Hort Innovation

Final Report

Developing knowledge and management of Strawberry Red Leaf Disorder

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Delivery partner:

Queensland Department of Agriculture & Fisheries

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Developing knowledge and management of Strawberry Red Leaf Disorder (BS19001)

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Content

Summary

This research project conducted by the Department of Agriculture and Fisheries (DAF) and the University of Queensland (UQ), and co-funded through Hort Innovation, has led to the identification of potential causal agents of the strawberry Red Leaf Disorder (RLD). A better understanding of the spread and economic impact RLD has on the commercial Queensland (QLD) strawberry industry has resulted.

RLD is characterised by reddish/maroon interveinal discolouration of the leaves and typically reduces plant vigour and yield. RLD is currently limiting the fruiting productivity and plant health of the major cultivars grown in QLD. Investigations into potential causes of RLD by DAF and UQ, have found no obvious single causal agent, suggesting that the disorder and mechanisms behind its transmission may be quite complex.

To identify the cause, a comprehensive approach was taken to explore the possible causes of RLD, that included Biological indexing, Transmission Electron Microscopy (TEM), fungal isolations, molecular, microbiological and microscopy studies to further explore or eliminate possible causal agents. A farm survey and yield trial were also undertaken to examine the impacts this disorder can have on the strawberry industry.

To better understand the threat that RLD poses for the strawberry industry and to continue the work DAF commenced in the 2019 season, the visual appearance and severity of RLD was examined through broadscale surveys of farms in South East Queensland. Eight farms (one a substrate farm) were surveyed once a month for RLD. Results from this survey showed cultivars Parisienne Kiss, Sundrench and Festival displayed the highest incidence of RLD with maximum percentages of 17%, 10% and 8% respectively. In 2019, a higher percentage of RLD was observed than in 2020 e.g., Scarlet Rose 39% RLD, Rubygem 15% and Festival 6%.

All the farms surveyed in 2020 had RLD present on their properties, with observations of RLD in both as runners and plug plants. A low percentage of cv. Red Rhapsody plants in substrate showed RLD symptoms and were identified only on water-stressed plants and symptoms later diminished when water supply issues were corrected. This suggests that stress, including water-stress, may play a major role in occurrence of the disorder.

In addition, a yield comparative study of 45 plants of cv. Red Rhapsody was undertaken to determine the effects of RLD on fruit quality and quantity and, overall plant health. Plants were examined weekly and rated for RLD severity, fruit weight, fruit numbers and quality. RLD rating (severity) increased progressively over subsequent weeks throughout the season. However, towards the end of the season some plants recovered to some extent, which coincided with what was observed on the eight farms surveyed in 2020. All 45 plants showed some degree of reddening, with six mortalities. There was an obvious progressive decline in yield and fruit quality as reddening developed. Differences in root length and browning were also apparent between plants both with and without RLD. We identified more root length variation in treatment plants with RLD, and plants with RLD had more root browning, and shorter roots. Plants without RLD had longer roots and less browning. Plants with no RLD had longer and more uniform leaf length (including petioles), all over 30 cm, compared to those with RLD which ranged from 18–28 cm. The number, weight and quality of 1st grade fruit progressively decreased as RLD severity increased. RLD rating, day (Time of observation), their interaction, and root browning (%) all showed statistically significant relationships with Class 1 (1st grade) fruit weight, and total fruit weight.

RLD affected plants were biologically tested by grafting suspect plants to indicator plants (genotypes free from RLD but susceptible) to determine if RLD is transmittable via vascular tissue transfer. Graft transmission with several varieties showed no RLD symptom transmission.

Laboratory fungal isolations of the crowns and leaves from healthy and RLD symptomatic plants were undertaken by DAF to determine the presence of fungal pathogens that could be associated with RLD. Pathology testing of plants with and without RLD symptoms have produced inconclusive results to support the notion that a fungal pathogen maybe the causal agent for RLD.

