Final report

Project title:

Rapid test and fungicide resistant screening for Stemphylium leaf blight in onion

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Public summary

Stemphylium leaf blight of onions (SLB) caused by *Stemphylium vesicarium* is becoming an increasing issue internationally and nationally. Certainly, within the Lockyer valley region this is the case, with multiple growers failing to harvest crops and moving away from onions because of the disease over the last two onion seasons. Downy mildew (DM) caused by *Peronospora destructor* and purple blotch (PB) caused by *Alternaria porri* are the two other major onion canopy diseases that have significant yield impact for onion growers. There was a lack of accurate diagnostic tools for these diseases, little understanding of the severity/incidence and poor data on fungicide resistance or management strategies. These issues were the motivation for this project which has had the following major outcomes:

- Developed accurate diagnostic tools for detection of the three major onion canopy diseases SLB, DM and purple blotch PB. These tests will be available next onion season for various diagnostic applications. The protocols for these tests will also soon be published for global use.
- These diagnostic tools were used to investigate the extent of each disease in the Lockyer valley region and determine any alternate plant hosts that might be harboring disease off season. We determined that SLB was indeed the major onion canopy pathogen in the region and that it can survive on alternate plant hosts over summer. DM was also a severe issue, however PB was a minor issue, if at all.
- We generated useful genome sequence data and phylogenetic information that has been uploaded to online databases and can now be accessed by the wider research community.
- We provided soil reports and disease information back to growers to keep them informed of what disease issues they had and how there soil chemistry and health may be affecting disease incidence.
- We investigated the state of fungicide resistance for SLB to help inform appropriate strategies for control. We found genetic evidence suggesting recent strains of *S. vesicarium* had evolved resistance to group 11 and 7 fungicides. This was confirmed by in vitro lab assays on azoxystrobin and penthiopyrad which demonstrated recent *S. vesicarium* isolates from the Lockyer valley were significantly less susceptible compared to historical isolates.
- We explored integrated disease management strategies by monitoring soil health, soil chemistry and thrips damage to determine any links that may exist between these factors and disease severity. We found some links to thrips damage and some soil chemistry factors.

This public summary only highlights the main results from this project. For a detailed breakdown of all the results generated from this project please see the results below and discussion section of this report as well as the appendices.

Technical summary

Spore analysis: *Stemphylium sp., Alternaria sp. and Peronospora sp.,* the genus of fungi that contains the three major onion canopy pathogen species have very distinct spores that can be easily identified down a microscope. Spore suspensions were made from any symptomatic leaf tissue samples and spore analysis was used as an initial diagnostic.

Pathogen isolations: Pathogen isolations were performed on all diseased plant samples to isolate and collect cultures of fungi infecting the diseased plant tissue. These fungi were then identified by ITS gene DNA sequencing. Genome sequencing was performed on some of these isolates.

PCR: The diagnostic tests that have been developed are a type of PCR test known as qPCR or real time PCR. This is a type of molecular diagnostic test that detects DNA from a target organism. The test works by detecting and quantifying a region of DNA that only the target organism has and no other organism has.

Genome sequencing: To develop the PCR tests, we had to find a region of DNA that only the target organisms had and to do that we had to perform genome sequencing. Genome sequencing generates data on all the DNA of an organism. The data was analyzed to find a unique region of DNA that only the target has and the tests were designed around these. Some genome data is already online on public databases and was accessed.

Phylogenetics: Phylogenetics is the science of analyzing DNA data to confidently confirm the species taxonomy and demonstrate how it is genetically and evolutionarily related to other species of fungi by generating a phylogenetic tree. We performed phylogenetic analysis using the generated genome sequencing data and other published data.

Fungicide resistance genes: These are specific gene regions within a fungal genome that are related to the mechanism of action of fungicides. Mutations in these genes can result in resistance to specific fungicides. There are known mutations that confer fungicide resistance and are published in online literature. We performed a literature review to identify common fungicide resistance mutations and looked in our genome sequencing data for these mutations.

Ec50 assays: Ec50 is a metric that represents the effective concentration of a chemical that inhibits growth of a target by at least 50% (ec50). It is commonly used in pharmacology and chemical resistance studies to benchmark and monitor the effectiveness of a chemical. Assays in a lab can be performed to determine the ec50 of a particular chemical. We performed these lab assays as part of the project and determined the ec50 of a range of fungicides for *S. vesicarium.*

Soil health testing: Microbiology is one of the most important aspects of soil health. Soil has certain microbiological indicators that can be used to assess soil health. Metagen has developed a soil health test based on measuring these microbial indicators. Soil was collected from 10 onion farms across the region with various levels of disease, and the Metagen soil health test was done to assess any microbial indicators of soil health that may be related to onion disease severity.

Soil chemistry: Soil chemistry is another important aspect of soil health that can often be directly related to disease severity. Soil chemistry testing was performed on 10 onion farms across the region to assess any correlation with disease severity.

Thrips assessment: Thrips are a common insect pest that causes problems in a range of crops. Research has shown thrips damage correlate with SLB disease severity. As part of this project, we assessed thrip numbers and disease severity to determine any relationship.

Keywords

<Onion, Stemphylium, downy mildew, purple blotch, plant disease, plant pathogen, diagnostics, fungicide resistance, disease management, disease monitoring, research and development, plant pathology, PCR.>

Introduction

Stemphylium leaf blight (SLB), caused by *Stemphylium vesicarium*, purple blotch (PB) caused by *Alternaria porri* and downy mildew (DM) caused by *Peronospora destructor* are the three major onion canopy pathogens globally. SLB has become an increasingly significant canopy disease affecting onions and other allium crops worldwide. The disease causes leaves to show necrotic lesions and premature leaf senescence, ultimately causing a reduction in bulb size and yield. Diagnoses can be difficult as other pathogens can cause similar symptoms and be present simultaneously. Infection can also be asymptomatic until conditions are conducive to the development of symptoms. The fungus has a wide alternate host range which allows it to persist in the environment between onion seasons. There are several concerning reports of fungicide resistance from the international community. Misdiagnosis and poor fungicide strategies will worsen the impact of SLB. In 2024, significant crop losses to this disease occurred in the Lockyer Valley. Multiple growers reported failing to harvest crops and that they will be moving away from growing onions because of the impact SLB has had over the last two onion seasons, this was again the case in 2025.

There is a significant opportunity for improved monitoring and control of onion canopy disease in Australia and in particular SEQ. As mentioned above diagnosis can be difficult and better molecular diagnostic tests will help ensure accurate and early diagnosis. Molecular tests provide opportunities for monitoring the prevalence of onion canopy diseases over time and can be used to detect asymptomatic infection, disease presence on alternate plant hosts and infected seed stock. There is no data on the state of fungicide resistance of *S. vesicarium* in Australia. This data would help improve disease outcomes through better informed chemical control programs. Currently there are no single dimension strategies such as fungicides or resistant varieties that effectively control SLB in any country. A multidisciplined approach is therefore the most likely pathway to improve control.

All of the above provides the basis for this project where we generated data on the incidence and severity of SLB in the Lockyer valley region, generated new genome sequence data for *S. vesicarium*, developed molecular diagnostic tests, investigated fungicide resistance, and lastly investigated other factors that may be related to disease such as soil chemistry, soil health and insect damage.

Methodology

The work completed for this project and the progression of the project is shown below in **Fig 1.** For more detailed methods regarding the different aspects of the project see relevant appendices.

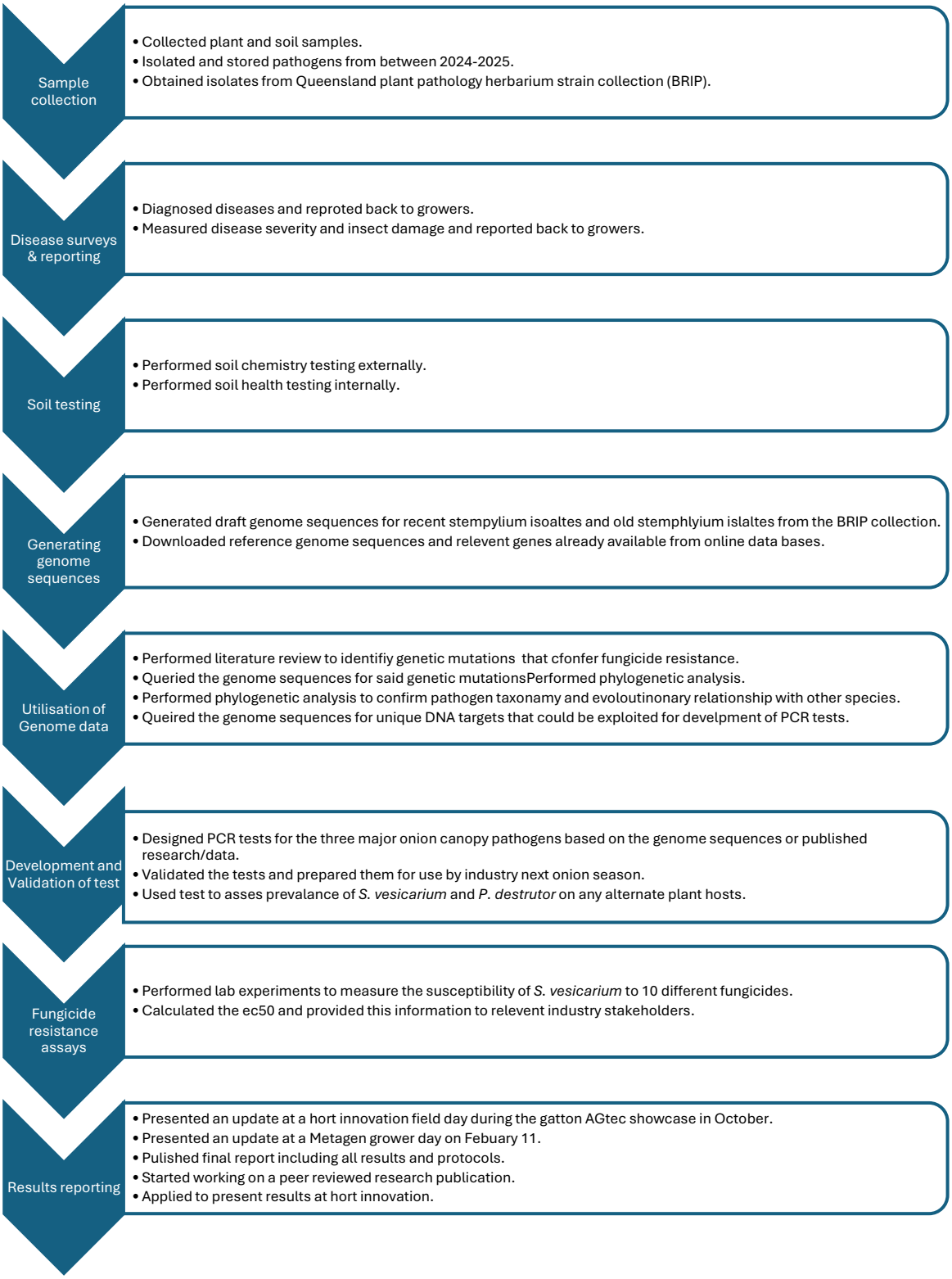

Figure 1. Flow chart showing the progression of the project and methodology employed.

