VG15010 Research Report

Damping off in spinach: Best bet fungicide and biologicals Trial 2016/2017

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VG 15010 A multi-faceted approach to soilborne disease management

'A multi-faceted approach to soilborne disease management' (Project VG15010) *is a three-year project (2015-2018) providing Australian vegetable growers with the tools and resources they need to manage the risk of crop losses due to soil-borne diseases.*

VG15010 delivers new information and resources about soilborne diseases to the vegetable industry through the established Soil Wealth and Integrated Crop Protection framework.

This project is a strategic levy investment under the Hort Innovation Vegetable Fund.

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Summary

A preliminary field trial was conducted to evaluate the efficacy of chemical and biological control treatments for damping off pathogens in spinach. We demonstrated that three fungicide treatments significantly reduced the area of diseased plants, however, they did not significantly increase spinach yield compared to untreated controls. This is most likely due to other variables affecting plant growth in the trial area.

Soil baiting and bioassays from the trial site taken prior to the experiment revealed two *Pythium* species: *P. irregulare* and *P. ultimum* var. *ultimum*, both of which are known to cause damping off diseases of a range of crop hosts including spinach. These results were supported by molecular tests of soil samples taken at sowing and at harvest. Consultation with crop protection companies (Syngenta and Bayer) assisted in the final choice of products with efficacy against *Pythium* spp. Suggested fungicide treatments were propamocarb + fosetyl-Al (Bayer; registered for vegetable seedlings in Europe) and metalaxyl-M + azoxystrobin (Syngenta; registered for seedling *Rhizoctonia* and *Pythium* control on cereals in Australia and similar formulations for vegetable crops in the USA).

A microbial biocontrol product containing a strain of the bacterium *Bacillus subtilis* was chosen as a stand-alone treatment and in combination with aforementioned fungicides. Although the chemical interventions in this trial were designed primarily for *Pythium* disease control, both chemical treatments contained actives that should suppress *Rhizoctonia* spp. (namely, fosetyl-Al and azoxystrobin).

During crop assessments, *Rhizoctonia solani* was detected causing spinach damping off and collar rot in the trial. The pathogen was confirmed by DNA analysis of soil samples taken at sowing and harvest. Two sub-species relevant to vegetable crops were determined from these soil molecular assays: *R. solani* AG2-1 and AG2-2 which can both affect a wide range of crops. AG2-2 is known to cause damping off of spinach.

Introduction

Baby-leaf spinach is an increasingly important crop nationally. Damping off is a major issue in spinach and other crops across all regions of Australia. Apart from crop losses it can cause problems with postharvest crop quality and shelf life as leaves from harvested diseased plants are physiologically stressed. Previous diagnostic pathology studies conducted by Dr Tesoriero have determined that spinach damping off, wilt, collar and root rots are caused by any one or a combination of several soil-borne pathogens (Table 1). The suite of pathogens can change with geography, cropping history, soil type and season. Therefore, any preventative chemical or biological treatments must have a wide spectrum of activity to protect seedlings from any or all of these pathogens.

Table 1. Key plant pathogens associated with spinach damping off, wilt, root and collar rot in Australia

Pathogen	Critical comment
Pythium aphanidermatum	A warm temperature and very aggressive pathogen that can grow at 40°C.
Pythium ultimum var. *ultimum*	A cooler to moderate temperature pathogen that can cause seed decay and damping off.
Pythium irregulare	A cooler to moderate temperature pathogen that can cause seed decay and damping off.
Phytophthora cryptogea *Phytophthora drechsleri*	Both cause damping off but not seed decay.
Rhizoctonia solani	This is a fungus which is favoured by a warm and wet soil surface; there are several different strains.
Fusarium oxysporum f.sp. *spinaciae*	This strain causes a vascular wilt disease and to date is only currently confirmed in Victoria.

The objectives of the study were:

- to assess biological and chemical products for the control/suppression of damping off in baby-leaf spinach,
- to assess the incidence and severity of damping off in spinach crops grown at Richmond, Tasmania in summer 2016-17 and quantify the impact on yield, and
- to identify pathogens associated with disease symptoms which will help to inform future research and control measures.