Strawberry plants with symptoms of RLD and comparative asymptomatic controls were analysed under the transmission electron microscope (TEM) and by PCR by DAF. A range of virus particles or virus-like particles were observed in the strawberry leaf samples. Possible fragments of closterovirus-like particles were detected in TEM analyses. Additionally, nucleorhabdovirus particles with dimensions of 47 x 330 nm were detected in one symptomatic sample and phage particles (viruses of bacteria) were observed in the root sample, likely originating from rhizosphere bacteria.

Preliminary molecular analysis from RLD plants prior to this project commencing did not indicate any strong causal

bacterial, fungal, or viral agents. A more comprehensive and controlled suite of molecular investigations were undertaken in this project using plants displaying varying degrees of RLD symptoms selected from farms and glasshouses in comparison to RLD free plants produced at the Maroochy Research Facility. While the molecular data did not identify any clear pathogens that were specific to only RLD infected plants, a number of organisms, particularly Phytoplasma, were present in all samples from 2021 sequencing data. It may be that these species, along with viruses they contain, may contribute to the RLD phenotype in the presence of other biotic or abiotic constraints. Ongoing molecular analysis is needed to verify the ongoing relationship between the potential targets and RLD in subsequent seasons. It should also be noted that the mite sequences identified prior to this project commencing were not identified in the subsequent analyses. Transcriptome analyses of plants demonstrating various stages of RLD symptoms compared to RLD-free controls should be undertaken to identify plant response genes or pathways that are differentially regulated based on disorder state.

Several industry engagement and communication activities were undertaken, including attendance and presentations at meetings publication in the industry newsletter, Acta Hort, and farm visits in South East QLD to ensure that growers and industry associates (including researchers) were up to date with the latest information about the progress of the RLD project. Project team members delivered five presentations, including a virtual presentation at the International Strawberry Symposium. This research project is relevant to growers, plant production providers, consultants, and service providers within the strawberry industry across Australia, in particular, to those individuals currently affected by RLD. The outputs will be beneficial to research scientists working on RLD by adding to our knowledge of this unknown disorder. The project utilised current communication programs in the strawberry industry to update stakeholders and deliver results of the project through farm visits, industry meetings, articles, and presentations.

This research study (Phase 1) has delivered data and useful information enabling future research to build upon existing foundations. Strawberry growers and industry can get closer to knowing the cause of RLD. In addition, this research has identified the extent of damage RLD can have on infected plants. At the commencement of this project, it was known that identifying a causative agent may not be achievable within the short time frame of the project, however the outcomes of our research thus far provide sufficient direction to aid in constructing a future PhD project (Phase 2).

Keywords

Fragaria × ananassa; fragariae; epidemiology; syndrome; pathogen; genomic.

Introduction

Red Leaf Disorder (RLD) was first observed in 2014, on plants growing in a fruit production field in South East Queensland (SEQ) (DAF). Since then, the occurrence and significance of RLD has increased, with RLD now reported in Western Australia and New South Wales. In Australia, RLD is most prevalent within the SEQ production region. SEQ is the largest strawberry producing region in Queensland, producing 34,570 t in 2019/20, approximately 42% of Australian production (Hort Innovation, 2020).

RLD is characterised by reddish/maroon interveinal discolouration of the leaves and typically reduces plant vigour and yield. RLD is currently limiting the fruiting productivity and plant health of the major cultivars grown in QLD. To better understand the scale and impact RLD poses for the strawberry industry, a survey of plants showing RLD commenced in the region of SEQ during the 2020 fruiting season. Fifteen percent of plants were surveyed in a field per cultivar, with over 220,000 plants viewed over the course of the season. We examined a diverse range of farm types, cultivars, and planting material to expand our data from the 2019 DAF RLD survey. A substrate production farm (Farm 3) was included in the survey to compare the incidence of RLD, in cv. Red Rhapsody plants, from both bare-root and plug style plants.

A comparison yield trial was also undertaken in 2020 to examine RLD severity, yield and root observations, total fruit weight (g), number of fruits and fruit quality between RLD-affected and non-RLD-affected plants. Plants were assessed throughout the season for RLD incidence and dug from the ground at the end of season to assess root, crown, and leaf ratings.