Results and discussion

Field surveys and pathogen reporting

Plant samples were collected in August (11 farms) and again in September (9 farms) from in and around the Lockyer valley region. Isolations and spore analysis was done on plant material collected to get an initial disease diagnosis. SLB was the most common disease found followed by DM. Very little PB was observed.

Findings from August Isolations and spore analysis:

- 8 farms had SLB
- 2 farms had PB
- 4 farms had DM

Findings from September spore analysis:

- 6 farms had SLB
- 1 farm had PB
- 6 farms had DM

Field surveys for thrips and disease severity were done on the farms visited in August. Previous research showed high thrips numbers have been associated with up to 3.6-fold increase in SLB infection through weakening the leaf cuticle. In our surveys no plants with a high thrips population had low disease levels, confirming the role thrips may play in SLB. All field survey results and pathogen results can be found in appendices 1 and 2.

Soil chemistry testing

Soil chemistry impact on disease presence was confounded with many variables including fungicide programs. However, in terms of boron and EC impact on disease no blocks with these on target were as badly impacted by disease. All blocks that lost yield were well below target on boron and EC. All soil chemistry tests can be found in appendices 3.

Soil health testing

10 soil health test reports were generated and provided back to the growers. We were unable to show any obvious link with soil health to disease severity. The only noteworthy result was that the two farms that initially had no SLB were the only two farms where *Trichoderma sp.* DNA was detected. Species in this genus are mycoparasites and are often associated with disease suppression. However, with such a small sample number and no repeats we cannot say for certain that the presence of *Trichoderma sp.* suppressed SLB. All soil health tests can be found in appendices 4.

Genome sequencing

Stemphylium sp. and *Alternaria sp.* isolates were collected during 2024 and 2025. Three *Stemphylium sp.* isolates and one *Alternaria sp.* isolate were obtained from the BRIP strain collection. Genome sequencing data was generated for the isolates shown in **Table 1** below. The genome sequencing data was uploaded to GenBank and can be accessed via the accession numbers in **Table 1.** Alternatively search the bioproject number "PRJNA1420467"

Table 1. Details of isolates with genome sequencing data generated.

Number	Crop	Location	Species	BRIP number	GenBank Accession
F110P	Apple	Shepparton	*Alternaria sp.*	N/A	Pending
F162	Wombok	Lockyer valley	*Stemphylium vesicarium*	N/A	Pending
F167	Tomato	Lockyer Valley	*Stemphylium lycoperseci*	N/A	Pending
F173	Onion	Lockyer Valley	*Stemphylium waikerieanum*	N/A	Pending
F174	Onion	Lockyer Valley	*Stemphylium vesicarium*	N/A	Pending
F175	Onion	Lockyer Valley	*Alternaria sp.*	N/A	Pending
F177	Onion	Lockyer Valley	*Stemphylium vesicarium*	N/A	Pending
F178	Onion	n/a	*Stemphylium vesicarium*	N/A	Pending
F187	Allium sativum	Lowood	Initially *Stemphylium botryosum* now *Stemphylium. vesicarium*	26714a	Pending
F188	Allium sativum	Gatton	*Alternaria sp.*	59593a	Pending
F189	n/a	Brisbane	*Stemphylium sp. aff. vesicarium*	5891a	Pending
F190	n/a	Gatton	*Stemphylium eturminium*	60383b	Pending
F86P	Broccolini	n/a	*Alternaria sp.*	N/A	Pending
F92P	n/a	n/a	*Alternaria sp.*	N/A	Pending

Diagnostic assay development:

We compared the genome sequencing data of all relevant organisms and found a species-specific DNA target for *S. vesicarium.* We designed two sets of primes for *S. vesicarium.* A PCR protocol for *P. destructor* was taken from a peer reviewed paper and validated. None of the isolates we generated genome sequence data for turned out to be *A. porri* which made designing a test more difficult. Using genome data published online we were still able to design a PCR however there may be some detection of off target *Alternaria sp.* Since we do not have an *A. porri* isolate we have not been able to validate this test PCR yet. Full details and the protocol for running these PCR tests can be found in appendices 5.

qPCR testing results:

DNA was extracted from the plant material collected in August and once the qPCR test was ready it was performed to confirm the findings from spore analysis and isolations. We again collected plant material in January 2026 from five farms which had SLB and DM previously. This time we did not collect onion samples but instead, grasses, weeds and other crops that may be alternate hosts of the fungal pathogens. We used the qPCR to confirm the presence on any alternative plant hosts.

qPCR detected *S. vesicarium* on 7/8 of the farms that were initially confirmed to have Stemphylium sp. Since the assay is highly specific for *S. vesicarium* it is possible that the remaining sample had a different *Stemphylium sp.* or that a mistake was made during isolations/spore analysis. There was one sample where *S. vesicarium* was detected when none was previously confirmed using spore analysis and isolations. It is likely that this was missed initially due to a lack of spores or low levels of inoculum making it difficult to isolate.

S. vesicarium was detected on alternate plant hosts from 3 out of the 5 farms sampled that previously had SLB. A fourth farm had very low detection on alternate plant hosts, we are not 100% confident this was a true detection due to the low signal. *S. vesicarium* was detected on crowsfoot grass (very low), barnyard grass, pigweed (very low), bean, cheeseweed, nutgrass, torpedo grass (very low), crab grass (very low). *Peronospora destructor* was detected on none of the alternate plant hosts as expected since it is not reported to infect alternate hosts.

Phylogenetics:

We performed phylogenetic analysis and confirmed that the main species of fungi being isolated from onion leaf lesions in the Lockyer valley region was *S. vesicarium* as expected. Analysis also revealed that one BRIP collection isolate recorded as *Stemphylium botryosum* was *S. vesicarium*. Genome sequencing data for the BRIP isolates has been provided to Dr. Yu pei who will update the information in the BRIP collection accordingly. We retrieved relevant phylogenetic genes from the genome data and downloaded reference genes already available from online data bases. With this data we generated a phylogenetic tree to show the evolutionary relationship between different *Stemphylium sp.* Some of the findings from this analysis are confidential so have only been discussed in the appendices. See appendices 6 for full details of phylogenetic analysis.

Fungicide resistance genetic analysis and assays:

Through extensive literature reviews we identified several genetic mutations that are known to reduce fungicide sensitivity. These were on genes CYTB, CYP51, SDH (B, C, D subunits), and HIS-KIN. We investigated these genes in the genome data generated for our *Stemphylium sp.* isolates and found The CYTB gene carried the G143A substitution in three *S. vesicarium* isolates and in the single *S. waikerieanum* isolate, consistent with QoI (Group 11) resistance. Sequencing of sdhC identified the S135R substitution in multiple isolates, associated with reduced sensitivity to SDHI (Group 7) fungicides.

To confirm whether reduced fungicide sensitivity existed we performed ec50 assays on both the recent and historic *Stemphylium sp.* isolates. These are experiments that are done to determine the effective concentration which the growth of a fungus is reduced by 50% (ec50). These metrics are commonly used in scientific research as a benchmark for monitoring fungicide sensitivity. We chose the 10 chemicals listed in **Table 2** for ec50 assays since they represent a diverse range of frac groups and chemicals that are commonly used. The results from ec50 assays are shown below in **Fig 2.** Cyproconazole and fludioxonil consistently showed low EC_{50} values (10 µg/mL)**.** Chlorothalonil and tebuconazole displayed moderate activity (EC_{50}s in the 1–10 µg/mL range), while mancozeb EC_{50} exceeded the tested range (>10 µg/mL), consistent with its potential lower potency. These findings provide useful preliminary insights but will require assessment across additional isolates and repeated tests to confirm patterns of sensitivity. These results confirm that recent *S. vesicarium* isolates have reduced sensitivity to some FRAC group 11 and FRAC group 7 fungicides, particularly azoxystrobin and penthiopyrad. These findings provide useful preliminary insights but will require assessment across additional isolates and repeated tests to confirm patterns of sensitivity.

Table 2. Details of fungicides used of ec50 assays.

Name	Multi or single site	Mechanism	FRAC group
Azoxystrobin	Quinone outside inhibitor (QoI). Blocks electron transfer at the Qo site of cytochrome b, halting mitochondrial respiration.	Single-site	11 – QoI (Quinone outside inhibitor)
Tebuconazole	Demethylation inhibitor (DMI). Inhibits sterol 14α-demethylase (CYP51), blocking ergosterol biosynthesis in fungal membranes.	Single-site	3 – DMI (Demethylation inhibitor, triazole)
Boscalid	Succinate dehydrogenase inhibitor (SDHI). Blocks succinate dehydrogenase (complex II) in mitochondrial respiration.	Single-site	7 – SDHI (Succinate dehydrogenase inhibitor)
Procymidone	Dicarboximide. Inhibits lipid peroxidation and signal transduction linked to osmotic stress pathways.	Single-site	2 – Dicarboximide
Chlorothalonil	Reacts with thiol groups in fungal enzymes and glutathione, disrupting multiple metabolic pathways.	Multi-site	M05 – Multi-site activity
Mancozeb	Chelates metal-containing enzymes, disrupting respiration and enzyme activity.	Multi-site	M03 – Multi-site activity (dithiocarbamate)
Fluopyram	Succinate dehydrogenase inhibitor (SDHI). Same target as boscalid—complex II of mitochondrial respiration.	Single-site	7 – SDHI
Penthiopyrad	Succinate dehydrogenase inhibitor (SDHI). Inhibits complex II, blocking mitochondrial respiration.	Single-site	7 – SDHI
Fludioxonil*	Phenylpyrrole. Interferes with osmotic signal transduction (MAP kinase pathway), leading to uncontrolled glycerol accumulation.	Single-site	12 – Phenylpyrrole
Cyprodinil*	Anilinopyrimidine. Inhibits methionine biosynthesis and secretion of hydrolytic enzymes, disrupting fungal penetration and growth.	Single-site	9 – Anilinopyrimidine

*These chemicals are often used in combination in commercial products so were tested individually and combined in ec50 assays.