The Trial

The trial was established at Harvest Farms at Richmond, Tasmania.

Trial design was a Latin square with six treatments and six replicates. Each plot was a 10 m length of bed.

Trial treatments were:

1. Control (6 L water/treatment unit)
2. Previcur® @ 2.6 L/ha + Aliette® @ 1.2 kg/ha
3. Serenade Prime® @ 7 L/ha
4. Uniform® (Metalaxyl-M 124 g/L + azoxystrobin 322 g/L) @ 400 mL/ha
5. Uniform® + Serenade Prime®@ 7 L/ha
6. Previcur® @ 2.6 L/ha + Aliette® @ 1.2 kg/ha + Serenade Prime®@ 7 L/ha.

Information about each product:

- Propamocarb (Previcur® is registered in Australia for control of *Pythium* in ornamental plants).
- *Bacillus subtilis* (QST 713 strain as Serenade Prime®) can both stimulate plant growth and suppress plant pathogens.
- Fosetyl-Aluminium (Aliette®) is registered for control of *Pythium* and *Phytophthora spp*. in perennial tree crops and ornamental crops. It is equivalent to phosphonic (=phosphorous) acid which also initiates a broad-spectrum plant defence response.
- Metalaxyl-M 124 g/L + azoxystrobin (Uniform®) is registered for suppression of *Rhizoctonia* and control of *Pythium* in wheat and barley crops. Metalaxyl also has Australian permits for *Pythium* control on various vegetables while axoxystrobin has wider efficacy to a range of soil and foliar pathogens.

The spinach was sown on 19th December 2016. The seed variety was 2157. Seed was dressed with Thiram and the effective sowing rate was 1,795 seeds / linear metre (1,600 seed / linear metre x 0.9 field factor[1]). The width of the sowing bed was approximately 1.5 m.

Trial treatments were applied as a soil drench using watering cans after sowing on the same day.

Paddock history was as follows:

- Winter 2016 - a 'sparse' rye corn cover crop (<10-15 plants / m^2). This was established by broadcasting and harrowing. It was established late due to autumn

[1] 'Field Factor' is a correction based on expected losses in the field for the species.

cropping and wet weather.

- The cover crop was killed with glyphosate, previous beds were deep ripped, ground was spring-tine harrowed to level the beds, new beds were formed with a stone burying bed-former to invert trash and clods. (Note that beds were reformed in new positions as part of the installation of a new solid set irrigation system.)
- October 2016 – a spinach crop was grown.
- 19th December 2016 – spinach crop sown (this trial).
- 19th January 2017 – spinach harvested using a commercial harvester (this trial).

Note that in addition to baby-leaf spinach, lettuce and brassicas are also grown at this farm in rotation with winter cover crops.

Sampling and data collection comprised:

- Soil samples taken on 19th December (sowing date) for:
  - standard pathology assessment
  - DNA assays (linkage to Hort Innovation project VG15009 test conducted by The South Australian Research and Development Institute [SARDI])
- Weekly observations
- Plant samples assessed by Dr Len Tesoriero to identify pathogen presence
- NDVI image of trial site 12th January 2017
- At harvest, 19th January 2017:
  - Assessment of area of disease patches (bare patches). A disease patch was defined as a 10 cm circular bare patch of ground. The number of disease patches in each plot was counted (2 assessors) and recorded.
  - Harvested weight per plot (a 9 m length of bed was harvested from each plot)
  - DNA assays from soil samples from 12 out of 36 plots.

Results

Soil Pythium *and* Rhizoctonia solani *levels at sowing*

At sowing, *Pythium* clade I and *Rhizoctonia solani* were detected in soil samples.

Soil samples taken prior to and at sowing were used in direct baiting and bioassays for pathogenic fungi. The two species of *Pythium*: *P. ultimum* var. *ultimum* (Figure 1) and *P. irregulare* were detected and caused root rot symptoms on spinach plants (Figures 2 & 3).

