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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: PS2125
2. Project title: Novel strategies for optimising powdery mildew management on outdoor cucurbits and protected herbs
3. Contractor organisation(s): ADAS UK Ltd
   Battlegate Road
   Boxworth, CAMBS
   CB23 4NN
4. Total Defra project costs (agreed fixed price): £ 218,560
5. Project: start date................. 1 January 2009
   end date.................. 30 April 2011

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

A previous Defra review (PS2117) collated a wide range of literature reporting that control of plant disease, in particular powdery mildew, can be achieved through use of a range of simple inorganic salts. For example, potassium bicarbonate (also known as potassium hydrogen carbonate; $KHCO_3$) currently has a commodity substance approval for use as a horticultural fungicide in the UK and is increasingly being used as an inexpensive component of integrated programmes for powdery mildew control in the soft fruit and ornamental sectors. There is increasing grower interest in this product, despite the need for frequent applications and occasional crop damage. Given grower interest in the use of non-conventional fungicides, there could be scope to extend the range of salt compounds that are available for disease control, by looking for products with greater efficacy than that provided by potassium bicarbonate, broad-spectrum activity against a range of key diseases and minimal crop damage.

In this project, inorganic salts (including bicarbonates, chlorides, nitrates, phosphates and silicates) were evaluated for powdery mildew control with the aim of reducing grower reliance on conventional fungicides. In addition to the inorganic salts, two further materials (as coded products) were included in the treatment list, selected based on published reports of their efficacy against powdery mildew, interest in the products in Europe, and potential for future registration in the UK via the biopesticides route. The research effort was targeted at high disease risk crops (protected parsley and outdoor courgettes) where there are limited modes of action in fungicides, intractable disease problems, and potential residue implications.

From a series of glasshouse bioassays (incorporating 15 salts and two coded products), it was concluded that sodium silicate at 10 g/L with a standard wetter (25% Tween 20) was consistently effective for control of powdery mildew on parsley (*Erysiphe heraclei*) and courgette (*Podosphaera fuliginea*), with minimal phytotoxic or spray residue effects. Higher doses increased phytotoxicity without improving disease control. Spray timing was most effective when the compound was applied curatively (post infection but prior to symptom development). Mixtures of sodium silicate with sodium bicarbonate and potassium phosphate (for parsley) and ammonium dihydrogen phosphate (for courgette) improved disease control compared with the single salt.

Practical use of sodium silicate alone or in mixtures for powdery mildew control on protected herbs and outdoor courgette was considered. Experiments on effective spray intervals for sodium silicate alone and in combination with sodium bicarbonate and potassium phosphate, suggested that for a short duration commercial crop of potted parsley, a single application of these compounds after infection or immediately after initial symptom development would likely provide sufficient disease control, although a later spray could provide further benefit under high disease pressure. Two timely applications of the mixture reduced

mildew to trace levels (1% plant area affected) at 21 d after infection (equivalent to crop duration). It was also noted that repeated sprays of water alone (after an infection event) could provide moderate disease control, offering a simple management option for growers, although this could increase risk of other diseases (eg grey mould caused by *Botrytis cinerea*). From the courgette glasshouse screens, it was found that a mixture of sodium silicate and ammonium dihydrogen phosphate was optimal, and that multiple sprays repeated within the latent period were most effective. From subsequent field experiments, it was found that the optimal approach devised from the screens was unable to provide mildew control as good as that obtained from approved fungicides. Further work to improve the formulation and rainfastness of the salt solution may lead to better powdery mildew control under field conditions. In addition, the use of salts in programmes with conventional fungicides to reduce the total fungicide input and reduce risk of resistance development, warrants further investigation.

Growers are increasingly reliant on use of biological control as a component of integrated pest management in protected cropping and therefore, novel fungicides need to be 'safe' for use with biological control agents. There was very limited information available prior to this project on the compatibility of inorganic salts with biological pest control. Studies in this project tested mortality effects of a range of inorganic salts and a coded plant extract on two biological control agents, *Aphidius colemani* and *Neoseiulus cucumeris*, which are used to control aphids and thrips on protected herb crops. The bioassays used worst case scenarios where the biological control agent was either dipped or exposed to leaves sprayed with the test compound. Based on the IOBC classification of plant protection products for their side-effects on beneficial arthropods, the inorganic salts and plant extract were 'non-toxic' against *N. cucumeris* and *A. colemani* adults. These potential alternative controls for powdery mildew would then be compatible with IPM programmes used on protected herbs.

The development and use of inorganic salts as fungicides for powdery mildew control on horticultural crops is currently limited in the UK for market and legislative reasons. Changes to EU legislation mean that the option for approval of compounds such as simple inorganic salts as fungicides via a commodity substance approval route is no longer available. Under EU Regulation 1107/2009 (in force from 14 June 2011), any active ingredient to be used for crop protection requires approval at the EU level (previously referred to as Annex 1 listing) followed by product registration. Compounds such as salts would likely be treated as 'basic substances' under the new regulation, possibly with potential to negotiate for reduced dossier requirements. However, there would seem little incentive for agrochemical companies to seek approvals for 'basic substances' that would be difficult to 'protect' by patent, with the added problem of offsetting approval and registration costs through product sales to a limited market. Similarly, grower groups would potentially have difficulty in bringing a 'basic substance' for approval because of cost implications, unless perhaps there was coordination at a European level. In comparison with one remaining commodity substance approval of an inorganic salt (potassium bicarbonate) in the UK (until 2019), there are at least 25 commercial products containing inorganic salts (bicarbonates, phosphates and phosphites) approved as biopesticides by the Environmental Protection Agency in the USA. A meeting being planned in conjunction with the HortLINK SCEPTRE project (project number HL01109) later in 2011 will further discuss the opportunities and difficulties of registration of this type of compound under new EU regulations.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

PS2125: Novel strategies for optimising powdery mildew management on outdoor cucurbits and protected herbs

Background to the project and overall purpose

Powdery mildew diseases affect the yield and quality of a wide range of edible and non-edible horticultural crops in outdoor and protected production (e.g. cucurbits, herbs, soft fruit, brassicas, carrots, cut flowers and ornamentals). Despite commitment by UK growers to minimise pesticide use, many are reliant on the use of a very small number of approved conventional fungicides on crops at risk from powdery mildew, in order to achieve marketable quality and yield.

There is good evidence of powdery mildew control by inorganic salts (bicarbonates, silicates, phosphates and chlorides), with several of these approved as 'bio-pesticide' products in the USA (Deliopoulos *et al*., 2010). The multiple modes of action of inorganic salts suggest that there may be a decreased risk of resistance development by these salts relative to conventional fungicides. In the UK, potassium bicarbonate is the only inorganic salt that has a commodity substance approval (valid until 31 August 2019) as a horticultural fungicide particularly for powdery mildew control. Potassium bicarbonate is now used widely in the soft fruit and ornamental industries as a component of integrated disease management. In addition to inorganic salts, several potential alternatives to conventional fungicides have been identified; the only biofungicide registered in the UK with reported activity against powdery mildew is Serenade ASO (*Bacillus subtilis*). Other potential products include extract of giant knotweed (*Reynoutria sachalinensis*) previously marketed as Milsana® in Germany, with research demonstrating that this project can control powdery mildew, reportedly stimulating plant defence responses (Daayf *et al*., 1995), SB Plant Invigorator (SB products), and Enzicur (Koppert).

The overall purpose of the project was to reduce grower reliance on conventional fungicides available for powdery mildew control. The research effort was targeted at high disease risk crops where there are limited modes of action in fungicides, intractable disease problems, and potential residue implications.

Scientific objectives

1. Identify the efficacy of inorganic salts, biological control agents (BCAs) and other products against powdery mildew via high through-put screens, using parsley and courgette as model crop systems.

2. Validate the efficacy of compounds and products on outdoor cucurbits and protected herbs under semi-commercial conditions and develop a programmed approach to the integration of products into commercial practice.

3. Evaluate selected novel products for their effects on invertebrate BCAs in protected parsley, using laboratory bioassays and crop experiments.

4. Develop appropriate linkages with key industry players in order to identify potential pathways for approval of effective products and future uptake by UK growers.

Objective 1. Identify the efficacy of inorganic salts, biological control agents (BCAs) and other products against powdery mildew via high through-put screens, using parsley and courgette as model crop systems.

Status: Complete

Introduction

Inoculated glasshouse experiments were done to determine product efficacy against powdery mildew. Selected products (with emphasis on inorganic salts) were evaluated using two crops (protected parsley and outdoor courgette) that are highly susceptible to powdery mildew, which are representative of different industry sectors and for which there are potential residue issues.

A list of 17 treatments selected for efficacy screening under objective 1 is shown in Table 1. The efficacy experiments focussed on screening of inorganic salts that from previous literature review (Deliopoulos *et al*., 2008) were considered to have potential for powdery mildew control. In addition to the inorganic salts, two further materials (listed as experimental products) were included in the treatment list, selected based on published reports of their efficacy against powdery mildew, interest in the products in Europe, and potential for future registration in the UK via the biopesticides route. Although reported to have activity against powdery mildew, the biological fungicide Serenade ASO (*Bacillus subtilis*) was not included in the screens, since it was already registered for use in the UK. Other potential biofungicides with activity against powdery mildew (such as AQ10; *Ampelomyces quisqualis*) were not included since they were considered too distant from UK registration.

Table 1. Compounds screened for efficacy against powdery mildew on parsley and courgettes

Compound	Formula / ingredients
Potassium bicarbonate	$KHCO_3$
Sodium bicarbonate	$NaHCO_3$
Ammonium bicarbonate	NH_4HCO_3
Potassium silicate	K_2SiO_3
Sodium silicate	Na_2SiO_3
Calcium silicate	$CaSiO_3$
Potassium phosphate	K_3PO_4
Dipotassium hydrogen phosphate	K_2HPO_4
Potassium dihydrogen phosphate	KH_2PO_4
Ammonium dihydrogen phosphate	$NH_4H_2PO_4$
Potassium chloride	KCl
Sodium chloride	$NaCl$
Manganese chloride	$MnCl_2$
Potassium nitrate	KNO_3
Potassium sulphate	K_2SO_4
Coded product: KBV99-01L	Enzymes, salts and additives
Coded product: MOI-106020	Plant extract

Methods

Inoculum of powdery mildew on both parsley (*Erysiphe heraclei*) and courgette (*Podosphaera fuliginea*) was sourced from naturally infected parsley and cucumber plants, respectively. Curly leaf parsley (var. Aphrodite) was used throughout. All courgette experiments used the powdery mildew susceptible variety President.

All inorganic salts were used as food grade materials (where appropriate) or at least 98% purity. They were screened in combination with a laboratory surfactant compound (polyoxyethylene (20) sorbitan monolaurate) as Tween 20. Previous research (e.g. Ziv & Zitter, 1992) has shown that inorganic salts are more effective against powdery mildew when used together with a wetter; in all of the screens Tween 20 was used as a standard wetting agent in combination with the inorganic salts. Coded products and appropriate wetters/adjuvants were supplied by the manufacturers, and were used according to their recommendations.

Protocols under this objective were devised between ADAS and HAUC to ensure that a similar approach was being used for each screen. There were variations in methodology due to different cropping requirements for parsley and courgettes. In addition, the specific products selected for individual screens on each crop varied slightly, due to differing performance on the two crops. Throughout these glasshouse screens, ADAS used potted parsley seedlings supplied by a commercial nursery (45-50 seedlings per pot, commercial specification) and HAUC used courgettes grown singly in pots and raised at HAUC. In each screen, disease incidence and severity, phytotoxicity and spray residues were assessed at intervals after inoculation and product applications. In each screen, product performance was assessed in comparison with an untreated control, a water only control and a water + 25% Tween 20 control (unless stated otherwise). Salts were used at 10 g/L (unless stated otherwise) and combined with 1 ml/L 25% Tween 20. Product KBV99-01L was applied as 1 ml/L each of part I and part II, and 2.5 ml/L adjuvant (as supplied by the manufacturer). Product MOI-106020 was applied at 2.5 ml/L. Individual method details for the screens on each crop are summarised below.

