> + amino acids</td>
</tr>
</tbody>
</table>

* Where biocontrol organisms are the same, products will be discussed in the same section.
Twenty-one products that achieved the highest overall ranking scores are deemed the most promising to control or suppress Psa and are presented in the following section. The order of priority is based on the overall score (Table 1), which was obtained by simple addition of the individual scores (see Appendix for details). Brief comments on each product / organism are included to give a quick overview of any issues that could be important with regard to their use as biocontrol agents against Psa in NZ.

3.2. Detailed description

1) Bacillus subtilis QST 713

**Product Name:** Serenade Max – BASF New Zealand Ltd (score 20)

**Serenade Max/ Serenade ASO** – AgraQuest, Inc., USA, www.agraquest.com

**Cease®** – BioWorks, USA; www.bioworksinc.com (score 16)

**Rhapsody®** – AgraQuest, Inc., USA, www.agraquest.com (score 16)

**Target pathogen/disease:**

Serenade: Powdery mildew; Gray mould (*Botrytis cinerea*); Downy mildew; Early Blight (*Alternaria solani*); Late Blight (*Phytophthora infestans*); Bacterial spot (*Xanthomonas* spp.); Walnut blight (*Xanthomonas campestris*); *Monilinia* spp., *Venturia* spp., Fire Blight; *Pseudomonas syringae*.

Cease®: *Pseudomonas syringae* pv. *tomato*; Powdery Mildew, Anthracnose, Rust, *Botrytis cinerea*, Downy Mildew, Early/ Late Blight; Bacterial Soft Rot (*Erwinia, Pseudomonas*), Bacterial Leaf Spot and Speck, Anthracnose; fungal soil diseases (*Rhizoctonia* spp.; *Pythium* spp.; *Fusarium* spp.; *Phytophthora* spp.).


**Mode of action:**

Nutrient and niche competition, site exclusion; colonization and attachment of the bacteria to the fungal pathogen; Antibiosis through production of a class of lipopeptide antibiotics (incl. iturins) that help out-compete the pathogenic organisms by either killing them, disrupting development or reducing their growth rate. May help foster plant’s natural defences by inducing natural systemic resistance or systemic acquired resistance.

**Registered for use on/Target crop:**

*Bacillus subtilis* strain QST 713 according to US Environmental Protection Agency (US EPA, 2011): wide variety of food crops, including cherries, cucurbits, grapes, leafy vegetables, peppers, potatoes, tomatoes, and walnuts.

Serenade Max in NZ: vegetables, fruit (Peach, apricot, plum, cherry, nectarine), nut and vine crops (grape vine, table grapes), tomato; kiwifruit, berries, avocado.
Cease®: foliar spray on agricultural greenhouse crops (incl. leafy vegetables, roots and tubers, herbs/ spices, fruiting vegetables; Cucurbits; bulb vegetables; brassica, strawberries).

Rhapsody®: turf, landscape areas, ornamental and greenhouse plants.

**Formulation:**
Serenade Max: wettable powder for spray/ foliar application; Serenade ASO: aqueous solution (lower concentration of biocontrol agent); Cease®: aqueous solution; Rhapsody®: aqueous solution.

**Efficacy:** (Raudales et al., 2010)
Serenade Max (US formulation): Evidence for disease control and/or yield increase when used on/against: Blueberry/Anthracnose fruit rot; Grape/Bunch rot and Powdery mildew; Hydrangea/Powdery mildew; Pansy/Cercospora leaf spot; Pumpkin, Cantaloupe, Honeydew/Powdery mildew; Lettuce/Powdery mildew; Broccoli/Downy mildew.

Evidence for disease control is mixed with some reports showing positive results and others not when used on/against: Blueberry/Mummy berry; Lettuce/Lettuce drop.

No obvious response to treatment in one or more published reports when used on/against: Cranberry/Fruit rot; Apple/Fireblight; Apple/Flyspeck; Apple/Sooty blotch; Apple Black pox; Apple/Brooks fruit spot; Red Raspberry: Fruit rot; Turnip greens/bacterial leaf spot.

Serenade ASO: Cranberry/Cotton ball: ± (evidence for disease control is mixed); Radish/Hypocotyl root rot and Clubroot: ±; Spinach/Stemphylium leaf spot: 0 (no obvious response to treatment); Snap bean/Rhizoctonia root rot: 0.

**Comments:** *Bacillus subtilis* strain QST 713 achieved moderate disease reduction of *Phytophthora infestans* on media (in vitro) and suppression of late blight on potato but not at levels observed for chemical compounds (Olanya and Larkin, 2006). Similarly, Gilardi *et al.* (2009) found that suppression of leaf spot (*Pseudomonas syringae* pv. *syringae*) on tomato was only partially effective. On the other hand, mixed or alternate applications of *Bacillus subtilis* QST 713 with chemical fungicides showed very good results in the control of *Alternaria* leaf blight on ginseng (Li *et al.*, 2008).