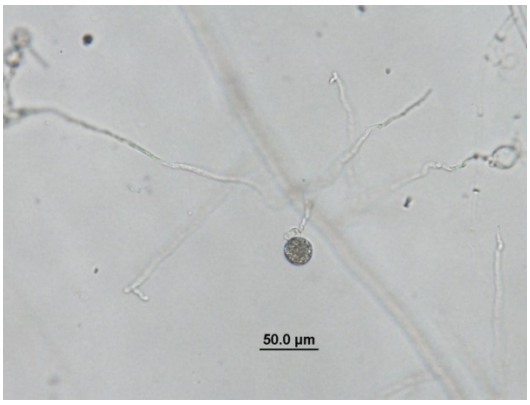

Figure 1. Photomicrograph of *P. ultimum* var. *ultimum* forming sexual structures in culture

Figure 2. Spinach roots from soil bioassay with root rot symptoms caused by *P. ultimum*

Figure 3. Spinach plants affected by *P. ultimum* in soil bioassay

DNA-based tests at sowing

One soil sample was taken from across the trial site for a DNA-based soil test. This test is still under development for vegetable crops and therefore data should be interpreted with care. Work to date has focussed on developing the test for carrots and brassicas rather than baby-leaf crops. So not all species relevant for lettuce or baby-leaf crops are included in the suite of pathogens tested.

Rhizoctonia solani AG2-1 and AG2-2 (Figure 4) and *Pythium* clade F and clade I (Figure 5) were detected at the sowing date tests. This shows that there were high levels of *Pythium* clade I. This test does tell us which *Pythium* species were present and if they are pathogenic but given the morphological taxonomy and bioassays we can assume that the Clade F detection includes *P. irregulare* and the Clade I includes *P. ultimum* of which some were shown to be pathogenic to spinach. Interestingly, other DNA tests from Tasmanian soils have also detected *Pythium* clade I (Michael Rettke, PIRSA, pers. comm.).

Of the other species tested for, the following were detected:

- *Plasmodiophora brassicae* was detected at low levels, 1,228 Copies/g.
- *Macrophomina phaseolina* was detected at low levels, 1,323 Copies/g.

See Appendix 1 for those tested for but not detected.