For parsley, spray treatments were applied in a polytunnel using a Mardrive pot sprayer with 02F110 nozzles, at 1000 L water/ha. Courgettes were taken outside the glasshouse and sprayed using a 2 m Oxford precision sprayer, with a F110 02 flat fan nozzle producing a medium spray quality at a speed of 0.2 m/second. The spray pressure was set to 2 bar with the water volume for each spray being calibrated to deliver 1000 L/ha.

Parsley and courgettes were inoculated by spraying to run-off with a spore suspension. The suspension was prepared by immersing infected foliage in distilled water in glass beakers. A sterile spatula was used to dislodge spores from the surface of the foliage. The resultant spore suspension was strained through a layer of sterile muslin into a conical flask to remove leaf debris. The concentration of the spores was determined using a haemocytometer and microscope, and the concentration amended to 2.5×10^5 spores/ml for parsley and 1×10^5 spores/ml for courgette. The spore suspension was applied to each of the plants to the point of run-off, late in the afternoon, and plants covered with a polythene ‘tent’ for at least 18 h in order to provide conditions of high relative humidity.

The glasshouse with parsley plants was maintained at 17^oC (night) and 22^oC (day); plants were watered by hand to capillary matting in trays containing the pots. Courgettes were maintained in a glasshouse at 18^oC (night) and 28^oC (day) and watering was by sub-irrigation to the capillary matting. Biological pest control was used during efficacy screens on parsley and cucurbits, to avoid the need for pesticide use.

For all parsley experiments, parsley seedlings were used at 14 days after sowing. Experiments were arranged in a randomised complete block design with 3-6 pots of seedlings per plot (depending on available bench space) and four replicate blocks. For all courgette experiments, the experimental units were 4-leaf stage plants (approx. 4-week-old), one per pot replicated four times in a randomised complete block design. Statistical analyses were by ANOVA in Genstat (with mean separation using Tukey's test at P=0.05 for the courgette experiments).

Screen 1: Product efficacy against powdery mildew
For both crops, the 17 products each at 10 g/L were applied 2 days before and 2 days after inoculation, and 3 days after initial symptom development. The products were applied at all three timings to enable efficacy to be tested, irrespective of product mode of action (protectant, curative or eradicant).

Screen 2: Product timing
Screen 2 was done in order to gain information on the optimum timing for product application in relation to infection. For parsley, this screen was run as two experiments, firstly looking at 15 products (those most effective from Screen 1) applied 2 days before or after inoculation, and secondly looking at the same products applied 4 days after inoculation or 3 days after first symptom development. For courgette, this screen was performed as a single experiment. The five most effective products from Screen 1 plus the coded plant extract and controls were applied 2 days before inoculation or 2 days after inoculation or 7 days after inoculation (which was 3 days after initial symptoms developed).

Screen 3: Dose response
For both crops, five salts selected from the previous screens were applied at 10 g/L (as in previous screens) or at double this rate (20 g/L), at either 2 days before or 2 days after inoculation.

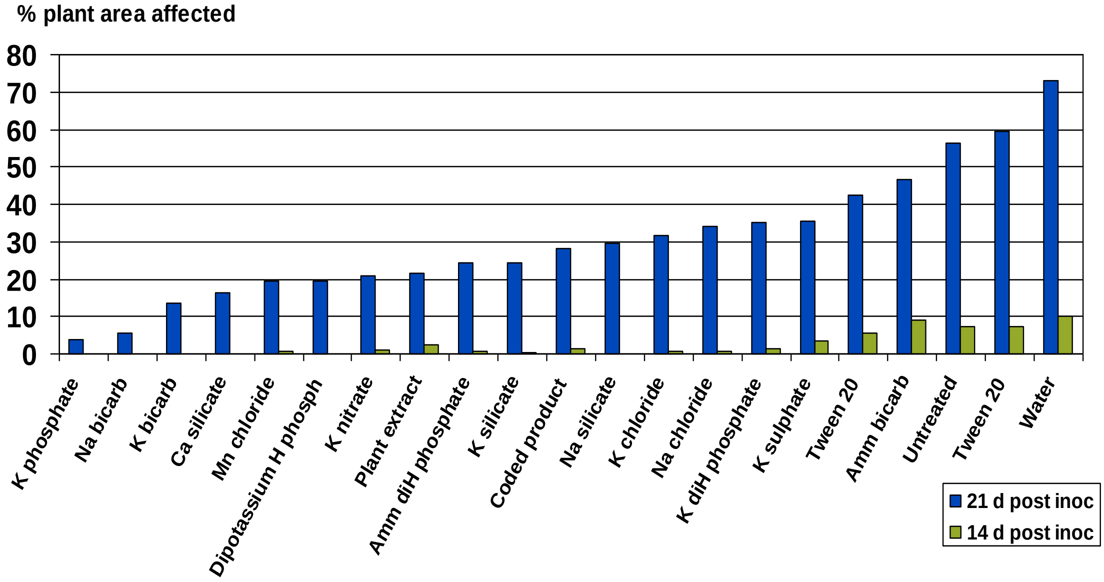
Screen 4: Efficacy of product mixtures
The efficacy of promising products in combination was tested in this screen. Sodium silicate (found to be effective on both crops) was tested alone or as 2-way and 3-way mixtures with the coded plant extract, sodium bicarbonate and selected phosphates, applied 2 days pre- or post-inoculation. For parsley, the phosphate used was potassium phosphate. For courgette, the phosphate used was ammonium dihydrogen phosphate.

Screen 5: Product persistence
This screen was done to determine the duration for which selected inorganic salts provided control of powdery mildew. For parsley, sodium silicate was tested alone or as a mixture with sodium bicarbonate and potassium phosphate. Sprays were applied singly at either 2, 7, or 14 days post-inoculation, or as repeated sprays at 2 and 7, 14 and 21, or 2 and 14 days after inoculation. For courgette, sodium silicate was tested alone or as a mixture with ammonium dihdrogen phosphate. Sprays were applied singly at 2 or 7 days post-inoculation, or as repeated sprays at 2 and 7 days, or 7 and 10 days post-inoculation.

Results

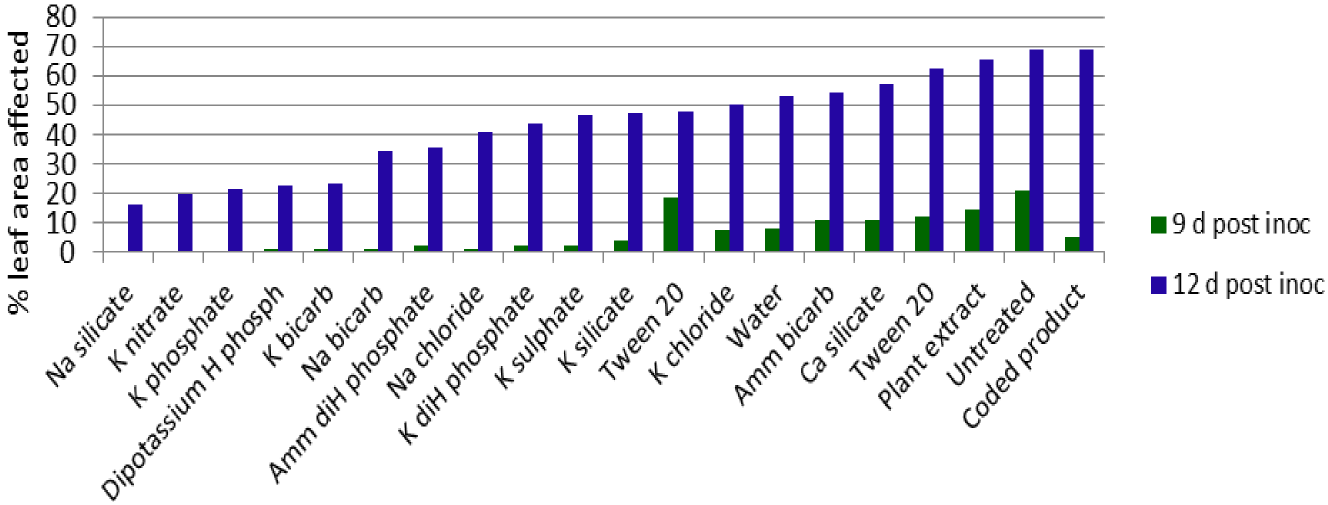
Screen 1: Product efficacy against powdery mildew
Parsley (March / April 2009) – First traces of powdery mildew were seen approximately 7 days after inoculation, and the disease affected 100% of plants in the untreated control by 14 days after inoculation. At 21 days after inoculation, 10 out of 17 products had reduced significantly (P<0.001) disease severity to <26% plant area affected, compared with 56% in the untreated control (Figure 1). Two particularly effective products (<6% severity) were potassium phosphate and sodium bicarbonate. The two least effective products (potassium sulphate and ammonium bicarbonate) were eliminated from subsequent screens. At an application rate of 10 g/L, manganese chloride was highly phytotoxic, leaving orange spots on foliage; spray residues remained after application of calcium silicate.

Courgette (September / October 2009) – All chloride salts, potassium nitrate and potassium phosphate were phytotoxic and excluded from the next screens. Of the other 12 products, all but one (ammonium bicarbonate) significantly suppressed powdery mildew 9 days after inoculation, with sodium silicate, potassium bicarbonate, dipotassium hydrogen phosphate, ammonium dihydrogen phosphate and sodium bicarbonate offering >90% protection (Figure 2). At day-12, mean efficacy was 50% lower than at day-9, but was still significant for four of the above mentioned products.



LSD (14 d post-inoculation) (60 df) = 4.3
LSD (21 d post-inoculation) (60 df) = 29.5

Figure 1. Product efficacy against parsley powdery mildew (21 d after inoculation), following applications 2 d before and 2 d after inoculation, and 3 d after initial symptom development



LSD (9 d post-inoculation) (57 df) = 10.4
LSD (12 d post-inoculation) (57 df) = 22.7

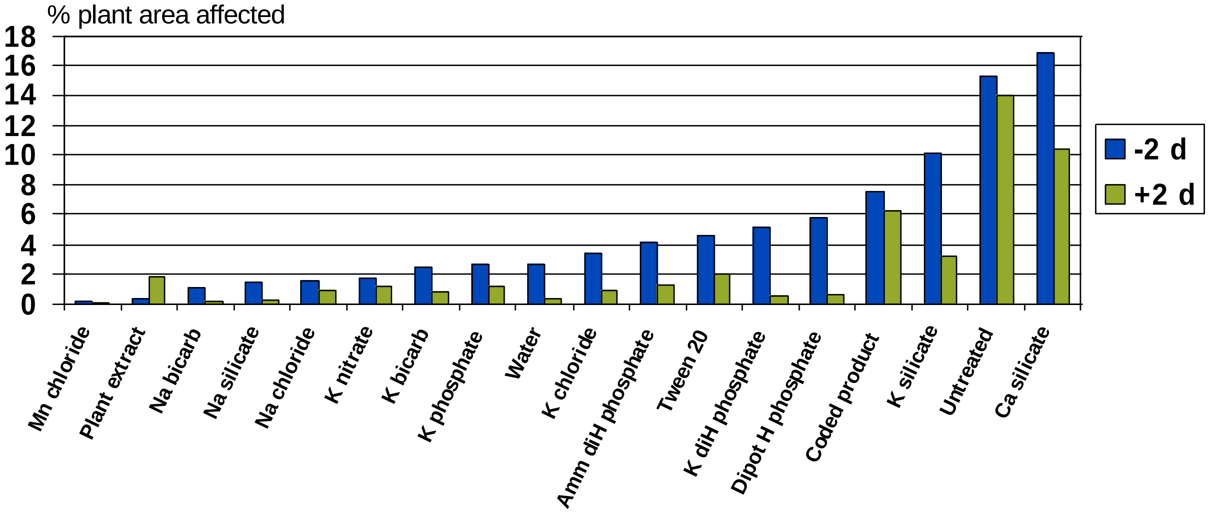
Figure 2. Product efficacy against courgette powdery mildew (9 and 12 d after inoculation), following applications 2 d before and 2 d after inoculation, and 2 d after initial symptom development

Screen 2: Product timing

Parsley (May to November 2009) – The first experiment indicated that overall, products were significantly more effective ($P<0.001$) when applied curatively (2 days post-inoculation), rather than preventatively (2 days pre-inoculation) (Figure 3). The exception to this was the coded plant extract. All of the products (except calcium silicate) significantly reduced disease severity compared with the untreated control. Interestingly, the water only control also gave effective control. In the second experiment, manganese chloride, sodium silicate, potassium and sodium bicarbonate, potassium and dipotassium hydrogen phosphate, were effective when applied either curatively (4 days after inoculation) or as eradicants (3 days after symptom development); manganese chloride was eliminated from subsequent screens due to phytotoxicity.