Two biological indexing methods were used in the previous RLD DAF project (i) biological indexing by petiole graft inoculation, and (ii) herbaceous indexing using *Chenopodium* quinoa onto the indicators *Fragaria vesca* clones UC4 and UC6 and *Fragaria virginiana* clone UC10. No transmission of the reddening occurred from these tests, indicating that RLD may not be transmitted by vascular tissue or transmission is temperamental or environment dependant. Thus, in this study we continued biological testing by grafting symptomatic leaves onto various susceptible cultivars.

Currently, a metagenomics analysis of 25 samples of different varieties has been completed by Dr Fiona Constable's team from The Biosciences Research Centre (AgriBio). To date no significant association has been found for common strawberry viruses including: Strawberry mild yellow edge, Strawberry crinkle, Strawberry necrotic shock, Strawberry pallidosis-associated virus, Strawberry mottle, Strawberry vein banding, or Beet pseudo yellows viruses. Samples were originally screened for the presence of viruses, phytoplasmas, rickettsia, fungal and bacterial pathogens. Internal transcribed spacers and 16S amplicon HTS were used to assess fungal and bacterial populations. In this project, a more thorough search for a potential candidate within the existing data was undertaken, using a larger cross-organisational project team incorporating a broader range of experts in the relevant fields. DAF colleagues, Dr David Innes, and Matthew Webb, and UQ's Reuben Brown each have extensive experience in bioinformatics, data analysis and information management of genomic, metagenomic, pangenomic and transcriptomic data for diverse applications including diagnostics and discovery. This allowed a deeper analysis of the pre-existing molecular work and built on the new molecular sequencing work carried out.

The objective of this project was to help address the challenges and build on the knowledge from the initial research work done by DAF and UQ, to identify the cause/s of RLD (Phase 1).

Methodology

Farm Survey

Eight strawberry fruiting farms in the SEQ region were selected to collect data on RLD incidence and severity in the 2020 fruiting season. Farms were visited every four weeks from May to September. Cultivars sampled included: Red Rhapsody, Scarlet Rose, Rubygem, Parisienne Kiss, Aussiegem, Florida Radiance (Fortuna) and Florida Festival.

To analyse the farm survey data, the Binomial GLMMs (Logistic Regressions) were performed using R package. The Akaike Information Criterion (AIC) method was used for assessing the quality of the model through comparison of related models. Odd ratio (OR) is a measure of association between an exposure and an outcome (least likely to develop RLD). OR or ORs are used to compare the relative odds of the occurrence of the outcome of interest (e.g., RLD), given exposure to the variable of interest (e.g., cultivar, farm). The odds ratio can also be used to determine whether a particular exposure is a risk factor for a particular outcome, and to compare the magnitude of various risk factors for that outcome. The proportion of plants with RLD per row was analysed. The best performing cultivar (Red Rhapsody) and farm (Farm 8) are used as reference values.

Plugs vs bare rooted plants

One farm (farm 3) with plants in substrate was included in our survey giving the opportunity to compare bare-rooted plantlets with plugs using the same cultivar. The farm consisted of two plots: Plot 1 consisted of bare-rooted plantlets of cv. Red Rhapsody, and plot 2 Red Rhapsody as plug plants. The distance between the plots was approximately 1.5 meters. These were planted at the same time in late March 2020, into coco peat bags in a tableponic substrate system. Plants were arranged with 8 plants per bag by 2 bags across a row, with approximately 1 m distance between rows. All plants received the same cultural practices (sprays, fertigation, watering, etc.).

Red Leaf Disorder, yield and root health data

We investigated the effect of RLD on crop yield and fruit quality in strawberry plants grown at Nambour, QLD. The plant leaves were scored on a symptom severity scale from 1-6, where rating 1 = healthy plant with no RLD symptoms, ratings 2 to 5 = progressively increasing of reddening and on an increasing number of leaves, rating 6 = plant death (Figure 1). We also measured fresh fruit weights (g), leaf length (cm), and fruit gradings (1st and 2nd class/grade, and 3rd class or waste). Measurements of leaf length (cm), root health and length (cm) were taken between two groups, a control group (no RLD), and the treatment group (showing RLD). Root ratings were determined on a scale from 1-6, where 1 = healthy roots, progressively increasing to a rating of 6 = shorter, unhealthy roots (Figure 2).