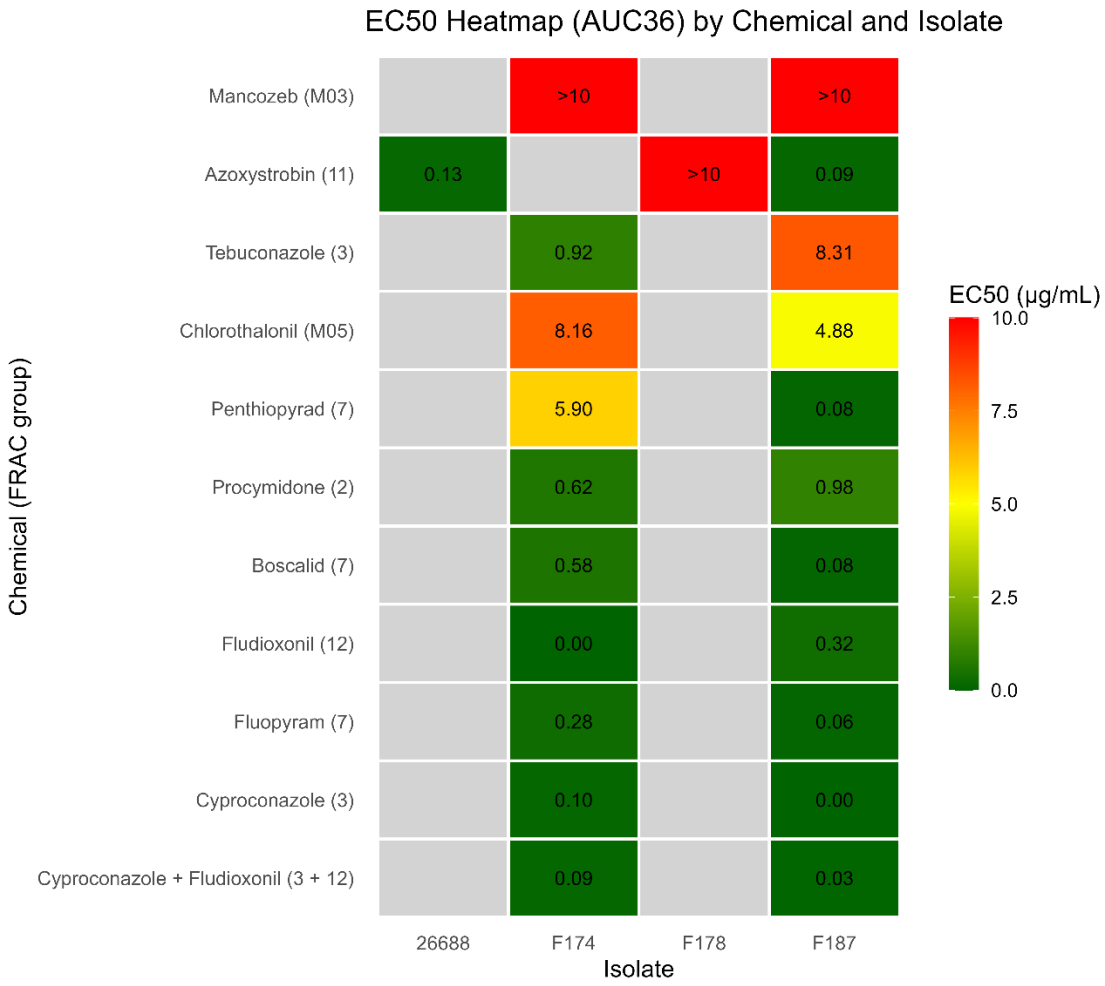

Figure 2. Heat map of preliminary EC_{50} values (µg/mL) across representative *Stemphylium* isolates for selected fungicides. The visualisation highlights variability in sensitivity between isolates. Grey tiles indicate isolate was not tested for that chemical.

Outputs

Table 3. Output summary

Output	Description	Detail
Diagnostic PCR tests developed for *S. vesicarium* and *P. destructo*r. A PCR test designed for *A. porri* with some limitations and not yet validated.	These are diagnostic tests that can be used as an accurate and rapid diagnostic, early detection, detection of asymptomatic infection and detection on alternate hosts.	These tests will be made available to onion growers as a commercial service by Metagen next onion season. The protocols for these tests will also be publicly available so that other labs may offer this service. Evidence of engagement can be obtained by reporting how many growers used this test at the end of next onion season. Also, by monitoring if any other labs or researchers decide to use this test.
Grower disease information, soil chemistry reports and soil health reports.	This is information on what diseases were detected on each farm as well as disease severity and thrips damage. Soil chemistry and soil health data for each farm was also generated.	This information and these reports have been provided back to the growers. Evidence of engagement and usefulness can be obtained by surveying the growers.
Genome sequence data	Genome sequence data was generated for 14 isolates 9 *Stemphylium sp.* isolates and 5 *Alternaria sp.* isolates.	This data will be uploaded and made publicly available on Genbank. This data was useful/necessary for other aspects of the project such as development of PCR tests, phylogenetic analysis and investigating fungicide resistance. Accurate publicly available genome sequence data is a useful resource for other researchers.
Phylogenetic analysis	This is the analysis of genetic data to accurately confirm a species taxonomy and determine its evolutionary/genetic relationship to other species.	See appendices 6 for useful results from this analysis.
Fungicide resistance data	We identified genetic mutations that confer fungicide resistance and confirmed with lab tests.	This information has been provided to Dorris Blessing to include in the Stemphylium R and D scan booklet. This information will also be provided to the appropriate onion industry stakeholders to help inform fungicide programs. Further fungicide resistance testing is necessary to validate these results and would help to monitor fungicide resistance over time. The usefulness of this data can be determined by surveying those in the onion industry who have been made aware of these results.
Alternate host qPCR test	These experiments confirmed that the qPCR test can be used to detect *S. vesicarium* on alternate plant hosts.	The engagement/usefulness of this can be determined by reporting how many growers use the test for this purpose before next onion season starts.
Published research	Dr Noel Knight is working	This will help make the results of this project more accessible

paper	on publishing the results from this project in a peer reviewed research paper.	to other research scientists. Engagement can be tracked over time by how often this research paper is cited.

Outcomes

Table 4. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
New diagnostic tools are accessible to onion growers.	Aligns to the onion fund SIP outcome 2: "Industry supply, productivity and sustainability". The key performance indicator (KPI) will be how often growers use the diagnostic test.	We developed this DNA test based on generated genome sequencing data and validated it on diseased plant samples and fungal pathogen isolates. Having accurate disease diagnostics will help boost productivity.	Evidence was collected in the form of experimental results from running the tests in the lab on diseased plant samples.
New data that will help inform disease management practices.	Aligns to the onion fund SIP outcome 2: "Industry supply, productivity and sustainability". The key performance indicators can be evaluated by surveying relevant industry stakeholders to see how useful they found this data.	Fungicide resistance genes identified and susceptibility tested in the lab. This information will inform management practices. Having good management practices boosts productivity.	Evidence was collected in the form of data generated from lab experiments and genome sequencing data analysis.
Contributed valuable resources and data to the research community and Hort innovation.	Aligns to the onion fund SIP outcome 3: "extension and capability". The key performance indicators can be evaluated by monitoring how often generated data and results are used in other research.	Genome sequencing data, Phylogenetics, fungicide resistance data is all published and can be accessed by other research groups.	GenBank accession numbers and communication with Yu Pei at BRIP. In progress research paper. This published report.
Growers more informed about onion canopy diseases.	Aligns to the onion fund SIP outcome 3: "extension and capability". The key performance indicators can be evaluated by surveying the growers who received reports and information about there diseases, soil chemistry and health.	Disease diagnoses were provided back to the growers so they are informed about what pathogens that are dealing with. Soil chemistry and soil health tests were also provided back to the growers.	Reports in appendices and communication with growers.

Monitoring and evaluation

Table 4. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
1. To what extent has the project achieved its expected outcomes? - *Has a new molecular test for Stemphylium leaf blight been developed?* *-Have we completed in vitro fungicide resistance assays and generated a list of fungicides which the disease has developed resistance to?* *- Have we published a research paper outlining the findings?*	The project has mostly achieved its expected outcomes. There are new molecular tests now available except that the one for PB has some limitations and is not yet validated. Fungicide resistance results have been achieved. A research paper is in progress.	Expansion of the fungicide resistance work would be beneficial since only a limited number of isolates were tested. Expansion of the PCR tests to detect different fungicide resistance mutations would be beneficial.
2. How relevant was the project to the needs of intended beneficiaries? *To what extent has the project met the needs of industry levy payers?*	The project has generated new tools and data directly available to onion levy payers. These tools and data will help them monitor and manage the major onion canopy pathogens.	More extension works to promote the testing would be beneficial to ensure its uptake.
3. How well have intended beneficiaries been engaged in the project? - *How many different growers have been involved/ had input on the project?* - *Have regular project updates been provided through linkage with the industry communication project?* - *How many different avenues were there for engagement?*	12 different growers were involved in the project. Multiple agronomists were consulted. Staff from the Lockyer valley grower association were consulted. Project updates were provided during grower events in October and February. Regular milestone reports were submitted in addition to this final report. Avenues for engagement were field days and individual farm visits.	More extension works to promote the testing would be beneficial to ensure uptake of the test.
5. What efforts did the project make to improve efficiency?	The project was collaborative and engaged different experts to help with different aspects of the project. See below list of the different groups/people involved: Metagen, UniSQ, ACE UQ, Yu pei (BRIP), Lockyer valley growers association, Hort innovation, multiple growers and agronomists.	Expanding the project to farms outside of Queensland would have been beneficial. Only farms in southeast Queensland regions were visited.

Recommendations

Extension and communication work:

- It is important that growers are made aware of the new diagnostic tools that are now available to them so that the testing is taken up to monitor disease and inform management practices. Ideally there should be an event in each key onion growing region where these new tools are brought to the attention of the growers.
- Present findings at Hort innovation and Ausveg forums, and other onion industry extension options. Generate new fact sheets to be sent out.