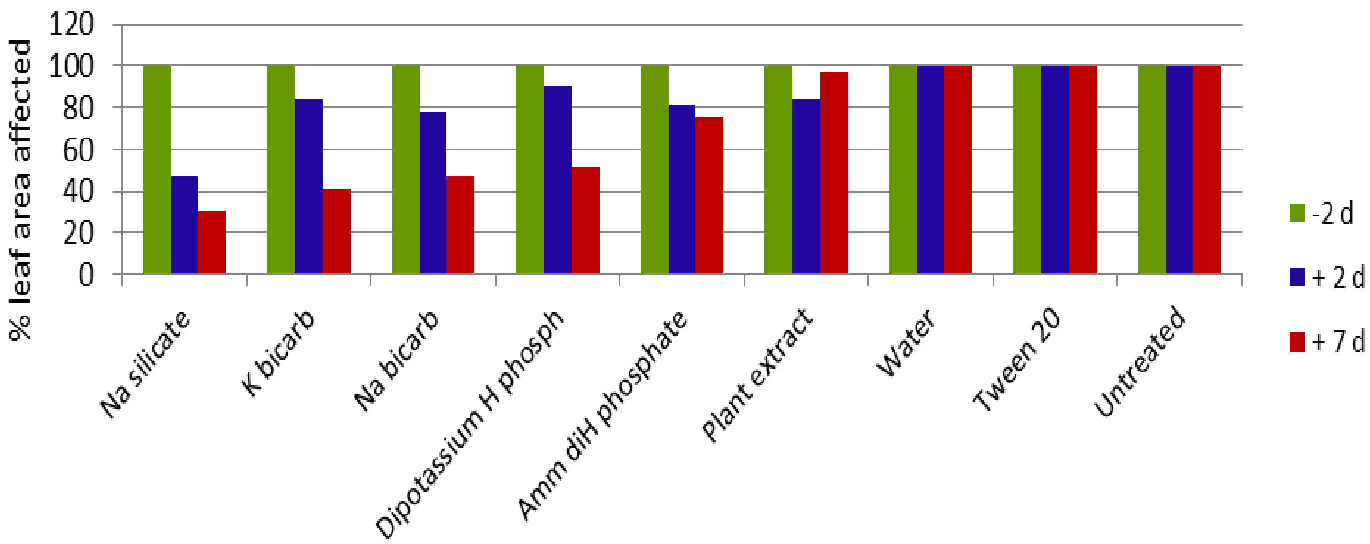
Courgette (November / December 2009) – All products, especially the five salts, induced significant reductions in disease severity compared with the untreated when applied 2 days post inoculation (Figure 4). Maximum recorded efficacies were 100% at day-7, 98% at day-9 and 53% at day-12, with sodium silicate the most efficacious product. As in the parsley experiment, the coded plant extract was the only effective product when applied before inoculation, with 8% (untreated: 41%) and 47% (untreated: 88%) leaf area affected at day-7 and day-9, respectively. Post-symptom spray had a minimal eradicant effect for four products, as shown by the 14-24% decrease in severity values from day-7 to day-9. None of the treatments were phytotoxic.

Based on the results of screens 1 and 2 for parsley and courgette, inorganic salts selected for further screening were: sodium silicate, sodium bicarbonate, potassium bicarbonate, dipotassium hydrogen phosphate. Because of observed differences in efficacy, the fifth salt differed in subsequent screens between parsley and courgette: potassium phosphate was used on parsley, and ammonium dihydrogen phosphate was used on courgette.



LSD (treatment.spray timing) = 3.9 (for different levels of treatment, 95 df)
LSD (treatment.spray timing) = 3.4 (for same levels of treatment, 54 df)

Figure 3. Product efficacy against parsley powdery mildew (14 days after inoculation), following applications either 2 d before (-2 d) or 2 d after (+2 d) inoculation



LSD (+2 d spray timing) = 17.29 (24 df)
LSD (+7 d spray timing) = 15.85 (24 df)

Figure 4. Product efficacy against courgette powdery mildew (12 days after inoculation), following applications either 2 d before (-2 d), 2 d after (+2 d) or 7 d (+7 d) after inoculation

Screen 3: Dose response

Parsley (January / February 2010) – As in screen 2, the salts were generally more effective when applied 2 days after inoculation rather than as protectants (P<0.001), reducing powdery mildew severity to <15% compared with 83% in the untreated control. For the post-inoculation timing, there was no evidence of improved efficacy with increased dose. Although generally less effective when applied pre-inoculation, there was a significant dose response for certain salts (potassium and sodium bicarbonate, potassium phosphate), with increased efficacy at the higher dose (notably for potassium phosphate). There was slight phytotoxicity for all treatments (none on the untreated, Tween 20 and water only controls) and slight spray residues were visible for sodium silicate (higher dose only). Phytotoxicty generally showed as white patches on leaves. Symptoms were greatest with potassium bicarbonate and potassium phosphate (1-5% leaf area affected) and were significantly greater (p=0.012) at the higher dose rate for these salts. At the lower dose rate, only dipotassium hydrogen phosphate resulted in leaf damage affecting more than 1% leaf area. As previously in screen 2, excellent disease control was obtained (<1% severity) with water applied 2 days post-inoculation, and also with the Tween 20 control.

Table 2. Effect of product dose and timing on parsley powdery mildew (21 days after inoculation)

Treatment	Salt dose (g/L)	% powdery mildew severity	
		2 days before inoc	2 days after inoc
1 Untreated		87.5	83.3
2 Water only control		52.1	0.1
3 Tween control		50.0	1.5
4 Potassium bicarbonate	10	81.3	10.3
5 Potassium bicarbonate	20	43.8	14.3
6 Sodium bicarbonate	10	42.1	3.3
7 Sodium bicarbonate	20	15.6	0.6
8 Sodium silicate	10	32.1	0.8
9 Sodium silicate	20	42.5	4.5
10 Potassium phosphate	10	58.3	2.0
11 Potassium phosphate	20	0.8	6.7
12 Dipotassium hydrogen phosphate	10	48.8	5.4
13 Dipotassium hydrogen phosphate	20	48.8	3.0

SED treatment (dose) / timing interaction:	
Comparison of different treatment levels (74.5 df)	7.9
Comparison of same treatment levels (39.0 df)	7.7

Courgette (January / February 2010) – Post-inoculation foliar sprays of the five tested salts (potassium bicarbonate, sodium bicarbonate, ammonium dihydrogen phosphate, dipotassium hydrogen phosphate and sodium silicate) suppressed powdery mildew at day-15 from 59% in the untreated to between 7-16%. There were no differences between low and high dose or among treatments for either post- or pre- inoculation sprays (Table 3). At that time, leaf area affected on pre-inoculation treated plants was greater than for post-inoculaion treatments, and averaged 38% across the five salts (no differences between low-high doses). Disease severity on foliage sprayed with 10 g/L sodium silicate remained significantly less than the untreated up to 19 days after inoculation. All treatments caused minor phytotoxic injury and sodium silicate produced slight spray residues at the higher dose of 20 g/L only.

Table 3. Effect of product dose and timing on courgette powdery mildew (15 days after inoculation)

Treatment	Salt dose (g/L)	% powdery mildew severity	
		2 days before inoc	2 days after inoc
1 Untreated		59.4	59.4
2 Water only control		63.8	50.6
3 Tween control		62.5	12.5
4 Potassium bicarbonate	10	25.0	10.6
5 Potassium bicarbonate	20	33.8	11.2
6 Sodium bicarbonate	10	41.9	16.2
7 Sodium bicarbonate	20	41.9	8.2
8 Sodium silicate	10	53.1	10.8
9 Sodium silicate	20	30.0	7.5
10 Ammonium dihydrogen phosphate	10	23.1	13.8
11 Ammonium dihydrogen phosphate	20	40.6	8.1
12 Dipotassium hydrogen phosphate	10	45.0	13.1
13 Dipotassium hydrogen phosphate	20	44.4	8.2
SED treatment (dose)/ timing interaction:		8.55 (72 df)	

Screen 4: Efficacy of product mixtures

Parsley (February / March 2010) – Salts treatments (and the water only control) applied before inoculation significantly reduced powdery mildew severity, although levels still exceeded 30% (Table 4). Mixtures with MOI-106020 (plant extract), sodium bicarbonate or potassium phosphate did not increase control compared with sodium silicate alone when applied preventatively. Sodium silicate was significantly more effective when applied 2 days post-inoculation compared with the earlier timing. It was particularly effective when applied in combination with both sodium bicarbonate and potassium phosphate, reducing powdery mildew severity to 2.5% at 20 days after inoculation, compared with 89% in the untreated control. There were only trace levels of phytotoxicity in this experiment, mainly from treatments containing sodium bicarbonate.

Table 4. Effect of product mixtures and timing on parsley powdery mildew (20 days after inoculation)

Treatment	Timing	% powdery mildew
1 Untreated control		88.5
2 Water only control	2 d before inoculation	66.0
3 Tween control	2 d before inoculation	72.9
4 Sodium silicate	2 d before inoculation	50.2
5 Sodium silicate + MOI-106020	2 d before inoculation	55.2
6 Sodium silicate + MOI-106020 + sodium bicarbonate	2 d before inoculation	44.2
7 Sodium silicate + MOI-106020 + potassium phosphate	2 d before inoculation	52.5
8 Sodium silicate + sodium bicarbonate + potassium phosphate	2 d before inoculation	31.3
9 Sodium silicate	2 d after inoculation	29.3
10 Sodium silicate + sodium bicarbonate	2 d after inoculation	9.4
11 Sodium silicate + potassium phosphate	2 d after inoculation	15.7
12 Sodium silicate + sodium bicarbonate + potassium phosphate	2 d after inoculation	2.5
SED treatment (33 df)		10.61

Courgette (February / March 2010) – The product mixtures were the same as tested on parsley except that for courgette, the phosphate used was ammonium dihydrogen phosphate instead of potassium phosphate. All post-inoculation sodium silicate mixtures were significantly more effective than those applied before inoculation (Table 5). In the case of single sodium silicate, both timings were effective. However, the pre-inoculation only application of sodium silicate was this time phytotoxic. At day-11, leaf area affected on post-inoculation treated plants ranged between 8-12%, with the respective value for the untreated being 84%. Unlike the other mixtures, the sodium silicate plus ammonium dihydrogen phosphate further improved the protection offered by the sodium silicate alone by 35% at day-14 and 21% at day-16 (Figure 5). The 4-way pre-inoculation mixture and all post-inoculation treatments formed slight deposits on the sprayed foliage.