Figure 1 Rating scale for RLD. From L to R: 1 = healthy plant with no RLD, 6 = plant mortality (rating 6 not shown).

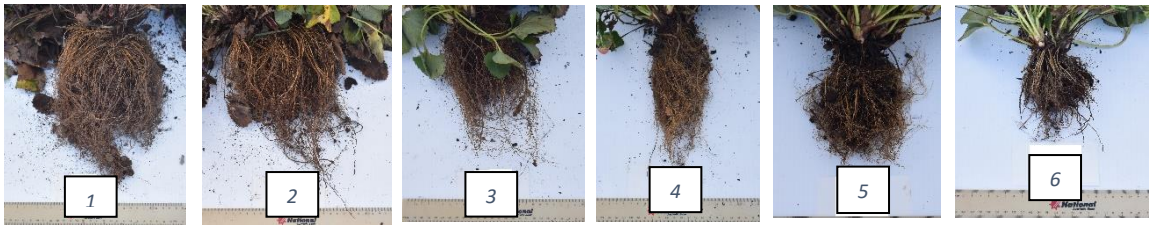

Figure 2 Root rating scale. 1 = healthy long roots, 6 = unhealthy short roots.

The relationships between Fruit Weight (the response) and RLD and some root & health (R&H) variables (used as predictors) during the trial, were obtained using Linear Mixed Models (LMMs). LMMs were implemented using the R package 'ASREML-R' (Butler, et al., 2017). The relationships between RLD and the root and health variables (used as predictors), were obtained using CLMs, GLMs, and GLMMs.

Red leaf fungal isolations

Laboratory fungal isolations of the crowns and leaves from healthy and RLD symptomatic plants were undertaken using methods described in Hutton et al. (2013). Leaf, crown, and root tissue pieces with and without necrotic lesions were placed on a Petri dish containing quarter-strength potato dextrose agar (PDA) amended with 50 ppm streptomycin sulphate (Sigma-Aldrich, St. Louis, MO, USA).

Biological indexing

In 2020 further tests were performed using graft inoculation of DAF candidate cultivar selections 2016-030, 2014-013, 2017-017, 2017-054, 2017-031, 2014-049-104 and Rubygem, using RLD symptomatic leaves from cv. Red Rhapsody (Figure 3). Petiole grafting was completed using the technique developed by Frazier (1974) and modified by the Department of Environment, Land, Water and Planning (DELWP) Victoria and DAF. The grafted RLD plants were maintained in a screened aphid-proof cage in a glasshouse, isolated from non-grafted controls. Graft unions were examined two weeks after inoculation to ensure graft success. Three grafts were made per plant. The grafted plants were examined weekly for symptoms over a four-month period. As no diagnostic test for RLD has yet been achieved, and due to symptomatic plants not always showing reddening symptoms, obtaining a confirmed negative control is problematic. Thus, we define 'negative controls as plants that have been keep in a secure high health glasshouse and have shown no RLD symptoms. Negative controls were also obtained from the Maroochy Research Facility tissue culture laboratory.

Figure 3 RLD leaf from cv Red Rhapsody grafted onto an indicator plant.

Microscopy analysis and PCR (DAF)

Samples from plants with symptoms of Strawberry RLD and comparative asymptomatic controls were analysed under a transmission electron microscope (Table 1). Samples were sent in two batches on 20th May 2020 and 19th October 2020. Symptomatic tissue (1-2 g) underwent partial purification and concentration before negative contrast staining with 1% ammonium molybdate and viewing under a JEM-1400 microscope (JEOL Inc, Japan). Micrographs were captured using an Orius CCD camera (Gatan Inc, USA).