Testing a range of biocontrol agents individually (incl. preparations of *Bacillus subtilis* QST 713 (Serenade Max), *Pseudomonas fluorescens* A506 (BlightBan® A506), *Pantoea agglomerans* C9-1 (BlightBan C9-1 – currently not registered) and *Pantoea agglomerans* E325 (Bloomtime®)) showed that they were not consistently effective in reducing fireblight in apple (average reduction between 9.1 to 36.1%, compared to 59.0 to 67.3% when using streptomycin). The main reason for this seems to be inconsistent blossom colonisation by the biocontrol organisms. However, blossom infection could be controlled more effectively when the biocontrol agents were used in combination with streptomycin as part of an integrated disease management program, thus leading to a reduction of necessary streptomycin applications while maintaining similar control levels (Sundin et al., 2009).
Serenade Max achieved the highest ranking score in our evaluation (closely followed by the other formulations of *Bacillus subtilis* QST 713) due to their wide range of target pathogens/diseases, target crops and various modes of action. This combination makes this biocontrol agent a promising potential candidate for the control or suppression of Psa.

Serenade Max is part of the New Zealand Kiwifruit Industry's *in vitro* & greenhouse testing programme.

http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_006479.htm
http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/tech_006479.htm
http://www.agro.basf.com/agr/AP-Internet/en/content/solutions/solution_highlights/serenade/bacillus-subtilis

2) *Bacillus subtilis* var. *amyloliquefaciens* D747

**Product Name:** BacStar™ – ETEC Crop Solutions Limited, NZ; www.elliottchemicals.co.nz (score 19)

**Target pathogen/disease:** Powdery mildew, *Botrytis* and sour rot, fireblight.

**Mode of action:**
Competition for space and nutrients; antibiosis (production of antibiotic compounds such as iturin A as observed for other *Bacillus amyloliquefaciens* strains (Yu et al., 2002)); possibly active as a plant growth regulator (as for *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24).

**Registered for use on/Target crop:** Grapes, kiwifruit, pipfruit, berryfruit, persimmon, onions, avocado, citrus, lettuce, turf.

**Formulation:** Water dispersible granules

**Comments:** This formulation of *Bacillus amyloliquefaciens* (strain D747) is intended for the control of fungal pathogens, such as *Botrytis*, powdery mildew and sour rot as well as fireblight on fruit (incl. kiwifruit) and other crops. Its alleged range of target crops and target diseases together with its various modes of action make this an interesting candidate for the management of Psa. While a literature search did not reveal any evidence that the product or organism had been tested for their efficacy in rigorous scientific experiments, products based on other strains of *Bacillus amyloliquefaciens* are marketed as biocontrol and plant strengthening agents (e.g. FZB24® *Bacillus subtilis*, Bayer CropScience AG/ www.bayercropsience.com and TripleX, BioStart Limited, NZ).

BacStar™ is part of the New Zealand Kiwifruit Industry's *in vitro* & greenhouse testing programme.
3) *Pseudomonas fluorescens A506*

**Product Name:** BlightBan® A506 – NuFarm Inc., USA; www.nufarm.com/USAg/Agriculture (score 18)

**Target pathogen/disease:** Frost damage, *Erwinia amylovora*, and russet-inducing bacteria.

**Mode of action:**
Reduces/ suppresses growth of frost forming and fireblight inducing bacteria; competition for space and nutrients by colonising blossoms of apples and pears before pathogenic bacteria can get a foothold.

**Active on or Registered for use on/Target crop:** Almond, apple, apricot, blueberry, cherry, peach, pear, potato, strawberry, tomato.

**Formulation:** wettable powder

**Comments:** Testing a range of biocontrol agents individually (incl. *Pseudomonas fluorescens* A506 (BlightBan® A506), *Pantoea agglomerans* E325 (Bloomtime®), *Pantoea agglomerans* C9-1 (BlightBan C9-1 – currently not registered) and preparations of *Bacillus subtilis* QST 713 (Serenade Max)) showed that they were not consistently effective in reducing fireblight in apple (average reduction between 9.1 to 36.1%, compared to 59.0 to 67.3% when using streptomycin). The main reason for this seems to be inconsistent blossom colonisation by the biocontrol organisms. However, blossom infection could be controlled more effectively when the biocontrol agents were used in combination with streptomycin as part of an integrated disease management approach, thus leading to a reduction of necessary streptomycin applications while maintaining similar control levels (Sundin et al., 2009).

Vanneste *et al.* (2004) found that *Pseudomonas fluorescens* A506 has limited ability to colonise apple and pear leaves, which suggests that it might not be a suitable candidate for the control of Psa, which manifests itself in leaves, shoots and trunks.

BlightBan® A506 is part of the New Zealand Kiwifruit Industry's *in vitro & greenhouse* testing programme.

http://www.nufarm.com/USAg/BlightBanrA506
4) *Pantoea agglomerans* P10c

**Product Name:** Blossom Bless™ – AgriNova NZ Ltd, NZ; www.grochem.co.nz (score 18)

**Target pathogen/disease:** fire blight

**Mode of action:**
Nutrient competition (nectar, stigma); exclusion by pre-emptive occupation of flower infection sites (competition for space); antibiosis.