Table 5. Effect of product mixtures and timing on courgette powdery mildew (14 days after inoculation)

Treatment	Timing	% powdery mildew
1 Untreated control		99.9
2 Water only control	2 d after inoculation	99.5
3 Tween control	2 d after inoculation	99.2
4 Sodium silicate	2 d before inoculation	52.1
5 Sodium silicate + MOI-106020	2 d before inoculation	97.7
6 Sodium silicate + MOI- 106020 +sodium bicarbonate	2 d before inoculation	97.2
7 Sodium silicate + MOI+ ammonium dihydrogen phosphate	2 d before inoculation	89.6
8 Sodium silicate+MOI+ sodium bicarb+ammonium dihydrogen phosphate	2 d before inoculation	87.8
9 Sodium silicate	2 d after inoculation	63.3
10 Sodium silicate + sodium Bicarbonate	2 d after inoculation	63.3
11 Sodium silicate + ammonium dihydrogen phosphate	2 d after inoculation	40.4
12 Sodium silicate+sodium bicarbonate+ammonium dihydrogen phosphate	2 d after inoculation	73.1
SED treatment (76 df)		6.05

Y-axis: % powdery mildew severity; X-axis: Days after inoculation

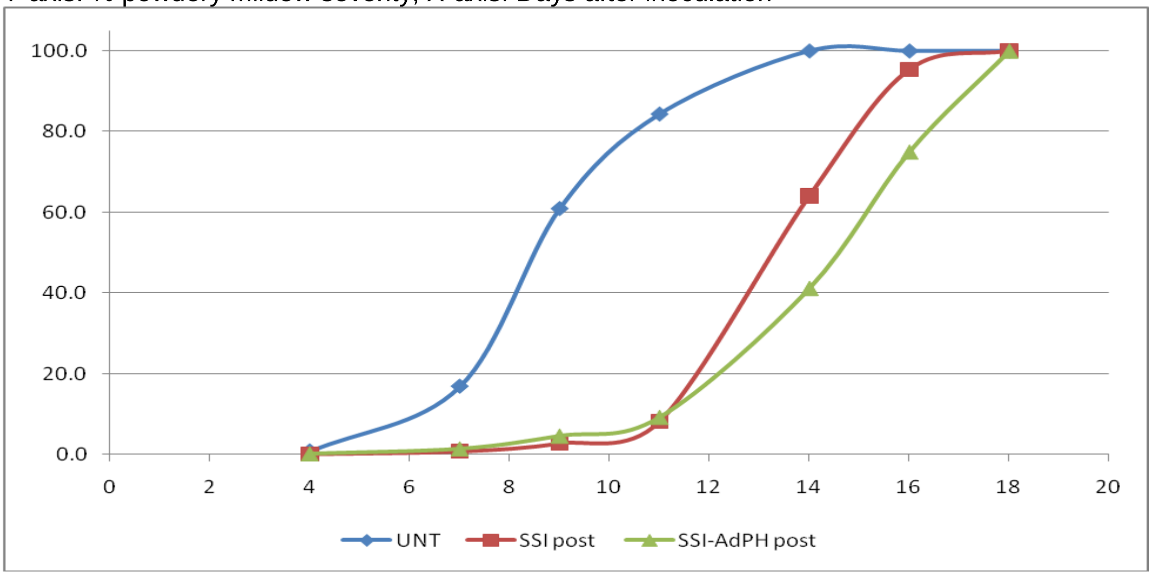


Figure 5. The progression of powdery mildew up to 18 days after inoculation on untreated (UNT) leaves or leaves sprayed 2 days post-inoculation with either sodium silicate (SSI post) or sodium silicate-ammonium dihydrogen phosphate (SSI-AdPH post) mixture (10 g/L of each).

Screen 5: Product persistence

Parsley (April / May 2010) - In previous experiments, products were applied either side of an infection event, or immediately after symptom development. In this experiment, products were applied at different timings after inoculation, including an extreme scenario at 21 d which would actually be close to harvest/packing on a commercial nursery. Assessments were continued up to 28 d, to enable monitoring of the late spray. Water was included as one of the spray treatments at different timings because of interesting results in previous screens.

At 14 d, disease severity did not exceed 13% (untreated control). Spray treatments significantly reduced disease in comparison with the controls, but there was no difference between spray treatments (even for treatments 15-17 that were due to receive a spray) (Table 6). At 21 d after inoculation (representing the stage at which parsley would be taken off the production line and packed), water and the salts treatments were similarly effective in reducing powdery mildew severity. Certain sodium silicate and mixture treatments maintained disease severity at less than 5% compared with 35% in the untreated control: sodium silicate applied at 2 d, 2+7 d, 14+21 d or 2+14 d; mixture treatment at 2 d, 2+7 d or 2+14 d. This result emphasises the importance of an early spray with an appropriate salt product. At the last assessment, under conditions of high inoculum pressure (90% in the untreated control), single applications and the 2+7 d timing no longer maintained disease control. Four treatments reduced disease severity to less than 10%: sodium silicate at 14+21 d and the mixture treatment at 14+21 d or 2+14 d.

Table 6. Effect of inorganic salt treatments at different intervals after inoculation on severity of parsley powdery mildew

Treatment	Spray timings (days after inoculation)	Powdery mildew severity (%) after inoculation		
		14 days	21 days	28 days
1 Untreated control	-	12.9	34.8	90.0
2 Tween control	2	3.6	16.7	65.0
3 Water	2	6.6	23.3	80.0
4 Sodium silicate	2	0.4	2.3	57.5
5 Sodium silicate + sodium bicarbonate + potassium phosphate	2	0.5	2.6	55.0
6 Water	7	2.8	5.6	54.0
7 Sodium silicate	7	3.3	15.7	63.8
8 Sodium silicate + sodium bicarbonate + potassium phosphate	7	2.9	5.5	75.0
9 Water	14	6.7	6.4	71.3
10 Sodium silicate	14	3.7	6.4	39.0
11 Sodium silicate + sodium bicarbonate + potassium phosphate	14	5.1	5.4	27.8
12 Water	2+7	3.5	5.2	52.5
13 Sodium silicate	2+7	0.2	0.4	47.3
14 Sodium silicate + sodium bicarbonate + potassium phosphate	2+7	0.5	1.0	45.5
15 Water	14+21	2.5	13.1	46.5
16 Sodium silicate	14+21	0.9	3.9	7.7
17 Sodium silicate + sodium bicarbonate + potassium phosphate	14+21	6.2	6.0	5.2
18 Water	2+14	0.8	5.8	34.3
19 Sodium silicate	2+14	3.4	2.4	23.1
20 Sodium silicate + sodium bicarbonate + potassium phosphate	2+14	5.8	3.2	8.9
SED treatment* (57 df)		0.9	4.82	9.75
F. prob		P<0.001	P<0.001	P<0.001
SED treatment.timing (57 df)*		-	-	12.77
F. prob		NS	NS	P=0.048

*comparison of untreated and Tween vs the rest; **comparison of water, sodium silicate and mixtures only

<u>Courgette (April / May 2010</u> - The sodium silicate mixture with ammonium dihdrogen phosphate gave better control than sodium silicate alone as found in Screen 4, and this mixture was therefore subsequently adopted in the field experiments. The results showed clearly that the mixture has little persistence, but delays disease progress. If sprayed within the latent period of the pathogen, that is to say before spores are visible (Programmes A and B in Fig. 6), there is a delay in the onset of symptoms. If sprayed after the latent period (Programmes C and D), symptom development is retarded for a few days, but then continues at a similar rate to that in the controls.

Y-axis: % powdery mildew severity; X-axis: Days after inoculation

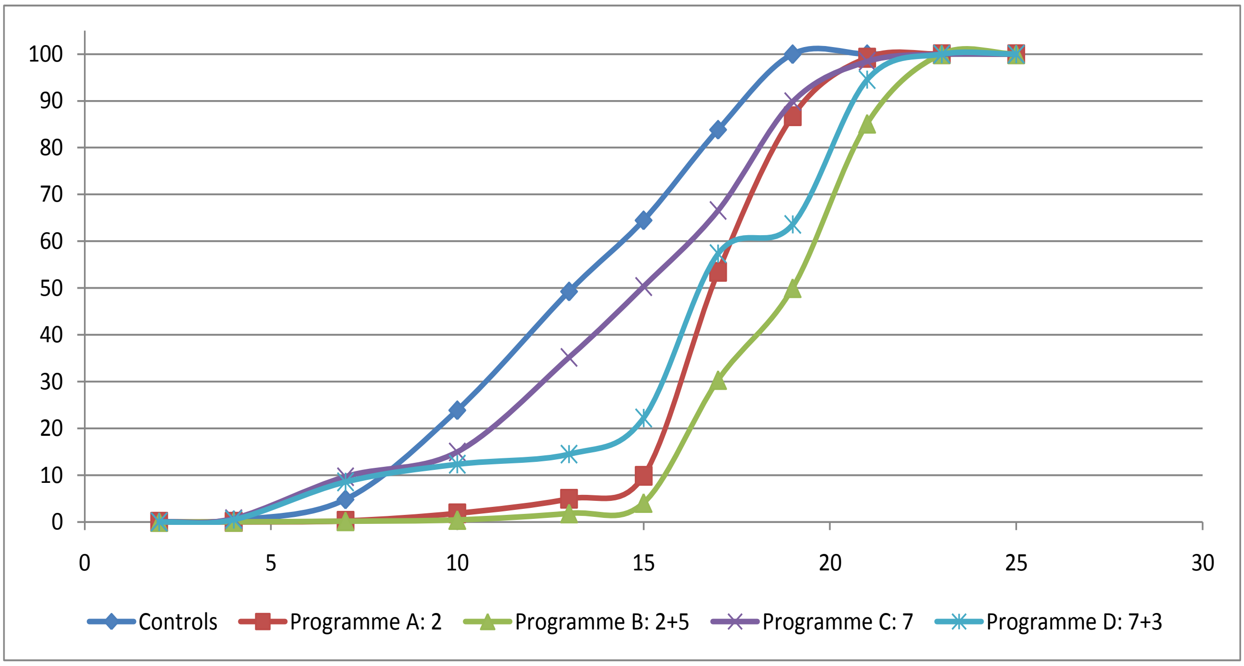


Figure 6. The progression of courgette powdery mildew up to 25 days after inoculation on untreated (Controls) leaves or leaves sprayed 2 days post-inoculation (Programme A), 2 days post-inoculation with a further spray at 7 days post-inoculation (Programme B), 7 days post-inoculation (Programme C), 7 days post-inoculation with a further spray at 10 days post-inoculation (mean of sodium silicate and sodium silicate-ammonium dihydrogen phosphate mixture (10 g/L of each).

Discussion

When considering the efficacy of treatments against powdery mildew, it should be noted that the level of disease that would be commercially acceptable on pot parsley would be very low (e.g. <3% plant area affected) because of the low tolerance of blemishes by retailers. In each screen, certain treatments achieved this level of control. In contrast, powdery mildew management on courgette would not require the same level of stringency, as long as green leaf area was maintained without a deleterious effect on fruit production.

In agreement with literature collated by Deliopoulous *et al*. (2010), results from initial screens indicated that specific salts within a range of inorganic salt types notably bicarbonates, chlorides, phosphates and silicates were effective for powdery mildew control (although with some resultant phytotoxicity). The curative and eradicant effect of sodium bicarbonate was in agreement with previous literature, although there are reported cases where bicarbonate salts gave more effective control when applied as protectants, for example by inhibiting fungal spore germination (Ilhan *et al*., 2006). It appears that multiple mechanisms are involved in the expression of anti-fungal effects by bicarbonate salts. Phosphates have more usually been associated with disease control by induction of systemic acquired resistance (Walters *et al*., 2005) such that a protectant application would be expected to be more effective. Although curative effects were more evident with phosphates in this project, results from screen 3 (dose response) on parsley indicated that potassium phosphate at the higher dose (20 g/L) was very effective when applied 2 d before inoculation. The mode of action of silicates in reducing fungal disease severity has been widely investigated, but has not yet been well defined; Deliopoulos *et al*. (2010) provide examples of both protectant and curative activity from the literature, while in our experiments foliar sprays with sodium silicate were clearly more effective when applied post-inoculation. Screens 4 and 5 provided new evidence that certain salt mixtures can be more effective than single salt compounds for powdery mildew control. This is likely to be because of the different modes of action that different salt groups can provide. This work warrants further investigation to determine combinations that would provide activity against a broader spectrum of plant pathogens.

In general there was consistency in the efficacy of different salts against the two powdery mildew pathogens on two crop hosts. For example, potassium and sodium bicarbonate were consistently more effective than ammonium bicarbonate for both pathogen/crop combinations. Sodium silicate was highly effective for both systems. While the phosphates varied in their performance for the two pathogen / crop combinations, there were still commonalities, with dipotassium hydrogen phosphate being effective for both.

The salts selected for the later screens all have uses related to the food industry. Sodium bicarbonate is widely used as a raising agent for example in baking powder. Sodium silicate can be used as a flocculant to clarify wine and beer by precipitating colloidal particles; it was also used extensively as an egg preservative in the early 20th Century. Potassium phosphate and ammonium dihydrogen phosphate are both used as food additives. Both silicates and phosphates are commonly used as agricultural fertilisers.