PCR details for *Nanoviridae* testing: Generic primer pairs nano-STL-dir/nano-SLT-rev (Kniermin et al. 2019) and F103/R101 (Kumari et al. 2009) were used with MangoTaq PCR reagents (Bioline Inc., Australia) to index strawberry samples from the first batch. Subterannean clover stunt virus isolates 5224 and 5597 from the DAF Plant Virus Isolate Collection were included in the assay as positive controls. Samples were electrophoresed through 1.5% agarose-TBE prior to visualisation.

PCR details for *Luteoviridae* testing: Generic primer pairs PLF/PLR, Pol3628F/Pol3982F and Pol3870/AS3 (M. Sharman, DAF) were used with Invitrogen cDNA synthesis and PCR reagents (ThermoFisher Inc, Australia) as per the manufacturer's protocols. A variety of polerovirus and luteovirus controls were included alongside the strawberry samples. Samples were electrophoresed through 1.5% agarose-TBE prior to visualisation. Amplicons of the expected size were cut from the gels and nucleic acids purified with the Isolate II PCR and gel purification kit (Bioline Inc, Australia).

Table 1 Samples sent for TEM analysis. All samples were leaf samples, except sample four in batch one which was roots

Batch	Sample number	Sample details	Comments
1	1	Ageratum sp RL GH	Growing next to red leaf glasshouse
1	2	Parisienne Kiss green leaf	
1	3	Parisienne Kiss red leaf	
1	4	Parisienne Kiss red leaf, roots	
1	5	2017-247 green leaf (field)	
1	6	2017-247 red leaf	
1	7	2019-215 green leaf (field)	Purification box dropped; sample lost
1	8	2019-215 red leaf	
1	9	2019-216 green leaf (field)	
1	10	2019-216 red leaf	
1	11	Red rhapsody green leaf (field)	
1	12	Red rhapsody red leaf	
2	1	Ageratum sp RL GH	Not checked by VMP-TEM
2	2	2017-107 green leaf	
2	3	2017-107 red leaf	
2	4	2017-031 green leaf	
2	5	2017-031 red leaf	
2	6	2016-032 green leaf	
2	7	2016-032 red leaf	
2	8	Rubygem green leaf	Purification box dropped; sample lost
2	9	Rubygem red leaf	Purification box dropped; sample lost

RNA Sequencing

RNA sequencing data was obtained from strawberry plant samples (cv. Red Rapsody, RR) from Queensland (Wamuran, Donnybrook, Glasshouse Mt, Elimbah, Donnybrook, Inglewood) (Table 2). These included plants with mild and severe red leaf symptoms, as well as plants without visible RLD symptoms. RNA based meta sequencing was chosen as this allows for detection of eukaryotic, bacterial, and viral organisms.

Table 2: Samples that were sent for RNA sequencing

Sample Accession	Location	Comments
1	Wamuran, Queensland	RR (three healthy plants pooled)
2	Wamuran, Queensland	RR (three infected plants pooled)
3	Donnybrook, Queensland	RR (three symptomatic plants pooled)
4	South West Queensland	RR (three asymptomatic plants pooled)
5	Glasshouse Mt, Queensland	RR (mild and severe symptom samples pooled)
6	Elimbah, Queensland	RR (mild and severe symptom samples pooled)
7	DonnyBrook, Queensland	From Previous SEQ work (concatenated data from 2 samples)
8	DonnyBrook, Queensland	From Previous SEQ work (concatenated data from 10 samples)

The emphasis was placed on identifying potential pathogens that were present in all samples. Additional RNA sequence data was incorporated from previous DAF project work to enable analysis across the biggest available dataset and across multiple seasons (2018, 2019, 2020). To enable the best comparison, DAF data was concatenated resulting in four major sample pools (OLDDAF_Healthy, OLDDAF_Infected, NEWDAF_1, NEWDAF_3).

For all samples, a mixture of plants displaying RLD symptoms or no symptoms where homogenised and extracted with a Promega SV total RNA kit according to the manufacturer's instructions, with some custom steps. Homogenisation included root, crown, stem, leaf material to ensure a comprehensive snapshot.