**Registered for use on/Target crop:** Pip fruit flowers

**Formulation:** wettable powder

**Comments:** *Pantoea agglomerans* P10c is an effective bacteria to suppress fire blight incidence on apples and pears blossoms. Under optimum conditions, experimental evidence suggests that 100% of all flowers randomly sampled in treated commercial orchards were colonised by P10c when the powder formulation was sprayed directly onto flowers or delivered to flowers using honeybees (Vanneste et al., 2002); ability of the organism to colonise apple and pear leaves, however, is low (Vanneste et al., 2004). This indicates that it might not be a suitable candidate for the control of Psa, which manifests itself in leaves, shoots and trunks.

Blossom Bless™ is part of the New Zealand Kiwifruit Industry’s *in vitro* & greenhouse testing programme.

http://www.grochem.co.nz/Portals%5C537%5Csds/BlossBless%20SDS.pdf
http://www.grochem.co.nz/Portals%5C537%5Clabels/BlossomBless_300g.pdf

5) *Bacillus subtilis* GB03

**Product Name:** Companion Gold – Zelam Ltd, NZ; www.nuchem.co.nz (score 17.5)

**Companion® –** Growth Products, Ltd., USA; www.growthproducts.com (score 15, ranked 12th)

**Target pathogen/disease:**
Companion Gold: powdery mildew, sour rot and *Botrytis*; Companion®: *Rhizoctonia, Pythium, Fusarium, Phytophthora*; Bacterial leaf spot *Xanthomonas campestris*.
Mode of action:
Competition for space and nutrients; antibiosis (B. subtilis produces the antibiotic Iturin, which inhibits the pathogen's growth by disrupting cell wall formation); Induced Systemic Resistance (ISR) (cf. Brannen and Kenney, 1997).

Registered for use on/Target crop:
Grapes and ornamentals (Companion Gold); turf, greenhouse, nursery, ornamental crops, field grown trees (preventative) (Companion®).

Efficacy: (Raudales et al., 2010)
Evidence for disease control is mixed with some reports showing positive results and others not when used on/against: Snap beans/Fusarium Root rot; Pea/Fusarium, Phoma, Pythium.

No obvious response to treatment in one or more published reports when used on/against: Snap bean/Rhizoctonia root rot; Wheat/Fusarium crown rot; cucumber/Damping off.

Formulation: liquid suspension.

Comments: B. subtilis GB03 is successfully used in many crops against disease of roots caused by soil fungal pathogens, such as Fusarium, Rhizoctonia and Pythium (Backman et al., 1998). When used to control Fusarium root rot of dry beans, seed application of B. subtilis GB03 resulted in the promotion of plant growth by increasing biomass and in a reduction of disease severity under greenhouse conditions and in the field (yield increase of 31%). However, the biocontrol treatment did not eradicate the disease (Estevez de Jensen et al., 2002). Although Companion Gold (registered in NZ) is primarily used against fungal diseases, such as powdery mildew, sour rot and Botrytis, Companion®, the US product containing the same strain of B. subtilis, is registered for use against bacterial leaf spot. This makes Bacillus subtilis GB03 an interesting candidate for the control / suppression of other bacterial diseases, incl. Psa.

Companion Gold is part of the New Zealand Kiwifruit Industry's in vitro & greenhouse testing programme.


6) Bacillus pumilus QST 2808
Product Name: Sonata® – AgraQuest, Inc., USA, www.agraquest.com (score 17)
Ballad® Plus – AgraQuest, Inc., USA, www.agraquest.com (score 15 due to different range of target crop)
Target pathogen/disease:


Mode of action:
Antibiosis: antifungal amino sugar compounds disrupt the cell metabolism and destroy the cell walls of the Target pathogen; competition for space and nutrients: Bacillus pumilus QST 2808 acts as a physical barrier between the leaf and the pathogen spores; may stimulate plant’s own defence mechanisms by inducing systemic resistance.

Registered for use on/Target crop:
Sonata®: Many food and non-food crops, including trees susceptible to sudden oak death syndrome; cucurbits, grapes, hops, vegetables, peanuts, pome fruits, stone fruits, black sigatoka in bananas, cereal. For use outdoors, including nurseries, landscapes, and rights-of-way, and in greenhouses.

Ballad® Plus: Barley, corn, millets, oat, rice, rye, sorghum, triticale, wheat and other cereal grain crops; grass seed, canola, castor, cotton, flax, oil palm, olive, peanut, rapeseed, safflower, sesame, sunflower, soybeans and other oilseed crops, sugar beets, sweet corn.

Formulation: emulsifiable concentrate

Efficacy of Sonata®: (Raudales et al., 2010)
Evidence for disease control and/or yield increase when used on/against (crop/disease): Lettuce/Powdery mildew; Broccoli/Downey mildew; Radish/Downey mildew.