Conclusions

- Results from Objective 1 demonstrated that under small-scale glasshouse screening conditions a range of inorganic salts in combination with a standard wetter were effective for reduction of powdery mildew on parsley and courgettes.
- Some salts (e.g. manganese chloride) were effective but were eliminated at an early stage of screening due to severe phytoxicity on the crop hosts.
- Commercial products included in the screens (MOI-106020 plant extract and KBV99-01L containing enzymes, salts and additives) were less effective for powdery mildew control than the best salt products.
- Similar groups of compounds were found to be effective for control of powdery mildew pathogens on both parsley and cucurbits, namely sodium silicate, sodium bicarbonate, potassium phosphate (for parsley) and ammonium dihydrogen phosphate (for courgette). These products generally caused nil or trace levels of phytotoxicity / spray residues at 10 g/L, but occasionally were more severely phytotoxic.
- Results from various experiments indicated that the selected compounds were more effective when applied curatively (2 d after inoculation) rather than preventatively (2 d prior to inoculation). They also showed an eradicant mode of action. Of all the products screened, only the plant extract was more effective as a protectant than as a curative.
- On both crops, increasing salt dose from 10 g/L to 20 g/L generally increased phytotoxicity without a significant increase in disease control.
- Mixtures of sodium silicate with sodium bicarbonate and potassium phosphate (on parsley) and with ammonium dihydrogen phosphate (on courgette) were more effective for powdery mildew control when applied curatively than with sodium silicate alone.
- On courgette, salt mixtures had little persistence, but delayed disease progress when sprayed within the latent period of the pathogen. On parsley, a spray using sodium silicate or a sodium silicate mixture within the latent period of the pathogen (e.g. 2 d after infection) or immediately after first symptom development was sufficient to manage powdery mildew for the commercial duration of the crop. Repeated sprays (e.g. 2 + 14 d) provided extended control.

Objective 2. Validate the efficacy of compounds and products on outdoor cucurbits and protected herbs under semi-commercial conditions and develop a programmed approach to the integration of products into commercial practice.

Status: Partially complete

Parsley

The initial intention had been to undertake experiments under Objective 2 on a commercial herb nursery. However, following discussions with nursery staff it was decided that this would not be suitable due to unpredictability of powdery mildew outbreaks at the nursery, and possible disruption to production. Experiments were instead planned for the temperature-controlled glasshouse at ADAS Boxworth, using artificial inoculation (as for Objective 1). Due to high temperatures in the glasshouse at the end of the screening experiments in Objective 1, inoculum of *E. heraclei* did not survive. Several attempts were made from summer 2010 until the end of the project to re-establish artificial inoculum for use in this objective. Repeated requests were made to commercial protected nurseries for infected samples, but none were observed by growers during the period required. Visits to field-grown parsley to collect inoculum were also unsuccessful. Attempts were made to infect parsley plants using inoculum of *E. heraclei* on parsley leaf material that had been kept frozen during Objective 1, however the spores were non-viable as they tended to lyse following freezing/thawing.

Due to lack of inoculum of *E. heraclei*, we were unable to further develop the information obtained from Objective 1 to evaluate a programmed approach for use of inorganic salts on protected herbs.

Courgette

Two field experiments were conducted in 2010 using the information gained from the glasshouse experiments, especially that a mixture of sodium silicate and ammonium dihydrogen phosphate appears to be optimal for control of powdery mildew on courgette, and that multiple sprays repeated within the latent period were most effective. The first experiment needed artificial inoculation, and the second experiment relied on natural infection.

Materials and Methods

The first experiment was sown in May and the second in July. Experiment 1 had seven replicates of five treatments, and Experiment 2 had eight replicates of five treatments, both arranged as randomised complete block designs.

There were six plants in each plot arranged in two rows of three plants with the two central plants being assessed. Each plot had three buffer plants at each side. Leaves selected to be monitored were marked using plant ties and as each plant grew and the infection killed the leaves, new leaves were tagged and monitored. Each time new leaves were tagged on experiment 1 the inoculum was re-applied.

For artificial inoculation (Experiment 1 only), spores from approximately 20 infected leaves were removed through rubbing and suspended in deionised water. The concentration of the spores was calculated to a density of 250,000 spore/ml using a haemocytometer and was then applied evenly to the Experiment 1 crop with a hand-held Killaspray on 21 July. To ensure infection a second inoculum spray was added the following day.

Sprays were applied to each corresponding plot at the intervals shown in Table 7. However, where weather conditions were unsuitable for spraying the spray was applied on the next suitable day. Timings were selected to correspond to before, at and after the expected latent period of the pathogen under the expected field conditions. The spray was applied with a 2 m Oxford precision sprayer, with a F110 02 flat fan nozzle producing a medium spray quality at a speed of 0.2 m/second. The spray pressure was set to 2 bar with the water volume for each spray being calibrated to deliver 1000 L/ha. The first spray was applied to Experiment 1 on 23 July and to Experiment 2 on 24 August.

Assessments were done three times a week with the powdery mildew being estimated using the following scale: 0%, 0.1%, 1%, 5%, 10%, 25%, 50%, 75%, 100%. Assessments were taken two days after the inoculum was applied in Experiment 1 and ended on each individual leaf once it was dead.

Table 7. Treatments in the field experiments

Experiment 1 (Artifical inoculation)

Treatment number	Spray product applied	Spray interval	Total number of sprays
1	None	N/A	N/A
2	Fungicide*	7 days	7
3	Salt solution	5 days	10
4	Salt solution	9 days	5+
5	Salt solution	13 days	6

Experiment 2 (Natural infection)

Treatment number	Spray product applied	Spray interval	Total number of sprays
1	None	N/A	N/A
2	Fungicide*	7 days	5
3	Salt solution	3 times per week	11
4	Salt solution	Twice per week	7
5	Salt solution	Once per week	4

*The fungicide treatment was either azoxystrobin (Amistar; 23.1% w/w; Syngenta Crop Protection UK Ltd, Cambridge) or bupirimate + n-butanol (Nimrod; 27.2% w/w; Makhteshim-Agan (UK) Ltd, Cambridge). Salt solution consisted of 10 g/L sodium silicate, 10 g/L ammonium dihydrogen phosphate and 0.25 ml/L of Tween-20.
+Fewer sprays applied than planned due to adverse weather preventing spraying

Results

Experiment 1. Powdery mildew symptoms increased markedly in late July and early August, but for most assessment dates only the fungicide gave a significant reduction in infection. The only significant effects of salts in reducing mildew were from the 13 day interval sprays on 28/0702010 and 30/07/2010 (data not shown). Results from the most frequent spray treatment are shown in Figure 7.

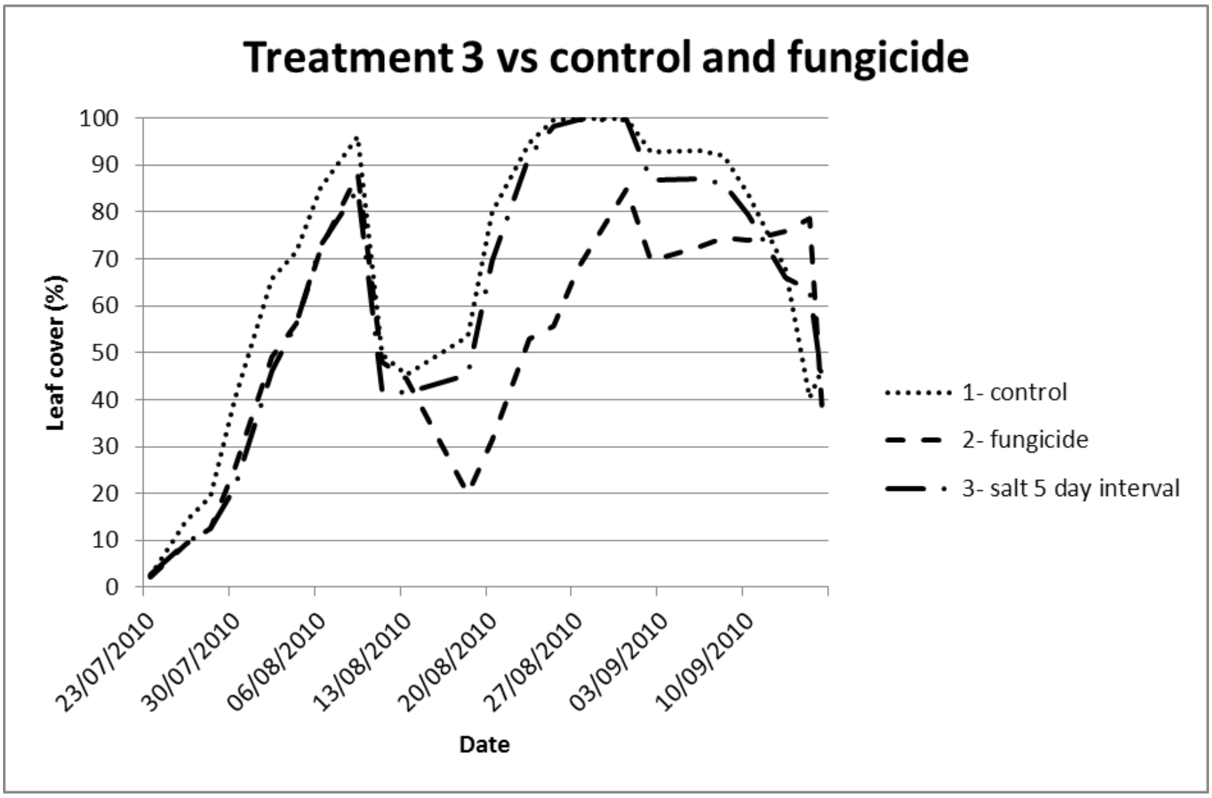


Figure 7. The progression of courgette powdery mildew from artificial inoculation in Experiment 1 on untreated (Controls) leaves or leaves sprayed with approved commercial fungicide or with sodium silicate-ammonium dihydrogen phosphate mixture (10 g/L of each).

Experiment 2. Powdery mildew symptoms increased throughout September. Although salt treatments showed some significant reductions in mildew infection at some assessments (all salt treatments on 10/09/2010, and the three times each week treatment on17/09/2010), in general the responses were small compared with the significant control provided by the commercial fungicide. The most frequent spray treatment (three times each week) gave numerically lower mildew infection than the less frequent spray treatements. Results from the most frequent spray treatment are shown in Figure 8.

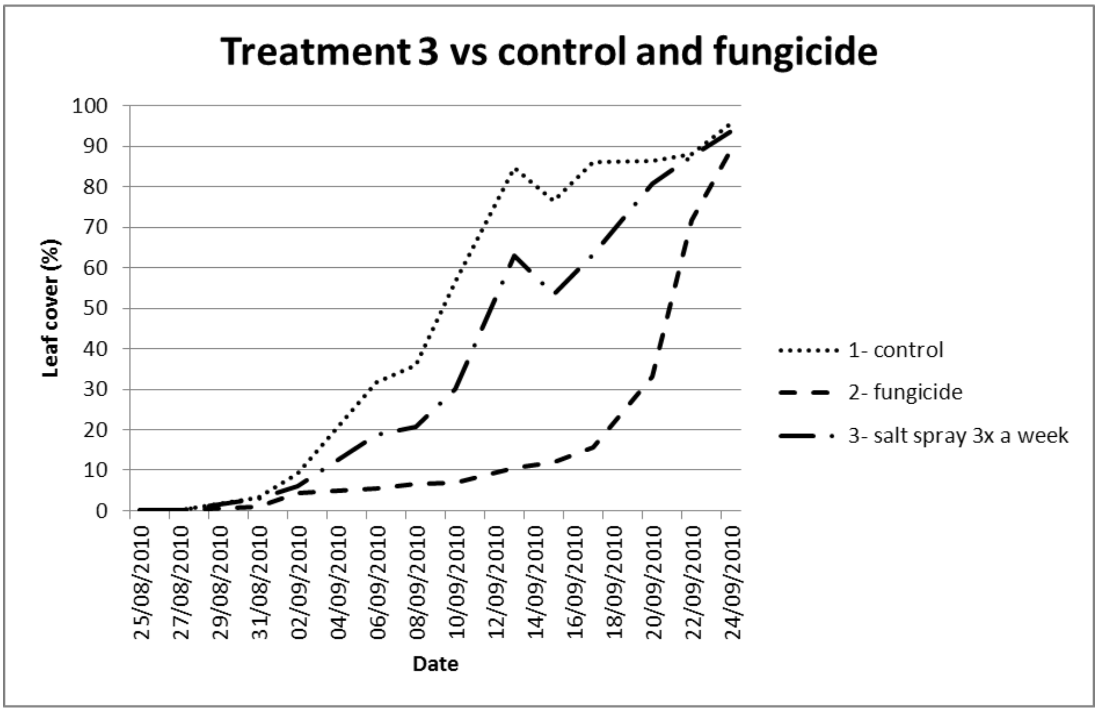


Figure 8. The progression of courgette powdery mildew from natural infection in Experiment 2 on untreated (Controls) leaves or leaves sprayed with approved commercial fungicide or with sodium silicate-ammonium dihydrogen phosphate mixture (10 g/L of each) three times each week.