RNA sequencing Data Analysis

The sequencing data (each sample comprising 40 million sequences) underwent several steps of data trimming and quality controls, as well as host read removal of Fragaria-like sequences. Taxonomy of the sanitised reads was then collated with taxonomic classifying programs Kraken (Wood and Salzberg, 2014) and Kaiju (Menzel et al, 2016). Both programs can compare the millions of microbial sequences to billions of microbial sequences that are available in public databases. The best matches are then shown at various taxonomical levels. Kraken is considered most accurate and is based on a curated database of DNA. Kaiju is less accurate but searches a more complete database including all the NCBI non-redundant (nr) protein collection. Due to the nature of data submission to the NCBI (https://www.ncbi.nlm.nih.gov/), nr contains incorrectly formatted sequences and some incorrect taxonomy; this however allows for the capture of sequences from newly identified species.

The tables are included in appendix 5; as a subset of the data displaying the best matches for each analysis. It should be noted that the higher the taxonomical level, the better the match. Matches at the genus or species level may not always be an exact match based on the limitations of available sequence data in public data bases. For example, a related species may show up as the best match rather than the actual species and this can differ for different samples and the different programs used. In some instances, this can mean that different, but strongly related, species matches may link to just one organism that was present in the samples.

It was determined that the most informative data can be obtained by applying two criteria:

(1) Identifying common microorganisms that are present in all (or nearly all samples). This is based on the observation that symptom-free plants can often develop symptoms after several weeks if plants underwent mild or severe stresses.

(2) Identify microorganisms that were only present (or more abundant) in plants with RLD symptoms but not in symptom-free plants.

Communication and engagement with industry stakeholders

Industry engagement is critical to capture the scale and impact of RLD, and to communicate project findings and suggested management strategies effectively and broadly. A stakeholder engagement and communication plan was developed and implemented for the project. Activities included:

- Publishing updates and sharing new information in each issue of the Australian Berry Journal
- Promoting a red leaf disorder identification leaflet for producers
- Presentations at QSGA meetings and other industry forums
- Team meetings with the project team and communication with Hort Innovation, to discuss the activities undertaken in the project and plan forward activities (occasionally through teleconferences to ensure key personnel were present)
- Engagement with QSGA and ability to work closely with Queensland strawberry industry development officer

A field day to visit an RLD-affected farm was initially proposed; however, due to COVID-19 restrictions, and other issues impacting growers, we were unable to implement this into Phase one of the project.

Results

Farm survey

From the eight farms surveyed in 2020, cultivars Parisienne Kiss, Sundrench and Festival displayed the highest incidence of RLD with maximum percentages across farms of 17.2%, 10.0% and 8.0% respectively. Cultivars Aussiegem (3.8%) and Scarlet Rose (2.3%) had lower RLD incidence, while Red Rhapsody (1.6%) and Fortuna (0.9%) had very little RLD incidence (Figure 4). All farms surveyed had RLD present on their properties, with observations of RLD in plants received both as bare-rooted and plug plants. A limited number of Red Rhapsody plants in substrate showed RLD, identified only on water stressed plants and later disappearing when water supply issues were corrected.

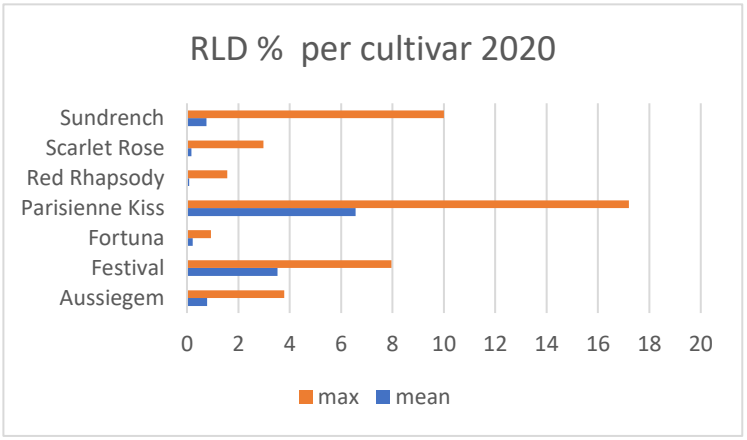

Figure 4 Mean and maximum percentage of Red Leaf Disorder occurrence for cultivars surveyed in SEQ region in 2020