Evidence for disease control is mixed with some reports showing positive results and others not when used on/against: Lettuce/Lettuce drop.

No obvious response to treatment in one or more published reports when used on/against: Lima Beans/White mould; Pumpkin/Powdery mildew; Radish/Clubroot and Rhizoctonia hypocotyl root rot.

Comments: In field experiments that compared the treatment of late blight on potato (Phytophthora infestans) using Sonata®, as well as a variety of other alternative products including the commercial biocontrol agents Actinovate, Serenade, Trichodex, sulphuric clays (e.g. Myco-Sin; EcoGrape, www.ecogrape.com) and
plant extracts with copper-based products none of the alternatives provided sufficient disease control on their own or in combination with each other to be an effective substitute for copper products (Dorn et al., 2007; Oregon State University, 2007; Syrovy et al., 2009). However, Sonata® is successfully used in integrated pest management to control Botrytis on strawberries in the United States (e.g. [http://www.ipmcenters.org/pmsp/pdf/TNstrawberry.pdf](http://www.ipmcenters.org/pmsp/pdf/TNstrawberry.pdf)).

Sonata®, Ballad® Plus or other formulations of Bacillus pumilus are not part of the New Zealand Kiwifruit Industry’s in vitro & greenhouse testing programme.

http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_006485.htm

7) **Pantoea agglomerans strain E325**  
**Product Name:** Bloomtime® – NorthwestAgricultural Products, USA; www.nap-chem.com (score 16)

**Target pathogen/disease:** fire blight in apples and pears *Erwinia amylovora*.

**Mode of action:**

- Competition for nutrients (for nectar, on the stigma) and space (exclusion by pre-emptive occupation of potential flower infection sites);  
- Antibiosis (by producing an antibiotic specific to *Erwinia amylovora*) (Pusey *et al*., 2011).

**Registered for use on/Target crop:** Apples and pears

**Efficacy:**

Evidence for disease control is mixed with some reports showing positive results and others not when used on/against: Apple/Fire blight (Pusey *et al*., 2008; Raudales *et al*., 2010; Stockwell *et al*., 2010).

**Comments:** Testing a range of biocontrol agents individually (incl. *Pantoea agglomerans* E325 (Bloomtime®), *Pseudomonas fluorescens* A506 (BlightBan® A506), *Pantoea agglomerans* C9-1 (BlightBan C9-1 – currently not registered) and preparations of *Bacillus subtilis* QST 713 (Serenade Max)) showed that they were not consistently effective in reducing fireblight in apple (average reduction between 9.1 to 36.1%, compared to 59.0 to 67.3% when using streptomycin). The main reason for this seems to be inconsistent blossom colonisation by the biocontrol organisms. However, blossom infection could be controlled more effectively when the biocontrol agents were used in combination with streptomycin as part of an integrated disease management program, thus leading to a reduction of necessary streptomycin applications while maintaining similar control levels (Sundin *et al*., 2009).
Bloomtime® is not part of the New Zealand Kiwifruit Industry's *in vitro* & greenhouse testing programme.

http://www.nap-chem.com/Product_Pages/yeoman_products.htm

8) *Bacillus subtilis MBI600*

**Product Name:** Clarity – Gro-Chem, T/A Agrinova NZ Ltd; grochem.co.nz (score 16); *Subtilex® NG* – Becker Underwood, Australia, www.beckerunderwood.com

**Target pathogen/disease:**
Clarity: *Botrytis, Powdery Mildew and Sour Rot; Sclerotinia; Subtilex®: Soil borne pathogens* *Fusarium* spp., *Rhizoctonia* spp., and *Pythium* spp. that cause seed and root rots.

**Mode of action:**
*Bacillus subtilis MBI600* colonises and protects plant roots by producing a metabolite that destroys the pathogen’s cell wall (antibiosis); suppressing numbers and viability of disease organisms by competition for space and nutrients.

**Registered for use on/Target crop:**
Clarity: grapes; kiwifruit; berryfruit; onions; citrus; lettuce; turf; Subtilex®: ornamentals, vegetable field and greenhouse crops.

**Formulation:** wettable powder

**Comments:** A field study showed that under conditions of severe disease pressure, yield increases and pathogen control could only be achieved through a combined application of *B. subtilis MBI600* and *Rhizobium tropici* (nitrogen-fixing, root nodulating bacteria that are known to promote plant growth). The co-application of *B. subtilis* with *Rhizobium* is considered a promising strategy to control root rot on beans (Estevez de Jensen et al., 2002).