Practical application of the project findings:

- Some funding so the test can initially be offered for free will likely help with uptake.
- Weather monitoring combined with testing to allow best timing of effective chemistry on the onion canopy pathogen mix.
- Development of an area wide fungicide resistance management protocol to extend the life of control options. Example rotation of different fungicide chemical groups used each season so resistance to frac groups does not build up.
- We would like to develop the test for early detection of the disease before symptoms appear. However, we need to work out how many non-symptomatic leaves can be combined into one sample and tested before losing sensitivity. We also need to develop some DNA extraction methods that allow for the DNA extraction of larger amounts of plant material.
- A great use of this test would be testing onion seed since SLB can be spread through infected seed. We would need to develop some DNA extraction methods for this and do some experiments to work out how much seed can be tested at once before losing sensitivity.

Future RD&E that directly flow from the results of this project:

- **See appendices 6 for one of our most relevant recommendations regarding pathogenicity pot trials.**
- Development of a standardised sampling protocol and DNA extraction protocol for the best use of the PCR tests.
- Development an additional PCR test that can detect the different fungicide resistance genes. This would allow the detection of *S. vesicarium* while simultaneously providing information on which fungicides the pathogen may be resistant to.
- Expansion of the fungicide resistance testing to cover a greater number of isolates and more geographic locations.

Refereed scientific publications

None published yet however one is in progress. The working title is **Diversity, fungicide sensitivity and species-specific detection of *Stemphylium* species from horticultural crops in Australia.**

Intellectual property

No project IP to report. The onion canopy PCR tests developed in this project will be offered by Metagen to growers as a commercial testing service. The details of these tests are publicly available so that other labs may also offer this as a service.

Acknowledgements

Metagen would like to acknowledge Hort Innovation, Ausvedge, the Lockyer Valley Grower Association and of course the onion growers themselves for their support regarding this project. We would also like to Acknowledge all the growers and agronomists that provided guidance during this project and allowed us onto their farms to collect samples.

Appendices

Appendix 1: Spore analysis and isolations

Appendix 2: Field survey results **Confidential**

Appendix 3: Soil chemistry results

Appendix 4: Soil health reports

Appendix 5: PCR protocol

Appendix 6: Phylogenetic analysis **Confidential**

Farm sample barcode		Isoaltions	Spores	Stemphylium qPCR 1	Stemphylium qPCR 2
	26681	Stemphylium, Alternaria	Stemphylium, Downy	Detected	Detected
	26682	none	none	negative or very low amounts	negative
	26683	none	none	Very low	Very low
	26684	Stemphylium	Downy, Alternaria and Stemphylium	Detected	Detected
	26685	Stemhpylium	Stemphylium	Detected	Detected
	26686	Stemphylium	Stemphylium and Downy	Detected	Detected
	26691	Alternaria	Alternaria	Detected	Detected
	26692	Stemphylium	stemphylium	Detected	Detected
	26690	None	downy	negative	Negative
	26688	Stemphylium	downy	Detected	Detected
	26693	Stemphylium	Stemphylium	Detected	Detected
	26976	N/A	downy and stemphylium	N/A	N/A
	26977	N/A	downy and stemphylium	N/A	N/A
	26978	N/A	downy, alternaria, little bit of stemphylium maybe	N/A	N/A
	26979	N/A	none	N/A	N/A
	26981	N/A	downy	N/A	N/A
	26982	N/A	none	N/A	N/A
	26984	N/A	stemphylium	N/A	N/A
	26985	N/A	downy and stemphylium	N/A	N/A
n/A		N/A	downy and stemphylium	N/A	N/A

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LABORATORY TEST RESULTS 13/08/2025 24/09/2025

	Target	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential	confidential
GENERAL																									
PH [1:5 CACL2]	5.5 - 7.0	7.4	7.7	6.7	7.7	7.4	6.1	7	7.7	7.6	7.3	7.3	7.9												
PH [1:5 H2O]	6.5 - 8.0	8.1	8.2	7.3	8.3	8.2	6.6	7.5	8.4	8.2	7.9	7.8	8.4												
ORGANIC MATTER %		2.0	2.0	2.4	2.0	1.7	2.5	2.9	1.6	1.8	1.8	1.8	1.3												
CEC		28.4	33.9	31.5	43.1	25.8	33.8	40.1	18.0	44.2	28.0	24.6	39.2												
TEC		28.4	33.9	31.5	43.1	25.8	33.8	40.1	18.0	44.2	28.0	24.6	39.2												
EC [1:5 H2O]		0.15	0.42	0.32	0.37	0.20	0.35	0.39	0.18	0.37	0.48	0.69	0.47												
CHLORIDE PPM	100 - 500	48	240	140	250	180	43	120	140	200	570	780	410		0.2	1.0	0.7	1.0	0.9	0.2	0.6	0.7	1.0	1.0	1.0	1.0
SULPHUR PPM	20 - 500	21	91	51	38	7	210	120	20	53	25	38	38												
PHOSPHOROUS																									
COLWELL PPM	20 - 500	140	180	130	210	66	170	130	110	190	91	110	180												
CATION BALANCE																									
CALCIUM PPM		3,264	3,264	3,264	3,876	2,244	4,080	5,304	2,244	4,488	2,448	2,448	3,264												
MAGNESIUM PPM		1,337	1,823	1,701	2,552	1,580	1,458	1,458	644	2,431	1,701	1,215	2,431												
POTASSIUM PPM		211	626	152	508	192	297	391	391	469	164	195	344												
SODIUM PPM		170.1	252.9	197.7	390.8	275.9	160.9	209.2	140.2	216.1	344.9	459.8	505.8												
NH4-N PPM		6.9	2.1	8.1	2.3	5.0	22.0	6	0.9	2.4	3.3	2.5	2.9												
ALUMINIUM PPM		0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0												
EXCHANGE CA %	62 - 80	56	47	51	44	43	59	65	61	50	43	49	41		0.9	0.8	0.8	0.7	0.7	1.0	1.1	1.0	0.8	0.7	0.8	0.7
EXCHANGE MG %	15 - 40	39	44	45	49	50	36	30	29	45	50	41	51												
EXCHANGE K %	3.5 - 8.0	1.9	4.7	1.2	3.0	1.9	2.2	2.5	5.5	2.7	1.5	2.0	2.2												
EXCHANGE NA %	0.0 - 4.0	2.6	3.2	2.7	3.9	4.6	2.1	2.3	3.4	2.1	5.4	8.1	5.6												
EXCHANGE H+ %	0.0 - 25.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0												
EXCHANGE AL %	0.0 - 0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0												
CA:MG RATIO		0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0												
OTHER NUTRIENTS																									
BORON PPM	2.5 - 3.5	1.0	3.2	2.8	0.9	0.7	1.2	1.4	0.9	1.4	1.0	0.9	1.4		0.4	1.0	1.0	0.4	0.3	0.5	0.6	0.4	0.6	0.4	0.4	0.6
IRON DTPA PPM	25.0 - 90.0	33.0	30.0	46.0	30.0	26.0	72.0	47	20.0	19.0	19.0	27.0	19.0												
MANGANESE PPM	15.0 - 90.0	8.0	9.8	8.7	5.5	5.0	19.0	12.0	9.0	7.9	4.4	4.4	2.8		1.0	1.9	1.8	1.2	1.1	1.1	1.4	1.2	1.5	1.3	1.3	1.4
COPPER DTPA PPM	1.0 - 10.0	1.1	5.1	1.6	1.7	1.2	1.7	1.9	1.1	1.8	1.1	1.2	0.9		49%	95%	89%	61%	55%	54%	70%	61%	74%	63%	63%	70%
ZINC DTPA PPM	1.2 - 15.0	1.8	5.2	2.5	1.5	3.2	3.4	3.1	4.2	2.3	3.8	1.4	2.6												
NITRATE N PPM		7.8	8.6	29.0	5.8	5.1	49.0	27	3.1	20.0	7.8	55.0	5.0												

metagen AUSTRALIA

THE SOIL HEALTH COMPANY

DNA Soil Health Report

Data Version: 2025-12-09 07:43:48

How the test works:

- **DNA is extracted and purified from your soil**
- **PCR assays are used to amplify genes of all the microbes present in that DNA**
- **We read this amplified DNA using advanced sequencing technology**
- **Our proprietary soil-informatics AI system analyses & interprets the DNA code to make this report**

1 Key Points

Enter your text here

Client: site 6,site 5,site 1,site 2,site 7,site 8,site 3,site 4,site 10,site 9 | Blocks: site 6 , site 5 , site 1 , site 2 , site 7 , site 8 , site 3 , site 4 , site 10 , site 9

Overview

Barcode	Benefical Microbes	Biodiversity	Carbon	Maturity	Pest Suppression	Soil Properties	Soilborne Pathogens
site 6	56	77	69	63	40	33	54
site 10	61	52	58	58	42	84	53
site 9	65	65	63	72	16	83	45
site 1	61	53	43	37	8	88	61
site 2	57	70	41	64	66	98	73
site 7	63	72	48	63	71	33	58
site 8	58	78	46	65	67	85	43
site 3	48	63	45	44	22	44	41
site 4	58	64	57	31	44	92	51
site 5	64	69	53	49	41	80	80

Client: site 6,site 5,site 1,site 2,site 7,site 8,site 3,site 4,site 10,site 9 | Blocks: site 6 , site 5 , site 1 , site 2 , site 7 , site 8 , site 3 , site 4 , site 10 , site 9