The proportion of plants with RLD was investigated for each cultivar, farm, and time as well as for interactions between these factors. The best model (that with the lowest AIC value) included the three individual predictors (cultivar, farm, time and a quadratic term for time i.e., 'time + time^{2}', both expressed as the number of days after commencement of surveying), as well as two interactions (cultivar:farm and cultivar:time) (Figures 5 and Appendix 1). The distribution of the percentage of plants with RLD for each farm are shown in Figures 6.

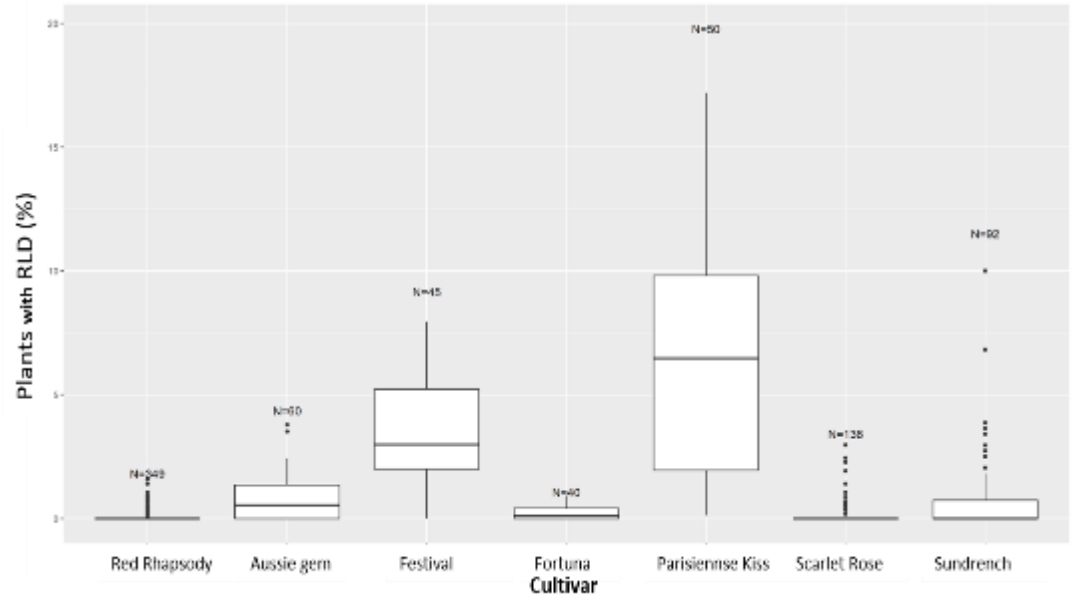

Figure 5 Box plots of distribution of the percentage of plant with Red Leaf Disorder for each Cultivar. Box plots display the data distribution and skewness through displaying the data quartiles (or percentiles) and averages. The lines extending parallel from the boxes "whiskers", indicate variability outside the upper and lower quartiles.