Clarity is part of the New Zealand Kiwifruit Industry's *in vitro* & greenhouse testing programme.

http://web.pppmb.cals.cornell.edu/resourceguide/mfs/01bacillus_subtilis.php
9) *Aureobasidium pullulans DSM 14941 and DSM 14940*

**Product Name:** Blossom Protect™ – bio-ferm GmbH, Austria, www.bio-ferm.com (score 15.5)

**Botector** – bio-ferm GmbH, Austria, www.bio-ferm.com (score 12: different target pathogen) (registered in Austria since 2009)

**Target pathogen/disease:**
Blossom Protect: fire blight (*Erwinia amylovora*); Botector: *Botrytis cinerea*

**Mode of action:**
Yeasts, such as *Aureobasidium pullulans* the main active ingredient in Blossom Protect, are naturally occurring on buds, leaves, flowers and fruit. They have the ability to readily colonise these plant surfaces and successfully compete with other microorganisms for space and nutrients and have antagonistic properties against fireblight (Seibold et al., 2004). *Aureobasidium pullulans* blocks the fire blight pathogen from colonising, for example, apple blossoms.

**Registered for use on/Target crop:**
Citrus, pome fruit, sugarcane, Florida greenway and trail lands (Blossom Protect); grapes (Botector).

**Formulation:** Water dispersible granules and powder for spray/ foliar application.

**Comments:** In field trials, Blossom Protect™ was the most effective out of 44 products tested against fireblight achieving on average 78% disease reduction. To avoid fruit russetting in susceptible (apple) varieties, Blossom Protect™ should be applied a day before conditions become conducive to disease development and used in combination with sulphur and sulphuric clay (e.g. Myco-Sin; EcoGrape, www.ecogrape.com) to control apple scab (Kunz and Mendgen, 2009). Botector’s efficacy against *Botrytis* bunch rot in grapes has been discussed but has not been established independently (Elmer and Reglinski, 2006). There is some evidence that the application of Botector might increase disease incidence (Landwirtschaftliches Versuchswesen Rheinland-Pfalz, 2011).

Blossom Protect™ is not part of the New Zealand Kiwifruit Industry’s *in vitro* & greenhouse testing programme. It is currently available in a range of European countries and the US; however, it’s high ranking score in our evaluation, which is based on its successful control of a bacterial disease in fruit trees, suggests that this candidate has potential for control of Psa and its use in NZ should be further investigated.

10) *Trichodema koningii, Trichodema hazianum*

**Product Name:** Tri-D25™ – Roots Shoots and Fruits Ltd, New Zealand, www.rd2.co.nz (score 15)

**Target pathogen / disease:** Phytophthora spp., Rhizoctonia spp., Botrytis spp.

**Mode of action:**
Hyperparasitism: Mycoparasitism, improved plant health; soil and / or root inoculant to increase beneficial microbial populations

**Registered for use on / Target crop:**
Avocados, Macadamias, Chestnut, Pistachio, Almonds & Kiwifruit; Brassicas; Carrot, Onion & Potato; Lettuce; Tomato, Capsicum, Eggplant & Strawberries; Fruit Trees; Tropical Fruits; Grapes

**Formulation:** wettable powder for soil or foliar application

**Comments:** A literature search did not reveal any evidence that Tri-D25™ has been scientifically tested in its application as foliar spray against Botrytis on grapes and its efficacy against the root rot fungi Phytophthora nicotianae and Pythium ultimum has been reported as lacking compared to conventional chemicals. Walker and Morey found that the formulation did not reduce isolation frequency of the fungi nor did it effectively reduce the level of root rot on citrus when studied in pot and field experiments. They concluded that their findings did not substantiate the manufacturer's claims. This suggests that despite its high score Tri-D25™ is not a promising candidate for the control/suppression of Psa on kiwifruit.

Tri-D25™ is included New Zealand Kiwifruit Industry’s in vitro & greenhouse testing programme, but has not been tested.

http://www.rd2.co.nz/uploads/TriD25%20105x100%20180907(1).pdf

11) *Ulocladium oudemansii*

**Product Name:** Botry-Zen® – Botryzen, New Zealand; www.botryzen.co.nz (score 14.5)

**Target pathogen/disease:** Botrytis cinerea, Sclerotinia sclerotiorum
Mode of action:
Competition for nutrients and space; the biocontrol organism competes with the pathogen for the same ecological niche.

Registered for use on/Target crop: Grapes, kiwifruit, blackcurrants, ornamentals

Formulation: water dispersible granules

Comments: Successful suppression of *Botrytis cinerea* on grapes using *Ulocladium oudemansii* has been reported, even under high disease pressure, however, biocontrol was much more effective when used in combination with a chemical plant defence stimulant (Reglinski et al., 2005).

*Botry-Zen®* is part of the New Zealand Kiwifruit Industry's *in vitro* & greenhouse testing programme but has not been tested yet.


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12) *Trichoderma harzianum*

Product Name: *Vinevax™* – Agrimm Technologies Ltd, New Zealand; www.vinevax.com (score 14.5)

Target pathogen/disease:
Wood-infecting fungal pathogens: *Eutypa lata*, *Botryosphaeria* spp., *Phaeomoniella chlamydospora*, *Cylindrocarpon destructans*, *Phomopsis viticola* and *Botrytis cinerea*.

Mode of action:
Niche exclusion and competition for nutrients; mycoparasitism - degradation of other fungi; antibiosis: Production of metabolites with antifungal activity; stimulation of the plant's defence mechanism (SAR).