site 6

	Score	Classification	Units	Response Type	Range
Soil Properties					
pH	6.42	Moderate	H+	Optimum	[5.59 - 7.97]
Carbon					
Fungi : Protists	2.65	High	Log Ratio	Positive	[0.13 - 3.35]
Bacteria : Cyano	12.19	Very-High	Log Ratio	Positive	[6.91 - 11.33]
Saprotrophic fungi	63.55	Moderate	% Abundance	Positive	[32.78 - 74.11]
Active carbon (POXC)	527.14	Moderate	mg / kg	Positive	[162.06 - 1184.44]
B-Glucosidase	5.09	Moderate	ug / g/ hr	Positive	[4.36 - 5.94]
Pest Suppression					
Entomopathogenic Fungi	0	Very-Low	% Abundance	Optimum	[0 - 0.11]
Trichoderma	0.19	Moderate	% Abundance	Optimum	[0 - 1.67]
Mycoparasitic Fungi	4.3	Moderate	% Abundance	Optimum	[0.69 - 13.12]
Maturity					
Annelids	0.58	Moderate	% Abundance	Positive	[0 - 11.06]
Rotifers	0.07	Moderate	% Abundance	Positive	[0 - 0.21]
Mites	0.56	High	% Abundance	Positive	[0 - 1.1]
Collembola	0.06	Moderate	% Abundance	Positive	[0 - 0.93]
Basidiomycota : Ascomycota	-3.04	Low	Log Ratio	Positive	[-3.58 - -0.8]
Bacterial efficiency	71.61	Very-High	Unitless	Positive	[9.97 - 54.75]
Benefical Microbes					
Proteobacteria	34.92	Moderate	% Abundance	Optimum	[23 - 53.22]
Actinobacteria	27.44	High	% Abundance	Optimum	[2.84 - 28.59]
Bacteroidetes	6.81	High	% Abundance	Optimum	[1.23 - 10.07]
Firmicutes	2.81	High	% Abundance	Optimum	[0.14 - 5.62]
Plant Benefical fungi	22.18	Moderate	% Abundance	Optimum	[10.62 - 50.92]
Endomycorrhizal fungi (AMF)	0.11	Moderate	% Abundance	Positive	[0 - 2.94]
Ectomycorrhizal fungi	0.1	Low	% Abundance	Positive	[0 - 4.35]
Biodiversity					
Fungal diversity	13.73	Moderate	Unitless	Positive	[7.03 - 19.2]
Bacterial diversity	87.47	Very-High	Unitless	Positive	[30.95 - 84.01]
Protozoan diversity	16.88	Moderate	Unitless	Positive	[6.82 - 24.55]
Mesofaunal diversity	15.12	High	Unitless	Positive	[4.08 - 15.8]
Nematode diversity	12.33	Very-High	Unitless	Positive	[3.49 - 12.28]
Soilborne Pathogens					
Sclerotinia	0	Very-Low	% Abundance	Negative	[0 - 0]
Macrophomina	0.1	Very-High	% Abundance	Negative	[0 - 0.01]
Fusarium	7.07	High	% Abundance	Negative	[0.04 - 10.62]
Verticillium	1.12	High	% Abundance	Negative	[0 - 1.65]
Rhizoctonia	0.08	Moderate	% Abundance	Negative	[0 - 0.93]
Phytophthora	0	Very-Low	% Abundance	Negative	[0 - 0.26]
Pythium	1.37	Moderate	% Abundance	Negative	[0 - 5.01]

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site 5

	Score	Classification	Units	Response Type	Range
Soil Properties					
pH	7.07	High	H+	Optimum	[4.83 - 8.43]
Carbon					
Fungi : Protists	2.65	Moderate	Log Ratio	Positive	[0.38 - 3.89]
Bacteria : Cyano	6.39	Low	Log Ratio	Positive	[6.34 - 10.63]
Saprotrophic fungi	62.95	Moderate	% Abundance	Positive	[26.94 - 78.4]
Active carbon (POXC)	746.14	High	mg / kg	Positive	[59.46 - 1269.13]
B-Glucosidase	5.29	Moderate	ug / g/ hr	Positive	[4.12 - 6.41]
Pest Suppression					
Entomopathogenic Fungi	0	Very-Low	% Abundance	Optimum	[0 - 1.64]
Trichoderma	0.33	High	% Abundance	Optimum	[0 - 0.62]
Mycoparasitic Fungi	3.13	Moderate	% Abundance	Optimum	[0.21 - 10.09]
Maturity					
Annelids	0.08	Moderate	% Abundance	Positive	[0 - 6.15]
Rotifers	0	Very-Low	% Abundance	Positive	[0 - 0.64]
Mites	0.01	Moderate	% Abundance	Positive	[0 - 0.63]
Collembola	1.01	High	% Abundance	Positive	[0 - 1.14]
Basidiomycota : Ascomycota	-2.73	Moderate	Log Ratio	Positive	[-3.97 - 0.07]
Bacterial efficiency	51.77	Very-High	Unitless	Positive	[5.37 - 44.58]
Benefical Microbes					
Proteobacteria	29.93	Moderate	% Abundance	Optimum	[24.87 - 51.29]
Actinobacteria	17.72	Moderate	% Abundance	Optimum	[3.88 - 48.51]
Bacteroidetes	6.66	High	% Abundance	Optimum	[0.5 - 7.52]
Firmicutes	3.17	High	% Abundance	Optimum	[0.31 - 4.8]
Plant Benefical fungi	30.51	Moderate	% Abundance	Optimum	[8.23 - 51.94]
Endomycorrhizal fungi (AMF)	0.44	Moderate	% Abundance	Positive	[0 - 0.82]
Ectomycorrhizal fungi	1.1	Moderate	% Abundance	Positive	[0 - 9.16]
Biodiversity					
Fungal diversity	14.31	Moderate	Unitless	Positive	[4.76 - 17.79]
Bacterial diversity	66.49	High	Unitless	Positive	[17.51 - 77.33]
Protozoan diversity	21.94	Very-High	Unitless	Positive	[3.65 - 21.77]
Mesofaunal diversity	9.37	Moderate	Unitless	Positive	[4.01 - 17.29]
Nematode diversity	7.32	Moderate	Unitless	Positive	[3.57 - 12.3]
Soilborne Pathogens					
Sclerotinia	0	Very-Low	% Abundance	Negative	[0 - 0]
Macrophomina	0.11	Very-High	% Abundance	Negative	[0 - 0.04]
Fusarium	10.16	Very-High	% Abundance	Negative	[0 - 6.91]
Verticillium	0.52	Very-High	% Abundance	Negative	[0 - 0.42]
Rhizoctonia	0.95	Very-High	% Abundance	Negative	[0 - 0.68]
Phytophthora	0.01	High	% Abundance	Negative	[0 - 0.04]
Pythium	5.01	High	% Abundance	Negative	[0 - 5.17]

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site 1

	Score	Classification	Units	Response Type	Range
Soil Properties					
pH	7.92	High	H+	Optimum	[5.7 - 7.98]
Carbon					
Fungi : Protists	2.18	Moderate	Log Ratio	Positive	[0 - 3.49]
Bacteria : Cyano	8.54	Moderate	Log Ratio	Positive	[6.25 - 11.14]
Saprotrophic fungi	62.94	Moderate	% Abundance	Positive	[30.33 - 79.19]
Active carbon (POXC)	370.97	Moderate	mg / kg	Positive	[250.61 - 1283.55]
B-Glucosidase	4.81	Moderate	ug / g/ hr	Positive	[4.2 - 6.05]
Pest Suppression					
Entomopathogenic Fungi	0	Very-Low	% Abundance	Optimum	[0 - 0.32]
Trichoderma	0	Very-Low	% Abundance	Optimum	[0 - 1.24]
Mycoparasitic Fungi	2.08	Low	% Abundance	Optimum	[0.76 - 11.47]
Maturity					
Annelids	0	Very-Low	% Abundance	Positive	[0 - 4.58]
Rotifers	0.06	Moderate	% Abundance	Positive	[0 - 0.23]
Mites	0.01	Moderate	% Abundance	Positive	[0 - 0.8]
Collembola	0	Very-Low	% Abundance	Positive	[0 - 0.96]
Basidiomycota : Ascomycota	-2.55	Moderate	Log Ratio	Positive	[-3.64 - 0]
Bacterial efficiency	45.49	High	Unitless	Positive	[10.45 - 53.56]
Benefical Microbes					
Proteobacteria	31.66	Moderate	% Abundance	Optimum	[20.29 - 50.67]
Actinobacteria	20.59	Moderate	% Abundance	Optimum	[2.59 - 37.44]
Bacteroidetes	4.23	Moderate	% Abundance	Optimum	[0.91 - 11.54]
Firmicutes	2.48	High	% Abundance	Optimum	[0.09 - 7.02]
Plant Benefical fungi	31.17	Moderate	% Abundance	Optimum	[10.7 - 57.94]
Endomycorrhizal fungi (AMF)	0.01	Moderate	% Abundance	Positive	[0 - 2.23]
Ectomycorrhizal fungi	6.11	Very-High	% Abundance	Positive	[0 - 4.76]
Biodiversity					
Fungal diversity	9.48	Moderate	Unitless	Positive	[4.63 - 19.86]
Bacterial diversity	64.43	Moderate	Unitless	Positive	[25.24 - 82.16]
Protozoan diversity	22.69	High	Unitless	Positive	[5.78 - 25.84]
Mesofaunal diversity	7.34	Moderate	Unitless	Positive	[3.49 - 16.45]
Nematode diversity	6.38	Moderate	Unitless	Positive	[3.07 - 11.7]
Soilborne Pathogens					
Sclerotinia	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Macrophomina	0	Very-Low	% Abundance	Negative	[0 - 0.04]
Fusarium	14.4	Very-High	% Abundance	Negative	[0 - 5.64]
Verticillium	0.71	Very-High	% Abundance	Negative	[0 - 0.71]
Rhizoctonia	0.06	Moderate	% Abundance	Negative	[0 - 0.7]
Phytophthora	0.84	Very-High	% Abundance	Negative	[0 - 0.01]
Pythium	2.17	High	% Abundance	Negative	[0 - 3.09]

Client: site 6,site 5,site 1,site 2,site 7,site 8,site 3,site 4,site 10,site 9 | Blocks: site 6 , site 5 , site 1 , site 2 , site 7 , site 8 , site 3 , site 4 , site 10 , site 9