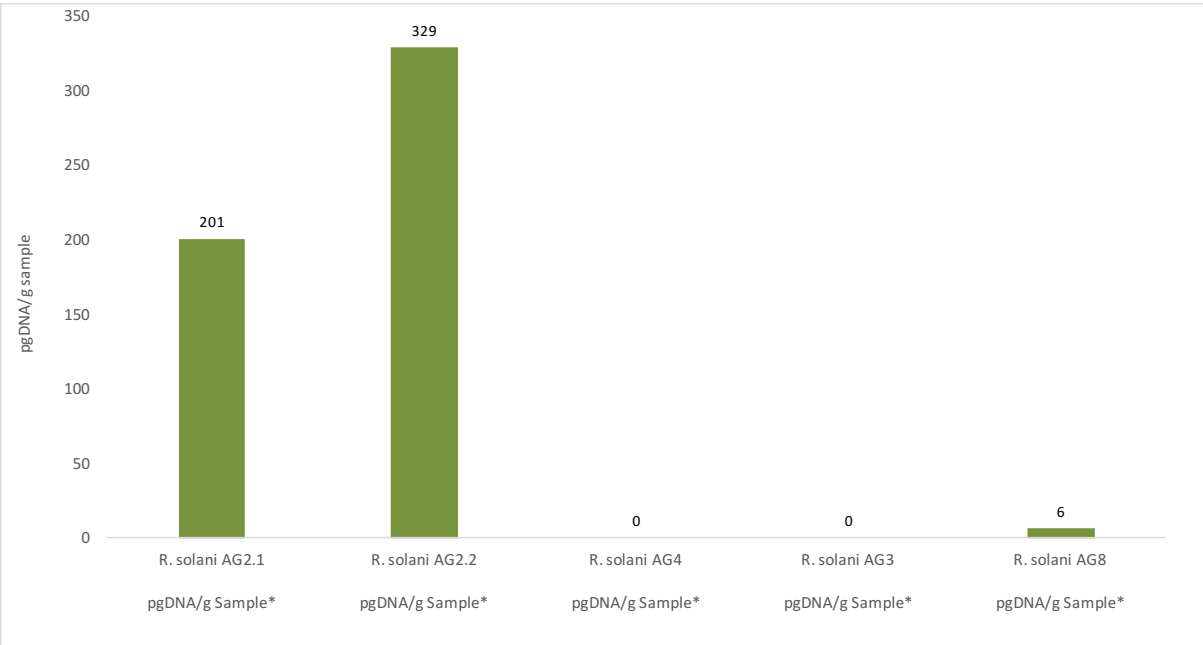

Figure 4. *Rhizoctonia solani* AG2-1 and AG2-2 detected at sowing date

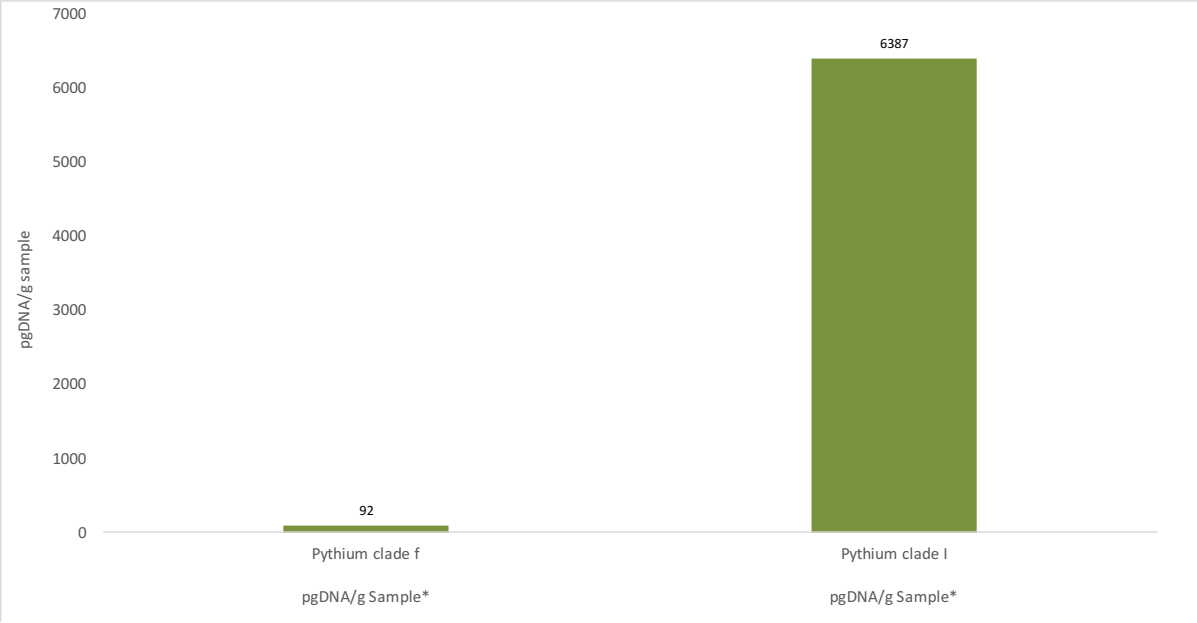

Figure 5. *Pythium* species detected at sowing date (data shown as pgDNA/g sample).

Field observations and identification of plant pathogens

Diseased plants were observed which showed typical symptoms of damping off. At early stages of plant development, individual plants were found to be wilting, dying or dead (Figure 6, 17 days after sowing). Then, as the crop developed, bare patches became evident, which is typical of damping off caused by *Rhizoctonia* spp. (Figure 7, 24 days after sowing). *R. solani* and *P. ultimum* were isolated when affected plants were sampled and plated.

Figure 6. Plants wilting and roots rotting (typical damping off symptoms) (17days after sowing).

Figure 7. Bare patches where plants have died (24 days after sowing).