Registered for use on/Target crop: Pruning wound dressing in vine

Formulation: paste or spray

Comments: Experimental evidence from glasshouse and vineyard trials suggests that commercial formulations of *T. harzianum*, including *Vinevax™*, can reduce the recovery of a fungal pathogen (*Eutypa lata*) from pruning wounds when the biocontrol agent is applied at least 1 day before the pathogen (John et al., 2005). John et al. (2008) also found that *T. harzianum* has the ability to colonise and persist on vine wood for at least 20 months indicating the biocontrol agent's potential to protect vines from inoculation with the pathogen. The manual
protection of pruning wounds, though labour intensive, might therefore be warranted. Whether the biocontrol agent is effective against bacterial diseases needs to be established.

Vinex™ is part of the New Zealand Kiwifruit Industry's *in vitro* & greenhouse testing programme but has not been tested yet.

http://www.vinevax.com/vinevax.html
http://www.vinevax.com/pdfs/BrochureVinevaxPWD.pdf
13) *Bacillus amyloliquefaciens BS 1b*

**Product Name:** TripleX – BioStart Limited, NZ; www.biostart.co.nz (score 14.5)

**Target pathogen/disease:** *Botrytis cinerea*

**Mode of action:**
Competition for space and nutrients; antibiosis (by breaking down peptide bonds of pathogen proteins).

**Registered for use on/Target crop:** Grapes, top fruit, vegetables, e.g. onions.

**Formulation:** liquid

**Comments:** This formulation of *Bacillus amyloliquefaciens* (strain BS 1b) is intended for use against *Botrytis*, a fungal pathogen, on fruit. Literature search did not reveal any evidence that this product/organism has been tested for its efficacy in rigorous scientific experiments; all current knowledge is based on manufacturer’s claims. However, products based on other strains of *Bacillus amyloliquefaciens* are also used as biocontrol and plant strengthening agents (e.g. FZB24® *Bacillus subtilis*, Bayer CropScience AG/ www.bayercropsience.com and BacStar™, ETEC Crop Solutions Limited/ www.elliottchemicals.co.nz) and have been suggested to be effective on other crops and diseases.

TripleX is part of the New Zealand Kiwifruit Industry's *in vitro* & greenhouse testing programme.

14) *Bacillus subtilis* strain ATCC6051 + seaweed extract

**Product Name:** Bio Sol B Sub Plus – Biological Solutions Ltd., NZ; www.biosolutions.co.nz (score 14.5)

**Target pathogen / disease:** Powdery Mildew, Botrytis, Sour rot

**Mode of action:**
Competition for nutrients and space; antibiosis through production of antifungal products; improved plant nutrient status and resistance.

**Registered for use on / Target crop:** grapes, fruits, vegetables, potatoes, ornamentals

**Comments:** A literature search did not reveal any independent evidence of the efficacy of this product based on rigorous scientific testing (only manufacturer’s information). This suggests that despite its high score this product is not a promising candidate for the control/suppression of Psa on kiwifruit.
Bio Sol B Sub Plus is part of the New Zealand Kiwifruit Industry’s *in vitro* & greenhouse testing programme.

http://www.biosolutions.co.nz/b-sub-plus
http://www.biosolutions.co.nz/assets/pdfs/bol-sol-b-sub.pdf

15) **Bacteriophages**

**Product Name:** AgriPhage™ – Omnilytics, USA; www.omnilytics.com (score 14)

**Target pathogen/disease:**
Bacterial diseases (*Xanthomonas campestris* pv. *vesicatoria* or *Pseudomonas syringae* pv. *tomato*).

**Mode of action:**
Predation: Bacteriophage destroy bacteria through lysis. Lysing begins the moment a phage comes in contact with a bacterium and results in a release of additional phages within 30 minutes. Over four billion phages are included in every ml of AgriPhage™. Each phage is capable of destroying bacteria, and can replicate as many as 100 additional phage.

**Registered for use on/Target crop:** Tomato, pepper (capsicum)

**Formulation:** concentrated liquid

**Comments:** The possibility of phage therapy in plants is of growing interest as phages are host specific viruses that destroy the pathogenic bacteria. The use of bacteriophages to treat Psa could therefore be an attractive and promising possibility.
For the existing Omnilytics product Agriphage™ there is scientific evidence for disease control when used on/against Bell Pepper/bacterial spot (Raudales et al., 2010). However, a specific phage type needs to be isolated from the location the product will be applied to (in order to guarantee its viability and effectiveness under prevailing environmental conditions) and, just as pathogens can develop resistance to chemical products and antibiotics, bacteria can become resistant to bacteriophages, which might lead to an uncontrolled spread of the disease (Goodridge, 2004; Evans et al., 2010; Mahony et al., 2011).

16) *Trichoderma atroviride* LC52

**Product Name:** Sentinel® – Agrimm Technologies Ltd, New Zealand; www.vinevax.com (score 14)

**Target pathogen/disease:** *Botrytis cinerea*

**Mode of action:**
Colonises the foliage, flowers and fruit competing with disease organisms for space and nutrients thus preventing invasion by *Botrytis*.