site 2

	Score	Classification	Units	Response Type	Range
Soil Properties					
pH	8.38	Very-High	H+	Optimum	[5.7 - 7.98]
Carbon					
Fungi : Protists	2.35	Moderate	Log Ratio	Positive	[0 - 3.49]
Bacteria : Cyano	6.57	Low	Log Ratio	Positive	[6.25 - 11.14]
Saprotrophic fungi	43.01	Moderate	% Abundance	Positive	[30.33 - 79.19]
Active carbon (POXC)	541.42	Moderate	mg / kg	Positive	[250.61 - 1283.55]
B-Glucosidase	5.04	Moderate	ug / g/ hr	Positive	[4.2 - 6.05]
Pest Suppression					
Entomopathogenic Fungi	0.12	High	% Abundance	Optimum	[0 - 0.32]
Trichoderma	0.16	Moderate	% Abundance	Optimum	[0 - 1.24]
Mycoparasitic Fungi	5.46	Moderate	% Abundance	Optimum	[0.76 - 11.47]
Maturity					
Annelids	0.52	Moderate	% Abundance	Positive	[0 - 4.58]
Rotifers	0	Very-Low	% Abundance	Positive	[0 - 0.23]
Mites	4.18	Very-High	% Abundance	Positive	[0 - 0.8]
Collembola	0.1	Moderate	% Abundance	Positive	[0 - 0.96]
Basidiomycota : Ascomycota	-0.93	High	Log Ratio	Positive	[-3.64 - 0]
Bacterial efficiency	57.19	Very-High	Unitless	Positive	[10.45 - 53.56]
Benefical Microbes					
Proteobacteria	27.69	Moderate	% Abundance	Optimum	[20.29 - 50.67]
Actinobacteria	17.62	Moderate	% Abundance	Optimum	[2.59 - 37.44]
Bacteroidetes	6.35	High	% Abundance	Optimum	[0.91 - 11.54]
Firmicutes	7.53	Very-High	% Abundance	Optimum	[0.09 - 7.02]
Plant Benefical fungi	19.09	Moderate	% Abundance	Optimum	[10.7 - 57.94]
Endomycorrhizal fungi (AMF)	0.36	Moderate	% Abundance	Positive	[0 - 2.23]
Ectomycorrhizal fungi	0.7	Moderate	% Abundance	Positive	[0 - 4.76]
Biodiversity					
Fungal diversity	17.23	High	Unitless	Positive	[4.63 - 19.86]
Bacterial diversity	91.4	Very-High	Unitless	Positive	[25.24 - 82.16]
Protozoan diversity	16.56	Moderate	Unitless	Positive	[5.78 - 25.84]
Mesofaunal diversity	5.12	Low	Unitless	Positive	[3.49 - 16.45]
Nematode diversity	15.2	Very-High	Unitless	Positive	[3.07 - 11.7]
Soilborne Pathogens					
Sclerotinia	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Macrophomina	0.02	High	% Abundance	Negative	[0 - 0.04]
Fusarium	3.49	High	% Abundance	Negative	[0 - 5.64]
Verticillium	1.94	Very-High	% Abundance	Negative	[0 - 0.71]
Rhizoctonia	0.17	Moderate	% Abundance	Negative	[0 - 0.7]
Phytophthora	0.63	Very-High	% Abundance	Negative	[0 - 0.01]
Pythium	1.63	High	% Abundance	Negative	[0 - 3.09]

Client: site 6,site 5,site 1,site 2,site 7,site 8,site 3,site 4,site 10,site 9 | Blocks: site 6 , site 5 , site 1 , site 2 , site 7 , site 8 , site 3 , site 4 , site 10 , site 9

site 7

	Score	Classification	Units	Response Type	Range
Soil Properties					
pH	6.35	Moderate	H+	Optimum	[5.7 - 7.98]
Carbon					
Fungi : Protists	2.73	High	Log Ratio	Positive	[0 - 3.49]
Bacteria : Cyano	5.99	Very-Low	Log Ratio	Positive	[6.25 - 11.14]
Saprotrophic fungi	47.13	Moderate	% Abundance	Positive	[30.33 - 79.19]
Active carbon (POXC)	748.5	Moderate	mg / kg	Positive	[250.61 - 1283.55]
B-Glucosidase	5.06	Moderate	ug / g/ hr	Positive	[4.2 - 6.05]
Pest Suppression					
Entomopathogenic Fungi	0.44	Very-High	% Abundance	Optimum	[0 - 0.32]
Trichoderma	0.59	High	% Abundance	Optimum	[0 - 1.24]
Mycoparasitic Fungi	3.75	Moderate	% Abundance	Optimum	[0.76 - 11.47]
Maturity					
Annelids	0.89	Moderate	% Abundance	Positive	[0 - 4.58]
Rotifers	0.07	Moderate	% Abundance	Positive	[0 - 0.23]
Mites	0.08	Moderate	% Abundance	Positive	[0 - 0.8]
Collembola	0.05	Moderate	% Abundance	Positive	[0 - 0.96]
Basidiomycota : Ascomycota	-2.37	Moderate	Log Ratio	Positive	[-3.64 - 0]
Bacterial efficiency	57.83	Very-High	Unitless	Positive	[10.45 - 53.56]
Benefical Microbes					
Proteobacteria	23.71	Low	% Abundance	Optimum	[20.29 - 50.67]
Actinobacteria	16.87	Moderate	% Abundance	Optimum	[2.59 - 37.44]
Bacteroidetes	7.06	High	% Abundance	Optimum	[0.91 - 11.54]
Firmicutes	12.29	Very-High	% Abundance	Optimum	[0.09 - 7.02]
Plant Benefical fungi	25	Moderate	% Abundance	Optimum	[10.7 - 57.94]
Endomycorrhizal fungi (AMF)	1.58	High	% Abundance	Positive	[0 - 2.23]
Ectomycorrhizal fungi	1.37	Moderate	% Abundance	Positive	[0 - 4.76]
Biodiversity					
Fungal diversity	17.52	High	Unitless	Positive	[4.63 - 19.86]
Bacterial diversity	82.74	Very-High	Unitless	Positive	[25.24 - 82.16]
Protozoan diversity	10.65	Low	Unitless	Positive	[5.78 - 25.84]
Mesofaunal diversity	14.25	High	Unitless	Positive	[3.49 - 16.45]
Nematode diversity	9.87	High	Unitless	Positive	[3.07 - 11.7]
Soilborne Pathogens					
Sclerotinia	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Macrophomina	0	Very-Low	% Abundance	Negative	[0 - 0.04]
Fusarium	4.35	High	% Abundance	Negative	[0 - 5.64]
Verticillium	0.82	Very-High	% Abundance	Negative	[0 - 0.71]
Rhizoctonia	0.29	High	% Abundance	Negative	[0 - 0.7]
Phytophthora	0.14	Very-High	% Abundance	Negative	[0 - 0.01]
Pythium	0.36	Moderate	% Abundance	Negative	[0 - 3.09]

Client: site 6,site 5,site 1,site 2,site 7,site 8,site 3,site 4,site 10,site 9 | Blocks: site 6 , site 5 , site 1 , site 2 , site 7 , site 8 , site 3 , site 4 , site 10 , site 9

site 8

	Score	Classification	Units	Response Type	Range
Soil Properties					
pH	7.85	High	H+	Optimum	[5.7 - 7.98]
Carbon					
Fungi : Protists	2.41	Moderate	Log Ratio	Positive	[0 - 3.49]
Bacteria : Cyano	7.53	Low	Log Ratio	Positive	[6.25 - 11.14]
Saprotrophic fungi	41.92	Low	% Abundance	Positive	[30.33 - 79.19]
Active carbon (POXC)	499.16	Moderate	mg / kg	Positive	[250.61 - 1283.55]
B-Glucosidase	5.45	Moderate	ug / g/ hr	Positive	[4.2 - 6.05]
Pest Suppression					
Entomopathogenic Fungi	0.56	Very-High	% Abundance	Optimum	[0 - 0.32]
Trichoderma	0.43	Moderate	% Abundance	Optimum	[0 - 1.24]
Mycoparasitic Fungi	2.93	Moderate	% Abundance	Optimum	[0.76 - 11.47]
Maturity					
Annelids	0.46	Moderate	% Abundance	Positive	[0 - 4.58]
Rotifers	0.08	High	% Abundance	Positive	[0 - 0.23]
Mites	0.29	High	% Abundance	Positive	[0 - 0.8]
Collembola	0.02	Moderate	% Abundance	Positive	[0 - 0.96]
Basidiomycota : Ascomycota	-2.28	Moderate	Log Ratio	Positive	[-3.64 - 0]
Bacterial efficiency	58.05	Very-High	Unitless	Positive	[10.45 - 53.56]
Benefical Microbes					
Proteobacteria	26.44	Moderate	% Abundance	Optimum	[20.29 - 50.67]
Actinobacteria	19.12	Moderate	% Abundance	Optimum	[2.59 - 37.44]
Bacteroidetes	5.05	Moderate	% Abundance	Optimum	[0.91 - 11.54]
Firmicutes	8.87	Very-High	% Abundance	Optimum	[0.09 - 7.02]
Plant Benefical fungi	20.94	Moderate	% Abundance	Optimum	[10.7 - 57.94]
Endomycorrhizal fungi (AMF)	1.41	High	% Abundance	Positive	[0 - 2.23]
Ectomycorrhizal fungi	0.44	Moderate	% Abundance	Positive	[0 - 4.76]
Biodiversity					
Fungal diversity	17.15	High	Unitless	Positive	[4.63 - 19.86]
Bacterial diversity	82.3	Very-High	Unitless	Positive	[25.24 - 82.16]
Protozoan diversity	12	Moderate	Unitless	Positive	[5.78 - 25.84]
Mesofaunal diversity	19.73	Very-High	Unitless	Positive	[3.49 - 16.45]
Nematode diversity	14.15	Very-High	Unitless	Positive	[3.07 - 11.7]
Soilborne Pathogens					
Sclerotinia	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Macrophomina	0	Very-Low	% Abundance	Negative	[0 - 0.04]
Fusarium	6.84	Very-High	% Abundance	Negative	[0 - 5.64]
Verticillium	0.3	High	% Abundance	Negative	[0 - 0.71]
Rhizoctonia	0.05	Moderate	% Abundance	Negative	[0 - 0.7]
Phytophthora	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Pythium	1.31	High	% Abundance	Negative	[0 - 3.09]

Client: site 6,site 5,site 1,site 2,site 7,site 8,site 3,site 4,site 10,site 9 | Blocks: site 6 , site 5 , site 1 , site 2 , site 7 , site 8 , site 3 , site 4 , site 10 , site 9