Conclusions

Despite lack of progress under this Objective for parsley, results from Objective 1 (particularly screen 5) and knowledge of commercial protected herb production can provide the basis for suggesting potential integrated programmes for powdery mildew control. Pot parsley is a short duration crop (approximately 34 days depending on season). Outbreaks of powdery mildew are sporadic but can severely affect production as disease spreads rapidly and symptoms result in rejections. Despite availability of approved effective fungicides for protected herbs, growers rarely apply protectant fungicides for powdery mildew control. When a disease outbreak occurs, fungicides are occasionally applied but are often ineffective because they lack a curative mode of action. Results from Objective 1 would suggest that an application of sodium silicate alone or in combination with other salts (sodium bicarbonate, potassium phosphate), immediately after infection, or more practically, immediately after appearance of first symptoms (following regular crop monitoring) could be effective for disease control on a short duration crop. Later sprays may be beneficial under high disease pressure. In situations where there has been an outbreak of powdery mildew (high risk conditions for a disease epidemic), a protectant application to plants on neighbouring benches could potentially be beneficial. Even if disease control is not complete, salt application could at least prevent development of an epidemic within a glasshouse compartment.

For parsley, it was also found that a spray of water alone (after infection) can provide moderate levels of disease control, particularly when repeated sprays are used. This could provide growers with a simple management tool for powdery mildew control, particularly in the current absence of registered alternatives. Growers would need to be aware, however, that water sprays will increase relative humidity and extend leaf wetness duration, thus providing conducive environmental conditions for other pathogens such as grey mould (*Botrytis cinerea*).

From the courgette glasshouse screens, it was found that a mixture of sodium silicate and ammonium dihydrogen phosphate was optimal, and that multiple sprays repeated within the latent period were most effective. From the field experiments, it was found that the optimal approach devised from the screens was unable to provide mildew control as good as that obtained from approved fungicides.

Future work

Use of sodium silicate alone or in salt mixtures applied immediately after first symptom development on protected parsley, warrants evaluation for powdery mildew control on commercial protected nurseries. Due to the short-term nature of the crop and high risk of residues, there is limited scope for integration within fungicide programmes. For field crops, however, there is more scope for evaluation of salts in combination with, or alternating with fungicides in managed programmes. For example, potassium bicarbonate is now used widely as an eradicant fungicide on soft fruit crops, alternating with other fungicides; it is also used in mixtures with fungicides such as myclobutanil on herbaceous and woody ornamentals for powdery mildew control. While not as effective as conventional fungicides, advantages are that it can be integrated with fungicides to reduce resistance risk and can be used close to harvest. Use of potassium bicarbonate on strawberries can help to maintain green leaf area (through powdery mildew control) and also to reduce the risk of disease spread to fruit.

Under field conditions, it is possible that further work to improve the formulation and rainfastness of salt solutions may lead to better powdery mildew control; little work has been done on this topic to date. In this project, inorganic salts were used together with the surfactant Tween 20, since previous work has shown that inorganic salts tend to be more effective for disease control when combined with a wetter. In the case of bicarbonates this is possibly due to the better coverage provided in the presence of an adjuvant, while for silicates and phosphates the adjuvant may help to increase salt uptake by the plant. Further work is needed to evaluate whether salt efficacy, persistence and phytotoxicity varies with different types of commercial wetters, although evidence with potassium bicarbonate suggests that a wide range of adjuvants could be appropriate.

Although Deliopoulos *et al.* (2010) listed the reported spectrum of activity of groups of salts against differerent pathogens, there is a need to define this activity more fully taking account of key pathogens on horticultural crop hosts. For example, the reported efficacy of phosphates and silicates against specific leaf spot diseases (anthracnose on cucumber and bean caused by *Colletotrichum* species) (Gottstein & Kuć, 1989; Moraes *et al*., 2009), could broaden the potential use of certain salts.

Objective 3. Evaluate selected novel products for their effects on invertebrate BCAs in protected parsley, using laboratory bioassays and crop experiments.

Status: Complete

Introduction

Biological pest control within Integrated Pest Management (IPM) programmes is increasingly used by growers of protected edible crops. Certain conventional fungicides used widely for powdery mildew can have a deleterious effect on biological control. For example, the use of sulphur burners reduces populations of *Aphidoletes aphidimyza* which are important for the control of key aphid pests on both strawberry and parsley, and is also harmful to *Encarsia* *formosa* and *Phytoseilus persimilis*, used for the control of whitefly and spider mites respectively on both cucumber and strawberry. For any alternative products that show promise against powdery mildew, further information is required on their compatibility with biological pest control. For example, research has shown that extract of *Reynoutria sachalinensis* (used in the product Milsana®) is not harmful to the parasitoid of lepidopteran eggs *Trichogramma cacoeciae* (Hafez *et al*. 1999), indicating potential for use of this product in IPM systems.

Side-effects testing

Replicated laboratory bioassays were used to test mortality effects of selected products (effective against powdery mildew) on two biological control agents used extensively on protected edible crops. These bioassays were done using worst case (Tier 1) scenarios where the biological control agents came into direct contact with the product. Worst case scenarios were achieved by dipping the biological control agents in the test solution or exposing them to direct sprays or to treated leaves. The species of biological control agent tested were *Neoseiulus* (*Amblyseius*) *cucumeris* and *Aphidius colemani*. *Neoseiulus cucumeris* is an oval, straw-coloured predatory mite used to control thrips, including western flower thrips (*Frankliniella occidentalis*) in protected edible crops including herbs and cucumber. *Aphidius colemani* is a small parasitic wasp (parasitoid) that lays a single egg inside an aphid host. The developing parasitoid larva kills the aphid. Once dead the aphid turns into a pale brown ‘mummy’ inside which the parasitoid pupates. After completing its development inside the aphid the adult parasitoid emerges by cutting a small exit hole in the top of the ‘mummy’. *Aphidius colemani* is used to control peach-potato aphid (*Myzus persicae*) and *Aphis gossypii*) in protected herbs and cucumber respectively. For both biological control agents the most vulnerable life-stage was tested i.e the immature stages of *N. cucumeris* and the adult stage of *A. colemani*. *Aphidius colemani* pupae inside the aphid ‘mummies’ were also tested in order to provide a less vulnerable life stage for comparison with the adult stage.

Methods

Each biological control agent was tested against six inorganic salts and a product (MOI-106020) based on a plant extract (Table 8), selected from the results of screening experiments in Objective 1. Decis (deltamethrin) is known to be harmful to a wide range of biological control agents including the two species tested and thus was included as a standard in each of the bioassays completed. Water was used as the control.

Bioassays were completed in a controlled temperature room at ADAS Boxworth. Each bioassay used a randomised complete block design and results were analysed using generalised linear models (GLM) within GenStat.

Table 8. Treatments tested against each biological control agent

Treatment no.	Product	Product rate
1	Water control	-
2	Potassium bicarbonate	10 g/L
3	Sodium silicate	10 g/L
4	Sodium bicarbonate	10 g/L
5	Potassium phosphate	10 g/L
6	Ammonium dihydrogen phosphate	10 g/L
7	Potassium dihydrogen phosphate	10 g/L
8	Plant extract (MOI-106020)	2.5 ml/L
9	Decis (deltamethrin)	500 mL/600 L (based on Specific Off-Label Approval (SOLA) 2007/1691 for protected herb crops)

Aphidius colemani

Aphidius colemani pupae were provided by BCP Certis (Ashford, UK) as mummified aphids. These mummified aphids were divided into two groups with one group being placed onto a Petri dish lid in a ventilated Perspex cage in a controlled temperature room (21°C, 16 hours light: 8 hours dark) and reared through to provide adult parasitoids. These aphid mummies were checked regularly in order to record when adult parasitoids had begun to emerge. A piece of cotton wool soaked in a 1:1 solution of honey and water was provided as a food source for the emerged adults. The remaining aphid mummies were used in the side effects experiment before the adult parasitoids had emerged.

Aphidius colemani pupae experiment – the aphid mummies were carefully divided into groups of five and placed onto tissue paper. Approximately 5 ml of one of the test solutions was decanted into a specimen tube, which in turn was screwed into the head of a standard garden mister. Taking one of the groups of five aphid mummies the modified garden mister was used to thoroughly wet the mummies. The tissue paper served to drain away any excess liquid. The aphid mummies were then transferred carefully to a ventilated plastic Petri dish (90 mm diameter), which was lined in the base with a piece of filter paper. The Petri dish was then placed in a controlled temperature room (21°C, 16 hours light: 8 hours dark) and checked regularly for adult emergence. The number of adult parasitoids emerging was recorded. The experiment was ended when no more adults were recorded to have emerged for successive days. Each treatment was replicated in this way a total of eight times.

Aphidius colemani adult experiment – the method used was based on that described by Longley & Jepson (1997) for *Aphidius rhopalosiphi*. Treatments were applied to a broad bean leaf (*Vicia faba* cv. Sutton Dwarf) placed onto tissue paper and sprayed using the modified garden mister. The upper and lower surfaces of the broad bean leaf were sprayed to run-off with the tissue paper soaking up excess liquid. Once the excess liquid had drained away the treated leaf was placed into the centre of a ventilated plastic Petri dish (90 mm diameter). A small piece of cotton wool soaked in a 1:1 solution of honey and water was also placed in the Petri dish, away from the treated broad bean leaf, to provide a food source for the parasitoids. Ten adult wasps (a mixture of female and male parasitoids <48 hours old) were collected from the Perspex cage using a 'pooter' (aspirator). The group of ten wasps was transferred to the Petri dish containing the treated broad bean leaf. The Petri dish was then placed in a controlled temperature room (21°C, 16 hours light: 8 hours dark) and mortality checked after 24 hours. In addition, the sex of dead or live parasitoids was recorded. Each treatment was replicated in this way a total of four times.

Neoseiulus cucumeris
Petri dishes with tight fitting lids were prepared for use in this bioassay by first sticking a small piece of blue-tack in the centre of the base. Next a 16 mm cork borer was used to cut discs from strawberry (*Fragaria* x *ananassa* cv. Elsanta) leaves. The strawberry plants selected for this purpose had not received any insecticide or acaricide applications prior to this experiment. A pin was pushed through the centre of each cut leaf disc before pushing the point of the pin into the blue-tack. In this way each leaf disc was held approximately 1 cm above the bottom of the Petri dish (Figure 9). A granule of freeze dried pollen was then carefully placed onto each leaf disc. Finally water was added to the Petri dish to a depth of approximately 0.5 cm so that the leaf disc was held 0.5 cm above the surface of the water.

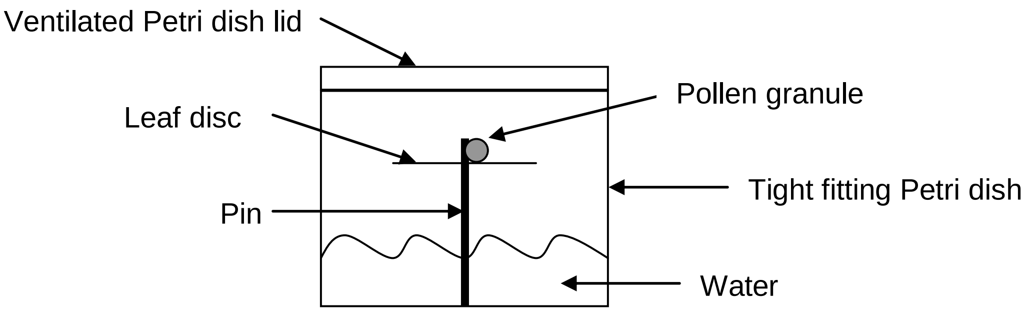


Figure 9. Preparation of Petri dishes used to test mortality of *Neoseiulus cucumeris* dipped in novel products used for control of powdery mildew

The method used to expose *Neoseiulus cucumeris* to the treatments was a simplified version of the method described by Dennehy *et al*. (1993) and described by Steiner (pers. comm.) which was used to immerse *Phytoseiulus persimilis* protonymphs in small volumes of a test solution.