Some plants were observed with symptoms that looked slightly different to typical damping off disease. Plants were stunted and yellowing but roots did not exhibit typical damping off rotting (Figure 8). *Colletotrichum* was isolated from leaves of these plants. Therefore, it was most likely anthracnose disease that caused the symptoms. These were observed in two or three small patches, but *Colletotrichum* may have also occurred in other areas within the trial that were not examined or tested in detail.

Figure 8. Plants wilting, yellowing and dying (17 days after sowing).

Disease patches

There was a significant treatment effect on the number of diseased (bare) patches. There were fewer bare patches in treatments 4, 5 and 6 compared to the control (Figure 9).

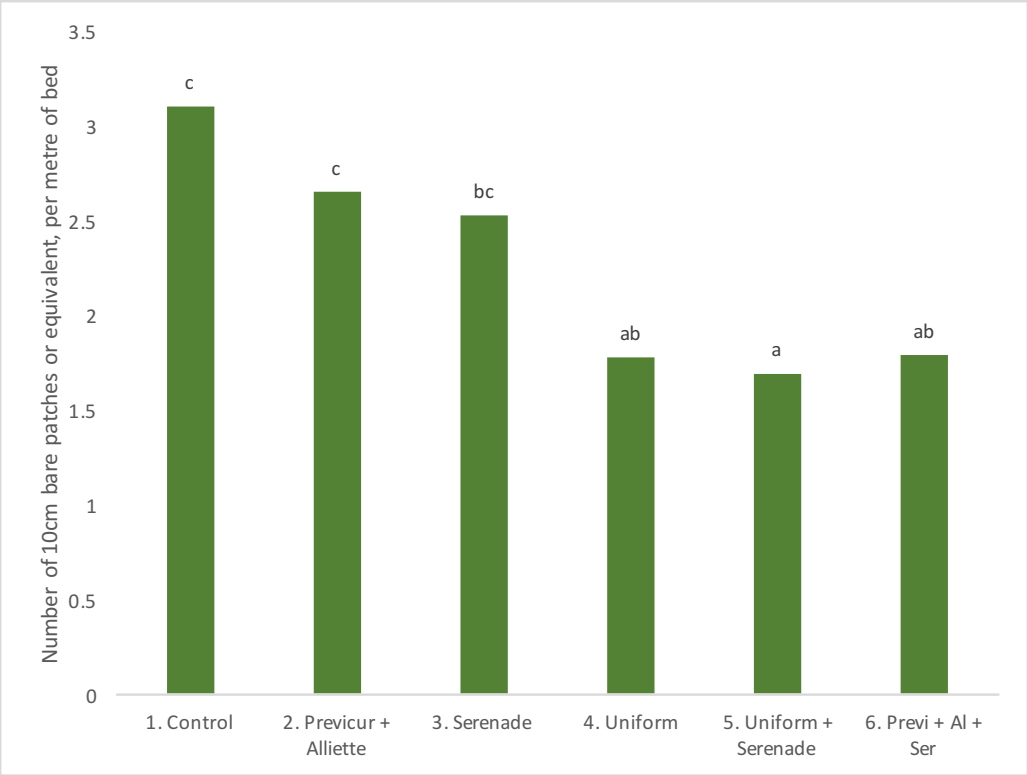

Figure 9. Number of 10 cm disease diameter patches per metre of bed (bars with different letters are significant at P=0.05).

Crop Yield

Yield and disease occurrence were very variable across the site. This may be due to variability in: soil structure (e.g. soil depth, compaction and drainage), irrigation coverage/uniformity and background variation in soil biology and pathogens present.

This variability is highlighted in the NDVI image (Figure 10).

There were no significant treatment effects on spinach yields. The average yield across all treatments was 2.5 kg per metre of bed. Data for each plot ranged from 1.6 to 3.9 kg per metre of bed.