site 3

	Score	Classification	Units	Response Type	Range
Soil Properties					
pH	6.67	Moderate	H+	Optimum	[5.7 - 7.98]
Carbon					
Fungi : Protists	1.63	Moderate	Log Ratio	Positive	[0 - 3.49]
Bacteria : Cyano	4.24	Very-Low	Log Ratio	Positive	[6.25 - 11.14]
Saprotrophic fungi	63.08	Moderate	% Abundance	Positive	[30.33 - 79.19]
Active carbon (POXC)	617.49	Moderate	mg / kg	Positive	[250.61 - 1283.55]
B-Glucosidase	5.02	Moderate	ug / g/ hr	Positive	[4.2 - 6.05]
Pest Suppression					
Entomopathogenic Fungi	0	Very-Low	% Abundance	Optimum	[0 - 0.32]
Trichoderma	0	Very-Low	% Abundance	Optimum	[0 - 1.24]
Mycoparasitic Fungi	5.87	Moderate	% Abundance	Optimum	[0.76 - 11.47]
Maturity					
Annelids	0	Very-Low	% Abundance	Positive	[0 - 4.58]
Rotifers	0.07	Moderate	% Abundance	Positive	[0 - 0.23]
Mites	0	Very-Low	% Abundance	Positive	[0 - 0.8]
Collembola	1.15	Very-High	% Abundance	Positive	[0 - 0.96]
Basidiomycota : Ascomycota	-5.22	Very-Low	Log Ratio	Positive	[-3.64 - 0]
Bacterial efficiency	68.86	Very-High	Unitless	Positive	[10.45 - 53.56]
Benefical Microbes					
Proteobacteria	27.18	Moderate	% Abundance	Optimum	[20.29 - 50.67]
Actinobacteria	19.52	Moderate	% Abundance	Optimum	[2.59 - 37.44]
Bacteroidetes	3.31	Moderate	% Abundance	Optimum	[0.91 - 11.54]
Firmicutes	7.21	Very-High	% Abundance	Optimum	[0.09 - 7.02]
Plant Benefical fungi	33.07	Moderate	% Abundance	Optimum	[10.7 - 57.94]
Endomycorrhizal fungi (AMF)	0.14	Moderate	% Abundance	Positive	[0 - 2.23]
Ectomycorrhizal fungi	0	Very-Low	% Abundance	Positive	[0 - 4.76]
Biodiversity					
Fungal diversity	12.64	Moderate	Unitless	Positive	[4.63 - 19.86]
Bacterial diversity	64.52	Moderate	Unitless	Positive	[25.24 - 82.16]
Protozoan diversity	20.41	Moderate	Unitless	Positive	[5.78 - 25.84]
Mesofaunal diversity	8.72	Moderate	Unitless	Positive	[3.49 - 16.45]
Nematode diversity	7.79	Moderate	Unitless	Positive	[3.07 - 11.7]
Soilborne Pathogens					
Sclerotinia	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Macrophomina	0	Very-Low	% Abundance	Negative	[0 - 0.04]
Fusarium	9.45	Very-High	% Abundance	Negative	[0 - 5.64]
Verticillium	2.06	Very-High	% Abundance	Negative	[0 - 0.71]
Rhizoctonia	0	Very-Low	% Abundance	Negative	[0 - 0.7]
Phytophthora	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Pythium	7.67	Very-High	% Abundance	Negative	[0 - 3.09]

Client: site 6,site 5,site 1,site 2,site 7,site 8,site 3,site 4,site 10,site 9 | Blocks: site 6 , site 5 , site 1 , site 2 , site 7 , site 8 , site 3 , site 4 , site 10 , site 9

site 4

	Score	Classification	Units	Response Type	Range
Soil Properties					
pH	8.03	Very-High	H+	Optimum	[5.7 - 7.98]
Carbon					
Fungi : Protists	2.97	High	Log Ratio	Positive	[0 - 3.49]
Bacteria : Cyano	7.51	Low	Log Ratio	Positive	[6.25 - 11.14]
Saprotrophic fungi	64.64	Moderate	% Abundance	Positive	[30.33 - 79.19]
Active carbon (POXC)	544.24	Moderate	mg / kg	Positive	[250.61 - 1283.55]
B-Glucosidase	5.31	Moderate	ug / g/ hr	Positive	[4.2 - 6.05]
Pest Suppression					
Entomopathogenic Fungi	0	Very-Low	% Abundance	Optimum	[0 - 0.32]
Trichoderma	0.52	High	% Abundance	Optimum	[0 - 1.24]
Mycoparasitic Fungi	4.72	Moderate	% Abundance	Optimum	[0.76 - 11.47]
Maturity					
Annelids	0.96	Moderate	% Abundance	Positive	[0 - 4.58]
Rotifers	0	Very-Low	% Abundance	Positive	[0 - 0.23]
Mites	0	Very-Low	% Abundance	Positive	[0 - 0.8]
Collembola	0	Very-Low	% Abundance	Positive	[0 - 0.96]
Basidiomycota : Ascomycota	-2.9	Low	Log Ratio	Positive	[-3.64 - 0]
Bacterial efficiency	63.21	Very-High	Unitless	Positive	[10.45 - 53.56]
Benefical Microbes					
Proteobacteria	32.5	Moderate	% Abundance	Optimum	[20.29 - 50.67]
Actinobacteria	17.65	Moderate	% Abundance	Optimum	[2.59 - 37.44]
Bacteroidetes	4.52	Moderate	% Abundance	Optimum	[0.91 - 11.54]
Firmicutes	3.98	High	% Abundance	Optimum	[0.09 - 7.02]
Plant Benefical fungi	32.43	Moderate	% Abundance	Optimum	[10.7 - 57.94]
Endomycorrhizal fungi (AMF)	0.1	Moderate	% Abundance	Positive	[0 - 2.23]
Ectomycorrhizal fungi	0.45	Moderate	% Abundance	Positive	[0 - 4.76]
Biodiversity					
Fungal diversity	16.63	High	Unitless	Positive	[4.63 - 19.86]
Bacterial diversity	65.82	Moderate	Unitless	Positive	[25.24 - 82.16]
Protozoan diversity	10.4	Low	Unitless	Positive	[5.78 - 25.84]
Mesofaunal diversity	11.58	Moderate	Unitless	Positive	[3.49 - 16.45]
Nematode diversity	9.34	High	Unitless	Positive	[3.07 - 11.7]
Soilborne Pathogens					
Sclerotinia	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Macrophomina	0	Very-Low	% Abundance	Negative	[0 - 0.04]
Fusarium	12.21	Very-High	% Abundance	Negative	[0 - 5.64]
Verticillium	1.49	Very-High	% Abundance	Negative	[0 - 0.71]
Rhizoctonia	0.24	Moderate	% Abundance	Negative	[0 - 0.7]
Phytophthora	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Pythium	3.57	Very-High	% Abundance	Negative	[0 - 3.09]

Client: site 6,site 5,site 1,site 2,site 7,site 8,site 3,site 4,site 10,site 9 | Blocks: site 6 , site 5 , site 1 , site 2 , site 7 , site 8 , site 3 , site 4 , site 10 , site 9

site 10

	Score	Classification	Units	Response Type	Range
Soil Properties					
pH	7.83	High	H+	Optimum	[5.59 - 7.97]
Carbon					
Fungi : Protists	3.67	Very-High	Log Ratio	Positive	[0.13 - 3.35]
Bacteria : Cyano	11.28	High	Log Ratio	Positive	[6.91 - 11.33]
Saprotrophic fungi	59.99	Moderate	% Abundance	Positive	[32.78 - 74.11]
Active carbon (POXC)	-155.24	Very-Low	mg / kg	Positive	[162.06 - 1184.44]
B-Glucosidase	5.11	Moderate	ug / g/ hr	Positive	[4.36 - 5.94]
Pest Suppression					
Entomopathogenic Fungi	0	Very-Low	% Abundance	Optimum	[0 - 0.11]
Trichoderma	0.42	Moderate	% Abundance	Optimum	[0 - 1.67]
Mycoparasitic Fungi	3.96	Moderate	% Abundance	Optimum	[0.69 - 13.12]
Maturity					
Annelids	3.94	Moderate	% Abundance	Positive	[0 - 11.06]
Rotifers	0	Very-Low	% Abundance	Positive	[0 - 0.21]
Mites	4.06	Very-High	% Abundance	Positive	[0 - 1.1]
Collembola	0.74	High	% Abundance	Positive	[0 - 0.93]
Basidiomycota : Ascomycota	-2.82	Moderate	Log Ratio	Positive	[-3.58 - -0.8]
Bacterial efficiency	41.24	Moderate	Unitless	Positive	[9.97 - 54.75]
Benefical Microbes					
Proteobacteria	23.96	Low	% Abundance	Optimum	[23 - 53.22]
Actinobacteria	24.56	High	% Abundance	Optimum	[2.84 - 28.59]
Bacteroidetes	3.6	Moderate	% Abundance	Optimum	[1.23 - 10.07]
Firmicutes	2.01	Moderate	% Abundance	Optimum	[0.14 - 5.62]
Plant Benefical fungi	42.15	Moderate	% Abundance	Optimum	[10.62 - 50.92]
Endomycorrhizal fungi (AMF)	0.68	Moderate	% Abundance	Positive	[0 - 2.94]
Ectomycorrhizal fungi	1.32	Moderate	% Abundance	Positive	[0 - 4.35]
Biodiversity					
Fungal diversity	17.1	High	Unitless	Positive	[7.03 - 19.2]
Bacterial diversity	73.82	High	Unitless	Positive	[30.95 - 84.01]
Protozoan diversity	7.45	Low	Unitless	Positive	[6.82 - 24.55]
Mesofaunal diversity	6.39	Moderate	Unitless	Positive	[4.08 - 15.8]
Nematode diversity	6.67	Moderate	Unitless	Positive	[3.49 - 12.28]
Soilborne Pathogens					
Sclerotinia	0	Very-Low	% Abundance	Negative	[0 - 0]
Macrophomina	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Fusarium	7.36	High	% Abundance	Negative	[0.04 - 10.62]
Verticillium	0.96	High	% Abundance	Negative	[0 - 1.65]
Rhizoctonia	0.38	High	% Abundance	Negative	[0 - 0.93]
Phytophthora	0.03	High	% Abundance	Negative	[0 - 0.26]
Pythium	0.27	Moderate	% Abundance	Negative	[0 - 5.01]