Neoseiulus cucumeris were provided by BCP Certis (Ashford, UK) in the form of mixed age individuals supplied in a bran carrier together with a prey mite, which acted as a food source. Having first gently rolled the container in which the *N. cucumeris* were delivered to ensure an even distribution of mites, a teaspoon of bran and mites was taken from the container and tipped into a coarse sieve. The bran and mites were carefully sieved so that the mites and only fine pieces of bran fell through onto a white tray beneath. Tapping the side of the tray brought the bran and mites together and this was then tipped into a specimen tube containing 5 ml of one of the treatments. The specimen tube was gently swirled for five seconds before tipping the contents onto tissue paper. The tissue paper rapidly absorbed the liquid and so avoided the risk of drowning the mites. Next the tissue paper was placed under a binocular microscope to allow five immature *N. cucumeris* mites to be carefully transferred to one of the leaf discs using a fine paintbrush. The lid of the Petri dish, which had a hole in the middle that was covered with fine gauze to allow ventilation, was then replaced. The Petri dish was then placed in a controlled temperature room (21°C, 16 hours light: 8 hours dark) and mortality checked after 24 hours. Each treatment was replicated in this way up to 10 times and a minimum of five times.

Results
Aphidius colemani
Pupae experiment – results from this bioassay showed high levels of adult emergence for all treatments and both the water and Decis controls (Table 9). Overall there was no significant treatment effect ($F = 1.57$, $P = 0.155$) following analysis by generalised linear models (GLM).

Table 9. Predicted proportions (%) of *Aphidius colemani* successfully emerging from aphid mummies sprayed with by each treatment

Treatment	Proportion (%) of adults emerging	Standard error	Confidence limit
Water control	80.0	7.47	14.94
Potassium bicarbonate	62.5	8.99	17.98
Sodium silicate	67.5	8.71	17.42
Sodium bicarbonate	85.0	6.68	13.36
Potassium phosphate	72.5	8.32	16.63
Ammonium dihydrogen phosphate	82.5	7.10	14.20
Potassium dihydrogen phosphate	70.0	8.53	17.06
Plant extract (MOI-106020)	90.0	5.62	11.24
Decis	87.5	6.19	12.38

Adult experiment – results from this experiment showed levels of mortality within the range of 0 to 17.5% for the novel powdery mildew control products tested (Table 10 & Figure 10). For the controls, no mortality was recorded for adult parasitoids exposed to water but when exposed to the Decis mortality was 95%. The analysis by GLM identified a highly significant treatment effect ($F = 34.49$, $P < 0.001$). Individual comparisons between the treatments were completed using the predicted means established through the GLM analysis and the confidence intervals calculated from the standard errors for each treatment mean. From individual comparisons between treatments, the mortality of parasitoids exposed to the water control was significantly lower than for sodium silicate, potassium phosphate, the plant extract product (MOI-106020) and the Decis control. All of the treatments tested had significantly lower levels of mortality than the Decis control. Numbers of female and male *A. colemani* were not controlled for when this bioassay was set-up but numbers were recorded at the end of the experiment. Females accounted for 76% of the total number of individuals tested while males accounted for 24% of the total. Mortality was similar for females (16%) and for males (17%) across all treatments. When the Decis treatment was excluded, mortality was also similar for females (5%) and males (9%).

Table 10. Predicted proportions (%) of total number of *Aphidius colemani* adults killed after exposure to each treatment

Treatment	Proportion (%) dead	Standard error	Confidence limit
Water control	0.0	0.00	0.00
Potassium bicarbonate	2.5	1.99	3.99
Sodium silicate	12.5	4.14	8.28
Sodium bicarbonate	2.5	1.99	3.99
Potassium phosphate	12.5	4.14	8.28
Ammonium dihydrogen phosphate	5.0	2.77	5.54
Potassium dihydrogen phosphate	0.0	0.00	0.00
Plant extract (MOI-106020)	17.5	4.71	9.41
Decis	95.0	2.68	5.36

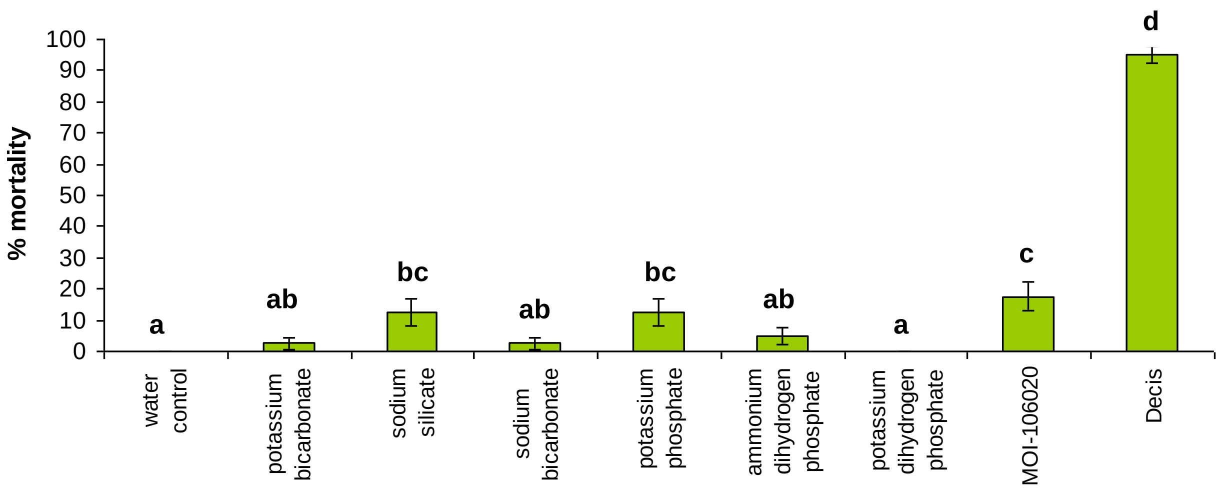


Figure 10. Predicted proportions (%) of total number of *Aphidius colemani* adults killed after exposure to each treatment (different letters indicate significant differences between treatments)

Neoseiulus cucumeris
Results from this bioassay showed levels of mortality of 5% or less for all treatments and controls tested with the exception of the Decis treatment. All immature mites dipped in the Decis solution died almost immediately and so this treatment was excluded from the analysis. Results for the seven treatments and the water control were analysed by GLM. This analysis identified a highly significant treatment effect ($F = 4.51$, $P < 0.001$). Individual comparisons between the treatments were completed using predicted means and confidence intervals calculated from the standard errors for each treatment mean (Table 11 & Figure 11)). Individual comparisons between treatments completed in this way confirmed that potassium bicarbonate, sodium silicate, sodium bicarbonate and potassium phosphate did not differ significantly from the water control. Where significant differences did occur, for ammonium dihydrogen phosphate, potassium dihydrogen phosphate and the plant extract (MOI-106020), the proportion dead was actually lower than for the water control.

Table 11. Predicted proportions (%) of *Neoseiulus cucumeris* killed by each treatment

Treatment	Proportion (%) dead	Standard error	Confidence limit
Water control	4.6	1.52	3.04
Potassium bicarbonate	2.6	1.26	2.52
Sodium silicate	2.3	1.10	2.20
Sodium bicarbonate	4.1	1.37	2.74
Potassium phosphate	3.5	1.72	3.44
Ammonium dihydrogen phosphate	0.0	0.00	0.01
Potassium dihydrogen phosphate	0.0	0.00	0.01
Plant extract (MOI-106020)	0.0	0.00	0.01

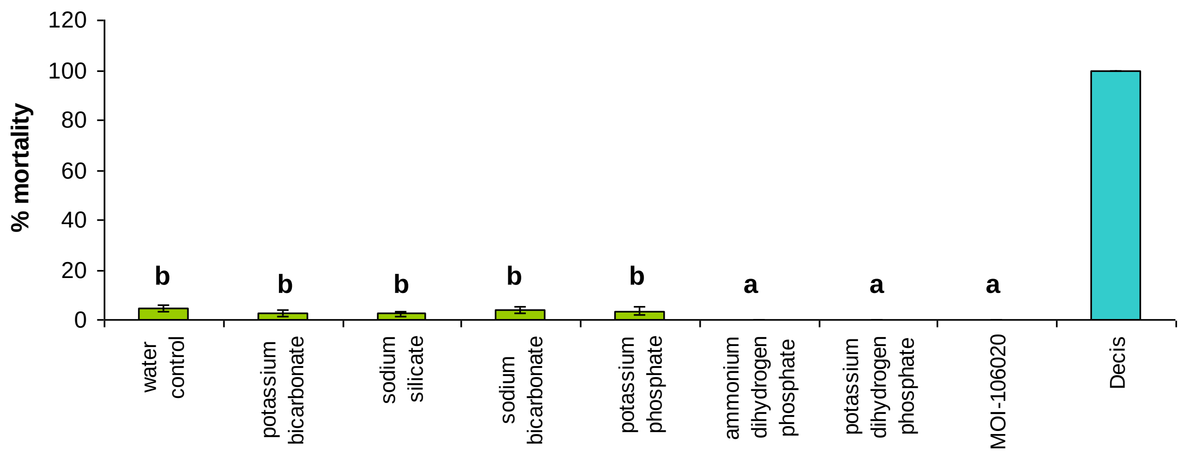


Figure 11. Predicted proportions (%) of *Neoseiulus cucumeris* killed by each treatment (different letters indicate significant differences between treatments, the Decis treatment was excluded from the analysis)

Discussion
The proportion of *Aphidius colemani* adults emerging from aphid mummies sprayed with each novel powdery mildew control treatment or one of the controls, including Decis (deltamethrin) was between 62.5 and 90%. The proportion of adult parasitoids emerging was not significantly affected by treatment application. In a similar study by Longley & Jepson (1997), emergence of *Aphidius rhopalosiphi* adults was reduced to 70% by applying deltamethrin at field rate but was similar to the control (91%) when deltamethrin was applied at half rate. By comparison with the present study, Longley & Jepson used a slightly higher concentration of deltamethrin and so the results would appear to be comparable with the present study. Both studies clearly indicate that the aphid ‘mummy’ protects the parasitoid within from applications of contact-acting insecticides such as pyrethroids. However, the degree of protection afforded by the aphid ‘mummy’ is likely to vary depending upon the properties of the insecticide.

Adult parasitoids are considered to be the most vulnerable to pesticide applications (Stary, 1970). This is supported by the current study in which 95% of adult *A. colemani* died following exposure to a broad bean leaf treated with Decis. However, only exposure to sodium silicate, potassium phosphate and the plant extract (MOI-106020) significantly increased adult mortality compared with the water control. Despite recording a significant increase in mortality with these treatments, the levels of mortality recorded remained low at only 2.5 to 12.5%.

Neoseiulus cucumeris bioassay results were similar to those for the adult parasitoids. Mortality of the immature mites ranged between 0 and 5% for the novel powdery mildew treatments tested and were comparable with the water control. The mortality of immature *N. cucumeris* dipped in potassium phosphate was 3.5%. This level of mortality is far lower than that recorded by Pertot *et al*. (2008) who recorded 32% mortality when the predatory mite *Amblyseius andersoni* was exposed to a product containing potassium monophosphate and sulphur. However, it seems likely that the presence of sulphur in the product tested by Pertot *et al*. was responsible for the higher mortality recorded. Sulphur as a foliar spray is classified as 'slightly toxic' (killing 25-50%) to *N. cucumeris and Amblyseius* species (Biobest 2011). The Decis treatment was again found to be harmful, killing all mites exposed to this control within a few minutes.

Potassium salts of fatty acids e.g. Savona are used as a contact insecticide. Savona is 'harmful' (kills over 75%) to adult and larval/nymphal stages of all biological control agents which it has been tested against (Koppert, 2011). However, once the spray residue is dry it is safe and so this product can be used in IPM programmes if timed carefully. The results presented here for adult parasitoids and immature predatory mites indicate that the potassium salts on their own are non-toxic even when dipped in the test solution or exposed to residues before they were dry. The plant extract (MOI-106020) was similarly confirmed as non-toxic to immature *N. cucumeris* as well as to adult and pupal *A. colemani*. These results support earlier studies, which showed a giant knotweed extract to be harmless to beneficial insects, including *Trichogramma cacoeciae* (Hafez *et al*., 1999) and insects and mites, including *Typhlodromus* and *Aphidius* species (Schuld *et al*., 2002).