**Registered for use on/Target crop:** Grapes and tomatoes

**Formulation:** wettable powder

**Comments:** Experiments under controlled environmental conditions showed that the efficacy of Sentinel® was very good and that the use of biocontrol product significantly reduced disease levels of *Botrytis cinerea* in strawberry plants (Robinson-Boyer et al., 2009).

Sentinel® is not part of the New Zealand Kiwifruit Industry's *in vitro* & greenhouse testing programme.

http://www.vinevax.com/sentinel.html
http://www.vinevax.com/pdfs/MSDSSentinel.pdf

17) *Bacillus subtilis, Pseudomonas putida, Trichoderma koningii & Trichoderma harzianum*

**Product Name:** Superzyme® – Roots Shoots and Fruits Ltd, New Zealand, www.rd2.co.nz (score 14)

**Target pathogen/disease:** plant growth promoter

**Mode of action:**
The mixture of selected fungi, bacteria and enzyme complexes provides beneficial microbes to the root zone, stimulates plant growth and reduces the risk of disease. Competition for space and nutrients might help suppress pathogen numbers.

**Registered for use on/Target crop:**
All crops; including all vegetables, potatoes, carrots, onions, all berries and strawberries, all brassicas, all fruit trees, avocados, persimmons, citrus, all nut
trees, vineyards, kiwifruit, tropical fruit, lettuce, asparagus, all melons, beans, ginger, ornamentals, cut flowers and pastures.

**Formulation:** wettable powder for soil or foliar application

**Comments:** Although literature search did not reveal any independent evidence of the efficacy of this product based on rigorous scientific testing (only manufacturer’s information), it is known that many saprotrophic fungi, such as isolates of Trichoderma species, positively influence plant growth similar to plant growth promoting rhizobacteria; and healthier, stronger plants might be able to better withstand pathogen attack. The plant growth promoting effect is most effective in the absence of any major pathogens (Whipps, 2001). There is also evidence that mixtures of fungi and bacteria can provide better biocontrol than individual control agents (Duffy et al., 1996; Mao et al., 1998; Duijff et al., 1999). For instance, suppression of take-all in wheat was greater when using a combination of *Trichoderma koningii* with *Pseudomonas* species compared to applying *T. koningii* by itself (Duffy et al., 1996). Based on the information available, soil application of Superzyme® might be useful in combination with other biocontrol agents as part of an integrated control strategy of Psa. Superzyme® is part of the New Zealand Kiwifruit Industry’s *in vitro* & greenhouse testing programme.

http://www.rd2.co.nz/uploads/Superzyme%20105x100%20180907(1).pdf

18) *Bacillus subtilis* (+ amino acids)

**Product Name:** Fulzyme Plus™ – Roots Shoots and Fruits Ltd, New Zealand; www.rd2.co.nz (score 14)

**Target pathogen/disease:** Biological plant growth promoter.

**Mode of action:**
According to manufacturer’s claims, Fulzyme Plus™ helps solubilise nutrients, incl. phosphorus, calcium, potassium, magnesium, to make them readily available for plant uptake; it aids in decomposing organic matter and plant material; and it produces natural and beneficial substances essential for plant productivity. These properties might assist in increasing the plant’s natural resistance and help the plant to withstand stress conditions as experienced during pathogen attack.

**Registered for use on/Target crop:** All crops

**Formulation:** liquid
Comments: Even though this product achieved a ranking score amongst the top 20 products based on the criteria chosen, no reports could be found of the product’s efficacy or whether it had been tested in a methodologically rigorous experiment.

Fulzyme Plus™ is part of the New Zealand Kiwifruit Industry’s in vitro & greenhouse testing programme.

http://www.rd2.co.nz/uploads/Fulzyme%20Plus%20100x100%200907(1).pdf

4. Conclusions and Recommendations

According to the New Zealand Kiwifruit Industry 'Psa-Actives Screening Status List' from 02 September 20112, 13 of our top 20 promising products/ biocontrol organism have already been tested as part of the New Zealand Kiwifruit Industry’s screening programme: Serenade Max (=Cease®/Rhapsody®), BacStar™, BlightBan® A506, Blossom Bless™, Companion Gold (=Companion®), Clarity, Vinevax™, TripleX, Bio Sol B Sub Plus, Superzyme® and Fulzyme Plus™.

The glasshouse screening tests indicate that Serenade Max (active ingredient: Bacillus subtilis), BlightBan® A506 (Pseudomonas fluorescens) and Blossom Bless™ (Pantoea agglomerans P10c) are the best microbial products tested so far. These also featured amongst the highest ranked on our list.

Products with the same active ingredients to those already tested (i.e. Bacillus subtilis, Pseudomonas fluorescens and Pantoea agglomerans P10c) are unlikely to provide substantially greater control than that already observed. Our evaluation and the screening test results suggest that there is limited value in screening other products based on Bacillus subtilis as Serenade Max is likely to be the best performer with this active ingredient and it is already being evaluated by Zespri.