Client: site 6,site 5,site 1,site 2,site 7,site 8,site 3,site 4,site 10,site 9 | Blocks: site 6 , site 5 , site 1 , site 2 , site 7 , site 8 , site 3 , site 4 , site 10 , site 9

site 9

	Score	Classification	Units	Response Type	Range
Soil Properties					
pH	7.81	High	H+	Optimum	[5.59 - 7.97]
Carbon					
Fungi : Protists	3.11	High	Log Ratio	Positive	[0.13 - 3.35]
Bacteria : Cyano	11.32	High	Log Ratio	Positive	[6.91 - 11.33]
Saprotrophic fungi	58.95	Moderate	% Abundance	Positive	[32.78 - 74.11]
Active carbon (POXC)	306.41	Moderate	mg / kg	Positive	[162.06 - 1184.44]
B-Glucosidase	5.33	Moderate	ug / g/ hr	Positive	[4.36 - 5.94]
Pest Suppression					
Entomopathogenic Fungi	0	Very-Low	% Abundance	Optimum	[0 - 0.11]
Trichoderma	0	Very-Low	% Abundance	Optimum	[0 - 1.67]
Mycoparasitic Fungi	3.42	Moderate	% Abundance	Optimum	[0.69 - 13.12]
Maturity					
Annelids	4.3	High	% Abundance	Positive	[0 - 11.06]
Rotifers	0.08	Moderate	% Abundance	Positive	[0 - 0.21]
Mites	1.12	Very-High	% Abundance	Positive	[0 - 1.1]
Collembola	0.93	Very-High	% Abundance	Positive	[0 - 0.93]
Basidiomycota : Ascomycota	-3.04	Low	Log Ratio	Positive	[-3.58 - -0.8]
Bacterial efficiency	45.19	High	Unitless	Positive	[9.97 - 54.75]
Benefical Microbes					
Proteobacteria	29.69	Moderate	% Abundance	Optimum	[23 - 53.22]
Actinobacteria	21.53	High	% Abundance	Optimum	[2.84 - 28.59]
Bacteroidetes	3.24	Moderate	% Abundance	Optimum	[1.23 - 10.07]
Firmicutes	2.62	Moderate	% Abundance	Optimum	[0.14 - 5.62]
Plant Benefical fungi	43.69	High	% Abundance	Optimum	[10.62 - 50.92]
Endomycorrhizal fungi (AMF)	1.43	High	% Abundance	Positive	[0 - 2.94]
Ectomycorrhizal fungi	1.36	Moderate	% Abundance	Positive	[0 - 4.35]
Biodiversity					
Fungal diversity	13.36	Moderate	Unitless	Positive	[7.03 - 19.2]
Bacterial diversity	70.74	Moderate	Unitless	Positive	[30.95 - 84.01]
Protozoan diversity	12.24	Moderate	Unitless	Positive	[6.82 - 24.55]
Mesofaunal diversity	17.16	Very-High	Unitless	Positive	[4.08 - 15.8]
Nematode diversity	9.42	Moderate	Unitless	Positive	[3.49 - 12.28]
Soilborne Pathogens					
Sclerotinia	0	Very-Low	% Abundance	Negative	[0 - 0]
Macrophomina	0	Very-Low	% Abundance	Negative	[0 - 0.01]
Fusarium	16.87	Very-High	% Abundance	Negative	[0.04 - 10.62]
Verticillium	0.95	High	% Abundance	Negative	[0 - 1.65]
Rhizoctonia	0.52	High	% Abundance	Negative	[0 - 0.93]
Phytophthora	0	Very-Low	% Abundance	Negative	[0 - 0.26]
Pythium	0.32	Moderate	% Abundance	Negative	[0 - 5.01]

Client: site 6,site 5,site 1,site 2,site 7,site 8,site 3,site 4,site 10,site 9 | Blocks: site 6 , site 5 , site 1 , site 2 , site 7 , site 8 , site 3 , site 4 , site 10 , site 9

Comparison Table

	site 6	site 10	site 9	site 1	site 2	site 7	site 8	site 3	site 4	site 5
Soil Properties										
pH	6.42	7.83	7.81	7.92	8.38	6.35	7.85	6.67	8.03	7.07
Carbon										
Fungi : Protists	2.65	3.67	3.11	2.18	2.35	2.73	2.41	1.63	2.97	2.65
Bacteria : Cyano	12.19	11.28	11.32	8.54	6.57	5.99	7.53	4.24	7.51	6.39
Saprotrophic fungi	63.55	59.99	58.95	62.94	43.01	47.13	41.92	63.08	64.64	62.95
Active carbon (POXC)	527.14	-155.24	306.41	370.97	541.42	748.50	499.16	617.49	544.24	746.14
B-Glucosidase	5.09	5.11	5.33	4.81	5.04	5.06	5.45	5.02	5.31	5.29
Pest Suppression										
Entomopathogenic Fungi	0.00	0.00	0.00	0.00	0.12	0.44	0.56	0.00	0.00	0.00
Trichoderma	0.19	0.42	0.00	0.00	0.16	0.59	0.43	0.00	0.52	0.33
Mycoparasitic Fungi	4.30	3.96	3.42	2.08	5.46	3.75	2.93	5.87	4.72	3.13
Maturity										
Annelids	0.58	3.94	4.30	0.00	0.52	0.89	0.46	0.00	0.96	0.08
Rotifers	0.07	0.00	0.08	0.06	0.00	0.07	0.08	0.07	0.00	0.00
Mites	0.56	4.06	1.12	0.01	4.18	0.08	0.29	0.00	0.00	0.01
Collembola	0.06	0.74	0.93	0.00	0.10	0.05	0.02	1.15	0.00	1.01
Basidiomycota : Ascomycota	-3.04	-2.82	-3.04	-2.55	-0.93	-2.37	-2.28	-5.22	-2.90	-2.73
Bacterial efficiency	71.61	41.24	45.19	45.49	57.19	57.83	58.05	68.86	63.21	51.77
Benefical Microbes										
Proteobacteria	34.92	23.96	29.69	31.66	27.69	23.71	26.44	27.18	32.50	29.93
Actinobacteria	27.44	24.56	21.53	20.59	17.62	16.87	19.12	19.52	17.65	17.72
Bacteroidetes	6.81	3.60	3.24	4.23	6.35	7.06	5.05	3.31	4.52	6.66
Firmicutes	2.81	2.01	2.62	2.48	7.53	12.29	8.87	7.21	3.98	3.17
Plant Benefical fungi	22.18	42.15	43.69	31.17	19.09	25.00	20.94	33.07	32.43	30.51
Endomycorrhizal fungi (AMF)	0.11	0.68	1.43	0.01	0.36	1.58	1.41	0.14	0.10	0.44
Ectomycorrhizal fungi	0.10	1.32	1.36	6.11	0.70	1.37	0.44	0.00	0.45	1.10
Biodiversity										
Fungal diversity	13.73	17.10	13.36	9.48	17.23	17.52	17.15	12.64	16.63	14.31
Bacterial diversity	87.47	73.82	70.74	64.43	91.40	82.74	82.30	64.52	65.82	66.49
Protozoan diversity	16.88	7.45	12.24	22.69	16.56	10.65	12.00	20.41	10.40	21.94
Mesofaunal diversity	15.12	6.39	17.16	7.34	5.12	14.25	19.73	8.72	11.58	9.37
Nematode diversity	12.33	6.67	9.42	6.38	15.20	9.87	14.15	7.79	9.34	7.32
Soilborne Pathogens										
Sclerotinia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Macrophomina	0.10	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.11
Fusarium	7.07	7.36	16.87	14.40	3.49	4.35	6.84	9.45	12.21	10.16
Verticillium	1.12	0.96	0.95	0.71	1.94	0.82	0.30	2.06	1.49	0.52
Rhizoctonia	0.08	0.38	0.52	0.06	0.17	0.29	0.05	0.00	0.24	0.95
Phytophthora	0.00	0.03	0.00	0.84	0.63	0.14	0.00	0.00	0.00	0.01
Pythium	1.37	0.27	0.32	2.17	1.63	0.36	1.31	7.67	3.57	5.01

Classes Very-Low Low Moderate High Very-High

***Stemphylium vesicarium*, *Peronospora destructor* and *Alternaria porri* qPCR protocol**

Primer details:

For the *S. vesicarium* assay CMD and GAPDH gene sequences from multiple isolates were aligned in Geneious, and SNPs unique to *S. vesicarium* were identified. For *A. porri* sequences of the Alt gene were retrieved from GenBank and aligned across multiple *Alternaria* taxa. The Alt gene was selected because it had the greatest representation of *A. porri* sequences in NCBI, providing a stronger basis for primer and probe design, however it should be noted sequences were of mixed quality. Primers and probes were designed in Primer3, incorporating discriminatory SNPs at the 3' terminus. Candidate sequences were tested for specificity using BLASTn. Primers for *P. destructor* were already available in the published literature.
https://apsjournals.apsnet.org/doi/10.1094/PDIS-05-20-1095-RE

All primer and probes were ordered from integrated DNA technologies. The details are shown in the below table. Due to the lack of an *Alternaria porri* isolate we have not yet validated this PCR.

Table 1. Primer and probe details.

Name	notes	3' Sequence 5'	Gene	Target species
SvCAL157F *	Foward	ACCAAAACCCTCACTAACCACAA	Calmodulin	*S. vesicarium*
SvCAL157R *	Reverse	TCATGACGTGACGCAGTTCA		
SvCAL157 P*	HEX probe	CGACTCTGAGGAGGAGATTCGGGA		
SvGA225F	Foward	CGTAAGTTTGATTGAGCCCGTT	GAPDH	
SvGA225R	Reverse	GTTAGCCTGCAATCCACTAGG		
SvGA225P	Fam Probe	CACTACGCCGTAGGTATCCCCG		
Pd ITSF	Foward	GGCTGTGAGTCCTTTGAAATGTATG	ITS	*P. destructor*
Pd ITSR	Reverse	CGAATCGAACACTCCTCCATTG		
Pd_ITSP	Fam probe	TGCTGGTTGTGAAGGCTGTCAGTA		
Ap_Alt_181F**	Foward	GACAAGCTCGAGGACCACA	Alt a1	*A.porri* *A. danida* *A. vanuatuensis* *A. anagallidis* *Alternaria sp.* (JD-2019/YZU strains)
Ap_Alt_181R**	Reverse	CGTAGGTGATGCTGGACAGAA		
Ap_Alt_181P**	Hex probe	TTGCCCTCGCGCTTTTCACTACCT		

*Both sets of Stemphylium primers work well and can be used. For our project we primarily used the SvCAL primers as they had better qPCR efficiency values.

** These primers may amplify other *Alternaria sp.* aside from *A. Porri*. Since we lacked a *A. porri* isolate we were unable to validate this assay.

DNA extraction and standard curve:

DNA was extracted from plant material or pure fungal cultures using the Qiagen DNeasy plant pro kit according to the manufacturer's instructions. DNA was quantified using the Promega quantiflour dsDNA system and a biotek plate reader as per the manufacturer's instructions. A standard curve was made using Stemphylium vesicarium genomic DNA at 50ng/ul, 5ng/ul, 0.5ng/ul, 0.05ng/ul, 0.005ng/ul and 0.0005ng/ul and was used as template DNA in triplicate for all qPCRs. Since *P. destructor* is an obligate plant pathogen DNA could not be extracted from pure cultures to make a standard curve, so PCRs were run as real time PCR without the quantitative aspect.

PCR set up and cycling conditions

Total volume of PCR reactions was 15ul using millennium science sensi fast qPCR probe mix as per manufacturer's instructions. Primers were used at a final concentration of 0.25uM. Fluorescent probe was used at a final concentration of 150mM. 2ul of template DNA was used from the standard curve or directly from kit DNA extractions of target samples. PCR cycling conditions are shown in **Table 2.**

Table 2. PCR cycling conditions.

	34 cycles		
Initial denaturation	Denaturation	Annealing	Extension *
95°C 3:00	95°C 0:10	59°C 0:10	72°C 0:20

*Fluorescent reads taken at this point.