Conclusions

Table 12 summarises the IOBC classification of plant protection products for their side-effects on beneficial arthropods. Based on this classification the inorganic salts and the plant extract (MOI-106020) would be classed as 'non-toxic' in terms of side-effects against *Neoseiulus cucumeris* and against *A. colemani* adults. Potassium bicarbonate, sodium silicate and potassium phosphate would be classed as 'slightly toxic' to *Aphidius colemani* pupae inside mummies, with the remaining inorganic salts and the plant extract being classed as 'non-toxic'. As such these potential alternative controls for powdery mildew would be compatible with integrated pest management programmes used on protected herbs and cucumber.

Table 12. Classification system for side-effects of plant protection products to beneficial arthropods (http://www.biobest.be/neveneffecten/3/none/)

Classification		% Effect observed
Class 1	Non-toxic	<25%
Class 2	Slightly toxic	25-50%
Class 3	Moderately toxic	50-75%
Class 4	Toxic	>75%

Future work

The results presented here are based on worst case scenario (Tier 1) tests. As each product tested, with the exception of Decis, was found to be non-toxic or only slightly toxic to *Neoseiulus cucumeris* and *Aphidius colemani* it is not considered necessary to test the salts in further semi-field (Tier 2) or field (Tier 3) i.e. crop experiments. However, growers may use a wide range of additional beneficial arthropods such as *Encarsia formosa* (whitefly parasitoid), *Phytoseiulus persimilis* (spider mite predator), *Hypoaspis miles* (sciarid predator) and *Anagrus atomus* (leafhopper parasitoid), and so it will important to extent this side-effect testing to include these and other species.

Objective 4. Develop appropriate linkages with key industry players in order to identify potential pathways for approval of effective products and future uptake by UK growers.

Status: Ongoing

Summary

The project participants have undertaken a range of knowledge transfer activities that have been used to communicate and discuss project findings and their implications with the scientific community, the horticultural industry (growers and consultants), the Horticultural Development Company (HDC), agrochemical manufacturers and distributors, and biological control companies. Completed technology transfer activities under this objective are listed below under 'Knowledge Transfer'.

The specific process of identifying pathways for product registration and future uptake has been difficult to achieve within the time-frame of this project. The reasons for this are two-fold. Firstly, because of the need to get detailed, quantitative data on the efficacy of inorganic salts products being screened, before the involvement of industry players. And secondly, because of changes to EU legislation which mean that the option for approval of compounds such as simple inorganic salts as fungicides via a commodity substance approval route is no longer available. Under

EU Regulation 1107/2009 (in force from 14 June 2011), any active ingredient to be used for crop protection requires approval at the EU level (previously referred to as Annex 1 listing) followed by product registration. We understand that compounds such as salts would likely be treated as 'basic substances' under the new regulation, possibly with potential to negotiate for reduced dossier requirements. However, there would seem little incentive for agrochemical companies to seek approvals for 'basic substances' because of the difficulty of offsetting approval and registration costs through product sales to a limited market. Similarly, grower groups would potentially have difficulty in bringing a 'basic substance' for approval because of cost implications, unless perhaps coordinated at a European level using a Task Force; this approach has been used to get some active substances registered in the EU.

This project report concludes that inorganic salts have potential for use in control of powdery mildew diseases within the horticultural industry. Now that we have useful information on specific inorganic salts, we would like to hold a meeting to present project results, and to further discuss the opportunities and difficulties of registration of this type of compound under new EU regulations.

Summary and implications of the project findings

A previous Defra review (PS2117) collated a wide range of literature reporting that control of plant disease, in particular powdery mildew, can be achieved through use of a range of simple inorganic salts. In this project, inorganic salts were evaluated for powdery mildew control with the aim of reducing grower reliance on conventional fungicides. The research effort was targeted at high disease risk crops (protected parsley and outdoor courgettes) where there are limited modes of action in fungicides, intractable disease problems, and potential residue implications.

From a series of glasshouse bioassays, it was concluded that sodium silicate at 10 g/L with a wetter (25% Tween 20) was consistently effective for control of powdery mildew on parsley and courgette, with minimal phytotoxic or spray residue effects. Higher doses increased phytotoxicity without improving disease control. Spray timing was most effective when the compound was applied curatively (post infection but prior to symptom development). Mixtures of sodium silicate with sodium bicarbonate and potassium phosphate (for parsley) and ammonium dihydrogen phosphate (for courgette) improved disease control compared with the single salt.

Practical use of sodium silicate alone or in mixtures for powdery mildew control on protected herbs and outdoor courgette was considered. Experiments on effective spray intervals for sodium silicate alone and in combination with sodium bicarbonate and potassium phosphate, suggested that for a short duration commercial crop of potted parsley, a single application of these compounds after infection or immediately after initial symptom development would likely provide sufficient disease control, although a later spray could provide further benefit under high disease pressure. Two timely applications of the mixture reduced mildew to trace levels (1% plant area affected) at 21 d after infection (equivalent to crop duration). It was also noted that repeated sprays of water alone (after an infection event) could provide moderate disease control, offering a simple management option for growers. From the courgette glasshouse screens, it was found that a mixture of sodium silicate and ammonium dihydrogen phosphate was optimal, and that multiple sprays repeated within the latent period were most effective. From subsequent field experiments, it was found that the optimal approach devised from the screens was unable to provide mildew control as good as that obtained from approved fungicides. Further work to improve the formulation and rainfastness of the salt solution may lead to better powdery mildew control under field conditions. In addition, the use of salts in programmes with conventional fungicides to reduce the total fungicide input and reduce risk of resistance development, warrants further investigation.

Growers are increasingly reliant on use of biological control for control of pests in protected cropping and therefore, novel fungicides need to be 'safe' for use with biological control agents. There was very limited information available prior to this project on the compatibility of inorganic salts with biological pest control. Studies in this project tested mortality effects of a range of inorganic salts and a coded plant extract on two biological control agents, *Aphidius colemani* and *Neoseiulus cucumeris*, which are used to control aphids and thrips on protected herb crops. The bioassays used worst case (Tier 1) scenarios where the biological control agent was either dipped or exposed to leaves sprayed with the fungicide. Based on the IOBC classification of plant protection products for their side-effects on beneficial arthropods, the inorganic salts and plant extract were ‘non-toxic’ against *N. cucumeris* and *A. colemani* adults. These potential alternative controls for powdery mildew would then be compatible with IPM programmes used on protected herbs.

The development and use of inorganic salts as fungicides for powdery mildew control on horticultural crops is currently limited in the UK for market and legislative reasons. In comparison with one commodity substance approval of an inorganic salt (potassium hydrogen carbonate) in the UK, there are at least 25 commercial products containing inorganic salts (bicarbonates and phosphates) approved as biopesticides by the Environmental Protection Agency in the USA. A meeting being planned in conjunction with the HortLINK SCEPTRE project (HL01109) later in 2011 will further discuss the opportunities and difficulties of registration of this type of compound under EU Regulation 1107/2009.

Knowledge transfer

Project start-up meeting
Held at ADAS Boxworth, Cambs, 27 January 2009 attended by Dr K. Green, Ms L. Kirkpatrick (ADAS) Dr T. Deliopoulos, Dr M.C. Hare, Dr P.S. Kettlewell (HAUC).

Project progress meetings
Held between ADAS (K. Green) and HAUC (T. Deliopoulos, P. Kettlewell and M. Hare) by meeting or conference call on three occasions (11 December 2009, 8 February 2010, 15 March 2010).

Scientific conferences
- Attendance by T. Deliopoulos and P.S. Kettlewell at the AAB conference on ‘Crop Protection in Southern Britain’, 10 February 2009, in Peterborough, UK. Each gave a platform presentation and published a paper in the Conference proceedings.
- Poster presentation by T. Deliopoulos at the 61st International Symposium on Crop Protection, 19 May 2009, in Ghent, Belgium: 'Utilisation of inorganic salts in fungal crop disease management in the UK'.
- 'Potential management of fungal potato diseases using inorganic salts': Paper presented by T. Deliopoulos as a platform presentation to ‘The Dundee Conference: Crop Protection in Northern Britain 2010’, West Park Conference Centre, Dundee, 24 February 2010, and published in the conference proceedings:
- 'Side-effects of inorganic salts on selected biological control agents': Results from Objective 3 were presented by Dr Tom Pope (ADAS) as an abstract and oral presentation at the AAB conference on Advances in Biological Control held on 17th November 2010 at Grantham. This meeting was attended by both researchers and IPM practitioners.

Industry meetings / visits
- A project outline was presented by Jude Bennison to members of the herb industry at a technical meeting of the British Herb Trade Association, National Herb Centre, Warwick, 17 March 2009.
- Kim Green met with Peter Glendinning (hops consultant) to discuss matters relating to novel products and commodity substance approvals (September 2009).
- A brief project overview was given by Thomas Deliopoulos to members of the British Herb Trade Association at a Technical Meeting held at HAUC, 7 October 2009. Kim Green also supplied a summary of results to consultant Claire Donkin, for presentation at this meeting.
- Growers from two commercial protected herb nurseries visited ADAS Boxworth, Cambs on three occasions to observe results from screens 1, 2 and 4 on parsley.
- A project summary was presented by Dr K. Green to the 'diseases' group of the SCEPTRE HortLINK project, 26 November 2011, ADAS Boxworth, Cambs.
- 'Optimising powdery mildew management on protected herbs': Oral presentation by Dr Kim Green (ADAS) to the British Herbs Trade Association (attended by growers, consultants, HDC, scientists, and representatives from agro-chemical and biocontrol manufacturers and distributors), 16 March 2011.

Publications
Deliopoulos T, Kettlewell PS, Hare MC. 2009a. Review of the use of inorganic salts as fungicides. *Aspects of Applied Biology* **91**, *Proceedings Crop Protection in Southern Britain*, pp. 23-26.

Deliopoulos T, Kettlewell PS, Hare MC. 2009b. Utilisation of inorganic salts in fungal crop disease management in the UK. *Communications in Agricultural and Applied Biological Sciences* **74**: 755-760.

Deliopoulos, T., Kettlewell, P. S. and Hare, M. C. (2010). Fungal disease suppression by inorganic salts: a review. *Crop Protection* **29**: 1059-1075.

Deliopoulos T, Kettlewell PS, Hare MC. 2010. Potential management of fungal potato diseases using inorganic salts. *Proceedings Crop Protection in Northern Britain 2010*: 237-240.

Pope, T., Maulden, K., Bennison, J. & Green, K. 2011. Side-effects testing of novel powdery mildew fungicides against biological control agents. Proceedings of the IOBC meeting on “Integrated Control in Protected Crops, Temperate Climate” (in press), Sutton Scotney, UK 19-22 Sep 2011.

Other
- An article: 'How salts can combat disease', 29 October 2009. Online: http://www.harper-adams.ac.uk/press/article.cfm?ID=3012&archive=true&year=2009
- A research video: 'Simple salts as disease control', 9 October 2009. Online: http://www.harper-adams.ac.uk/press/article.cfm?ID=3044

- Project Report on Landbased Library Online (OpenFields) at: http://www.openfields.org.uk/Library/content/GetDoc.axd?ctID=ZWVhNzBlY2QtZWJjNi00YWZiLWE1MTAtNWExOTFiMjJjOWU1&rID=MTIx&pID=MjI5&attchmnt=VHJ1ZQ==

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References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Publications:

Deliopoulos T, Kettlewell PS, Hare MC. 2009a. Review of the use of inorganic salts as fungicides. *Aspects of Applied Biology* **91**, *Proceedings Crop Protection in Southern Britain*, pp. 23-26.

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Other:

(Also see 'Technology Transfer' within the Project Report to Defra, Section 8.)

An article: 'How salts can combat disease', 29 October 2009.
Online: http://www.harper-adams.ac.uk/press/article.cfm?ID=3012&archive=true&year=2009

A research video: 'Simple salts as disease control', 9 October 2009.
Online: http://www.harper-adams.ac.uk/press/article.cfm?ID=3044

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