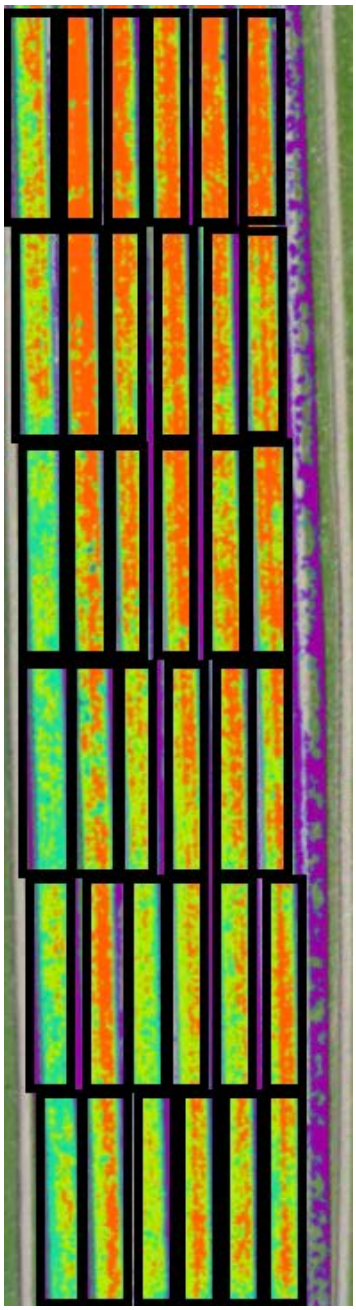

Figure 10. NDVI image and photograph (12th January 2017). In the NDVI image, purple indicates bare soil, red indicates denser growth, green indicates less dense growth.

Soil pathogen DNA levels at harvest

At harvest, 12 soil samples were collected for DNA-based testing; one sample for each of plots 1 to 6 and 31 to 36. These 12 plots were from the two outer beds of the trial which represented the areas that appeared to be more severely affected by disease. Data for *R. solani* and *Pythium* are shown in Table 2 (refer to Appendix 1 for full data set). The data cannot be compared directly to results from the 19th December (sowing date) because of different sampling strategies. However, it appears that inoculum levels may have dropped. There is no apparent or obvious difference between treatments, but it may be difficult to detect any treatment differences with a small number of tests (i.e. two plots for each treatment).

Table 2. DNA-based tests for *Rhizoctonia solani* and *Pythium* Clade F and Clade I (refer to Appendix 1 for full data set).

		R. solani AG2-1 pgDNA/g Sample	R. solani AG2-2 pgDNA/g Sample	R. solani AG-4 pgDNA/g Sample	R. solani AG-3 pgDNA/g Sample	R. solani AG-8 pgDNA/g Sample	Pythium Clade F pgDNA/g Sample	Pythium Clade I pgDNA/g Sample
At sowing, 19th December 2016								
		201	*329*	*0*	*0*	*6*	*92*	*6387*
At harvest, 19th January 2017								
Treatment	***Plot***							
1	*1*	*2*	*16*	*0*	*0*	*1*	*80*	*1508*
1	*36*	*2*	*0*	*0*	*0*	*0*	*122*	*2914*
2	*6*	*14*	*25*	*0*	*0*	*0*	*140*	*1311*
2	*35*	*4*	*0*	*0*	*0*	*0*	*131*	*2022*
3	*5*	*1*	*7*	*0*	*0*	*0*	*136*	*1669*
3	*31*	*4*	*121*	*0*	*0*	*7*	*111*	*1737*
4	*3*	*0*	*1*	*0*	*0*	*15*	*113*	*1008*
4	*32*	*3*	*6*	*0*	*0*	*0*	*119*	*1647*
5	*2*	*1*	*0*	*0*	*0*	*14*	*112*	*773*
5	*34*	*0*	*32*	*0*	*0*	*1*	*151*	*2035*
6	*4*	*12*	*46*	*0*	*0*	*1*	*119*	*1050*
6	*33*	*14*	*9*	*0*	*0*	*7*	*254*	*2154*

Discussion and implications

The wet conditions before sowing and just after sowing most likely worsened the occurrence of disease. These wet conditions made tillage and other operations difficult and as a result the soil in some areas within the trial was more cloddy than typical at sowing. Soil condition may also have influenced the occurrence and distribution of disease.