Similarly, other Pseudomonas fluorescens or Pantoea agglomerans-based products are unlikely to perform differently to or better than BlightBan® A506 and Blossom Bless™, which both came out as the No 3 priority in this evaluation and are currently being assessed by Zespri.

We, therefore, recommend to focus further screening efforts on those priority products with different biocontrol organisms. The following products would be worthwhile including in the screening programme and/or bringing into NZ for testing since they are distinct microorganisms with a range of different mechanisms of actions that ranked in our top 20:

- Sonata®/ Ballad® Plus (Bacillus pumilus QST 2808);
- Botry-Zen® (Ulocladium oudemansii);

• Blossom Protect™ (Aureobasidium pullulans DSM 14941, Aureobasidium pullulans DSM 14940);
• Sentinel® (Trichoderma LC52).

Of the products not rated amongst the top 20 in this evaluation the following could also be included in future screening tests as there is a limited chance of efficacy against Psa:

• Actinovate® / Microplus (Streptomyces lydicus WYEC 108);
• Prestop® Biofungicide WP (Gliocladium catenulatum strain J1446);
• Bio-save® 10LP (Pseudomonas syringae strain ESC-10).

Of these products Bacillus pumilus QST 2808 (Sonata®/ Ballad® Plus), Pantoea agglomerans strain E325 (Bloomtime®), Aureobasidium pullulans (Blossom Protect™), Prestop Biofungicide WP (Gliocladium catenulatum strain J1446) and Bio-save 10LP (Pseudomonas syringae strain ESC-10) are currently not commercially available in New Zealand according to the ACVM register (Registered Veterinary Medicines, Agricultural Chemicals and Vertebrate Toxic Agents3). The organisms, however, are recorded as being present in the country and are non-regulated 4. As agrichemicals, importation of the formulated products should thus be subject to approval under the Hazardous Substances and New Organisms (HSNO) Act 5.

To date, there is no strong evidence that commercial, microbial products will deliver standalone control or suppression of Psa. They are more likely to be successful when used as part of an integrated pest management strategy which incorporates tolerant kiwifruit cultivars, judicious crop hygiene and targeted pesticide applications.

It is possible that mixtures or combinations of two or more biocontrol products might be able to offer better disease suppression/control and greater reliability than individual products/organisms. However, compatibility issues would need to be addressed to ensure that individual agents in the mixture did not inhibit each other or interfere with the existing, non-pathogenic microbial community of the plant.

Similarly, integration of biological and chemical control systems could also lead to increased efficacy and better disease control.

We recommend that both approaches should to be considered when selecting biocontrol products for further evaluation of their potential to control Psa.

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5 http://www.epa.govt.nz/hazardous-substances/importing-manufacturing/Pages/Pesticide-Products.aspx
References


Appendix

Attached: Complete list and ranking of microbial and botanical candidates for control and/or suppression of Psa assessed as part of the desktop evaluation.
15.5 Complete list of microbial and botanical candidates for the control and/or suppression of Psa assessed as part of the desktop evaluation.
Cellulose-resistant organism: *Verticillium albo-atrum*, *SoilGard® 12 G*

- Foliar diseases on Turfgrass (pink snow mold).
- 63-28 *Pseudomonas chlororaphis*
  - *Rifai aggr* Tx-1
  - *Pseudomonas chlororaphis*
  - *Cedomon*
  - *Bacillus subtilis*
  - *AtEze* (dollar spot), *Talaromyces flavus* as *Dilophospora alopecuri Contans WG*
  - *EcoGuard*
  - *Bacillus pumilus Supresivit*

- Plant parasitic nematodes seed-borne diseases on barley and oats. (and anthracnose)
- *Pseudomonas chlororaphis* soybean, alfalfa, dry/snap beans, peanuts
- *spp.*

- Biocontrol agent/ organism: *); GN: *Pseudomonas aureofaciens* Trichodex and *MeloCon WG* HiStick® N/T or Peat (product no MA 342 Afla-Guard GR strain GB34 *Paecilomyces lilacinus* GL-21/

- Target crop: PIX PLUS Ornamentals; peas
- Annual ryegrass toxicity Twist Fungus
- *spp., Corn, field; Corn, popcorn; Corn, sweet; Peanuts bv. inhibits fungal growth.

- turf;  GN: greenhouses, nurseries, and ornamental gardens

- *Talaromyces flavus* (anthracnose), *Sclerotinia homeocarpa* as *Dilophospora alopecuri Contans WG* EcoGuard *Bacillus pumilus Supresivit*

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- *Pseudomonas chlororaphis* soybean, alfalfa, dry/snap beans, peanuts
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- turf;  GN: greenhouses, nurseries, and ornamental gardens

- *Talaromyces flavus* (anthracnose), *Sclerotinia homeocarpa* as *Dilophospora alopecuri Contans WG* EcoGuard *Bacillus pumilus Supresivit*
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