The variability in yields per plot was most likely affected by site variability including: irrigation and water application (irrigation affected by wind), soil structure, depth of top soil and natural spatial variability in abundance of soil pathogens. Compaction was evident at a depth of about 20 cm but was not consistent across the site or across beds. This may relate to variation in depth of top soil or due to beds being relocated on top of previous wheel tracks. Compaction can cause drainage issues which can make soil-borne diseases worse.

The treatment effect on disease bare patches is promising. However, further work is required to confirm this effect under different conditions. In both of the treatments that included Uniform®, there were fewer disease patches than in the control. The Previcur® +Aliette® + Serenade® treatment also had fewer disease patches than the control suggesting there may have been a positive interaction between these products given that treatments of Previcur® + Aliette® alone or Serenade alone did not have significantly fewer diseased patches than the control.

Conclusions and recommendations

It is promising that some of the products / product combinations examined in this trial reduced the bare patches in the crop.

This trial was conducted as a preliminary trial. Fungicide trials take considerable time and effort and require assessment under a range of conditions of not only efficacy but also different application rates, split applications and placement as well as other health and environmental aspects for registration purposes.

Future research should consider additional fungicide active ingredients and also consider results from current pot trials using seed treatments for spinach.

Next steps are to compile research from this and other trials to prioritise future research needs.

Seed treatment is preferable to soil drenches to reduce the number of operations in the field.

Following in Table 3 are some fungicide options that are being used on other crops (some as seed dressings) for control of *Rhizoctonia, Pythium, Phytophthora* and *Fusarium*. Given issues with resistance development and enhanced biodegradation experienced with various fungicides such as metalaxyl and azoxystrobin it might be prudent for the spinach industry to look at further options so that chemical seed dressings can be rotated. Note that some of these chemicals may not be appropriate for spinach diseases but this table can serve as a starting point in discussions with crop protection companies.

Table 3. Potential alternative agrichemicals for spinach diseases

Chemical active *Company* *Trade name*	*Activity group*	*Target(s)*	*Critical comment*
Fluopyram *Bayer* *Luna®*	*Gp7 SDHI*	*Rhizoctonia, Fusarium & Sclerotinia*	*Different binding site from other Gp 7 fungicides therefore lacks cross-resistance shown in other group members*
Penthiopyrad *Dupont* *Fontelis®*	*Gp7 SDHI*	*Rhizoctonia, Fusarium & Sclerotinia*	
Fluxapyroxad *BASF* *Imbrex®*	*Gp7 SDHI*	*Rhizoctonia, Fusarium & Sclerotinia*	
Flutolanil *Certis Aust.* *Monstar®*	*Gp7 SDHI*	*Rhizoctonia, Fusarium & Sclerotinia*	
Isopyrazam *Syngenta* *Reflect®*	*Gp7 SDHI*	*Sclerotinia*	
Mandipropamid *Syngenta* *Revus®*	*Gp 40 Carboxylic acid amide*	*Pythium? Downy mildew*	
Fluopicolide *Bayer* *Infinito®*	*Gp 43 Benzamides*	*Pythium, Phytophthora*	*Systemic in xylem*
Ametoctradin *BASF* *Zampro®* *formulated with Dimethomorph*	*Gp 45 Pyrimidylamines (QoSI)* *Gp 40* *Carboxylic acid amides*	*Phytophthora*	
Oxathiapiprolin *Dupont* *Zorvec®*	*New group* *Piperidinyl thiazole isoxazoline*	*Pythium? & Phytophthora*	*Translocates in both directions* *Seed dress*
GST-100 *ProBio Safeguard™*	*?*	*Damping off pathogens*	*Non-biological spinach seed treatment – establishes a barrier on roots*

Acknowledgements

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Soil DNA assays were conducted by SARDI, as part of Hort Innovation project VG15009.

The authors would like to acknowledge the team at Harvest Farms for sowing, managing and harvesting the trial.

Appendix 1. DNA-based soil tests

RDTS Samples				SARDI Plant & Soil Health Gate 2B, Hartley Gr. URRBRAE SA 5064	Note: Results are now reported as raw values only, and have not been log																				
SampleNo	Notes	Farmer		Paddock	R. solani AG2.1 pgDNA/g Sample*	R. solani AG2.2 pgDNA/g Sample*	R. solani AG4 pgDNA/g Sample*	R. solani AG3 pgDNA/g Sample*	R. solani AG8 pgDNA/g Sample*	Aphanomyces euteiches pgDNA/g Sample*	Colletotrichum coccodes pgDNA/g Sample*	Blackleg pgDNA/g Sample*	Common Scab pgDNA/g Sample*	Macrophomina phaseolina Copies / g soil	Plasmodiophora brassicae Copies / g sample	Pratylenchus crenatus nematodes/g soil	Pratylenchus neglectus nematodes /g soil	Pratylenchus thornei nematodes/g soil	Psul Copies / g sample	Pratylenchus penetrans nematodes /g soil	Pratylenchus zeae Copies / g sample	Pythium clade f pgDNA/g Sample*	Pythium clade l pgDNA/g Sample*	M yi
BB44234	19/12/2016 Harvest Farms, Richmond, Tasmania				201	329	0	0	6	0	0	0	0	1323	1228	0.0	0.0	0.0	0	0.0	0	92	6387	p S
BB52581	RCMG - supplied by D Lucas	Richmond	1	Plot 1	2	16	0	0	1	0	0	0	0	812	0	0.0	0.0	0.0	109	0.0	0	80	1508	
BB52592	RCMG - supplied by D Lucas	Richmond	1	Plot 36	2	0	0	0	0	0	0	0	0	2080	0	0.0	0.0	0.0	0	0.0	0	122	2914	
BB52586	RCMG - supplied by D Lucas	Richmond	2	Plot 6	14	25	0	0	0	0	0	0	0	903	0	0.0	0.0	0.0	0	0.0	0	140	1311	
BB52591	RCMG - supplied by D Lucas	Richmond	2	Plot 35	4	0	0	0	0	0	0	0	0	1094	0	0.0	0.0	0.0	0	0.0	0	131	2022	
BB52585	RCMG - supplied by D Lucas	Richmond	3	Plot 5	1	7	0	0	0	0	0	0	0	558	0	0.0	0.0	0.0	0	0.0	0	136	1669	
BB52587	RCMG - supplied by D Lucas	Richmond	3	Plot 31	4	121	0	0	7	0	0	0	0	716	0	0.0	0.0	0.0	0	0.0	0	111	1737	
BB52583	RCMG - supplied by D Lucas	Richmond	4	Plot 3	0	1	0	0	15	0	0	0	0	302	0	0.0	0.0	0.0	0	0.0	0	113	1008	
BB52588	RCMG - supplied by D Lucas	Richmond	4	Plot 32	3	6	0	0	0	0	0	0	0	1450	0	0.0	0.0	0.0	0	0.0	0	119	1647	
BB52582	RCMG - supplied by D Lucas	Richmond	5	Plot 2	1	0	0	0	14	0	0	0	2	682	0	0.0	0.0	0.0	0	0.0	0	112	773	
BB52590	RCMG - supplied by D Lucas	Richmond	5	Plot 34	0	32	0	0	1	0	0	0	0	1159	0	0.0	0.0	0.0	0	0.0	0	151	2035	
BB52584	RCMG - supplied by D Lucas	Richmond	6	Plot 4	12	46	0	0	1	0	0	0	0	507	0	0.0	0.0	0.0	0	0.0	0	119	1050	
BB52589	RCMG - supplied by D Lucas	Richmond	6	Plot 33	14	9	0	0	7	0	1	0	0	627	0	0.0	0.0	0.0	0	0.0	0	254	2154	

NOTES:

1. This data should be interpreted with care as the tests are still under development. Further, the tests have not been specially designed for lettuce or baby-leaf crops and therefore not all relevant pathogens are tested for.
2. Note that some data is reported in pgDNA/g sample and some is reported as Copies/g sample.