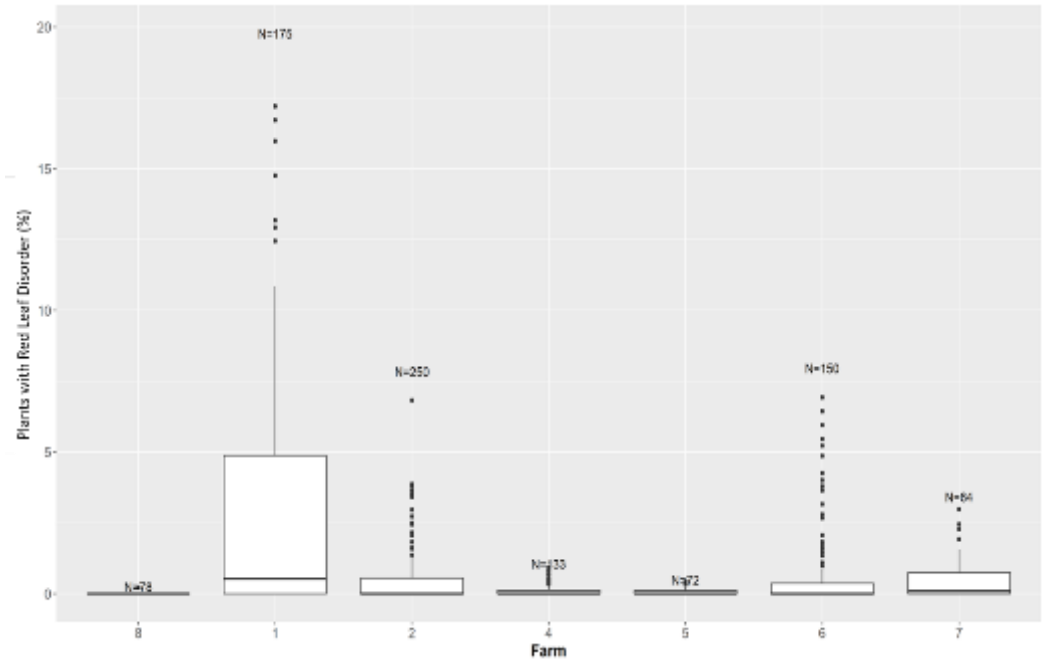

Figure 6 Distribution of the percentage of plants with Red Leaf Disorder for each farm.

The contribution to the ORs (Odd ratios) by all the cultivars were significantly different to that contributed by the cultivar used as reference, Red Rhapsody, except for Scarlet Rose (Figure 7). Some of the interactions between cultivar and time (linear and quadratic) were significant, indicating that RLD evolved at different rates in different cultivars. Our observations indicate that plants with RLD symptoms can recover, and the proportion of RLD often decreased over time after reaching a maximum. The OR of all cultivars and farms are low suggesting that plants were much less likely to develop RLD than not to develop it. The relative OR (proportion of probability) provides more insight. The relative ORs ranged between 405 for Fortuna to over 2 million for Scarlet Rose (Figure 8). That is, although still low, the proportion of the probability of developing RLD compared to not developing the disorder is over 2 million times greater in Scarlet Rose than Red Rhapsody. This suggests in the 2020 data, Red Rhapsody may be more genetically tolerant to RLD than Scarlet Rose.
Similarly, the relative ORs for the farms ranged from ~1.1 million for Farm #1, the second best performing farm, to over 506.5 million for Farm #7, the worst performing farm (Figure 8). The relative OR for four of the farms are not that dissimilar (Farms #2 to #5). However, Farm #8 seems to perform much better than the rest and Farms #6 and specially #7 performed much worse than the rest.

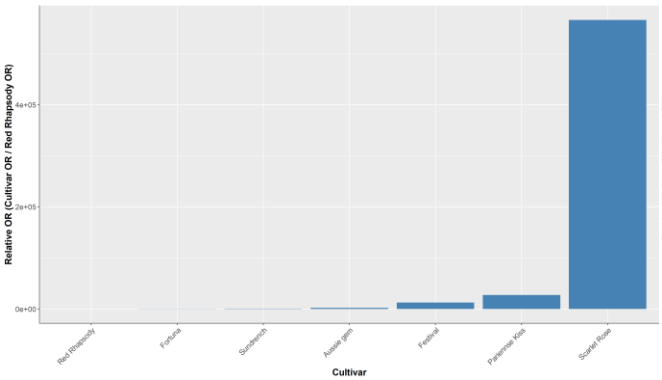

Figure 7 Cultivar relative Odds Ratios (OR)s obtained in the best fitting model for the proportion of plants with Red Leaf Disorder in the dataset for Farm Visits in 2020

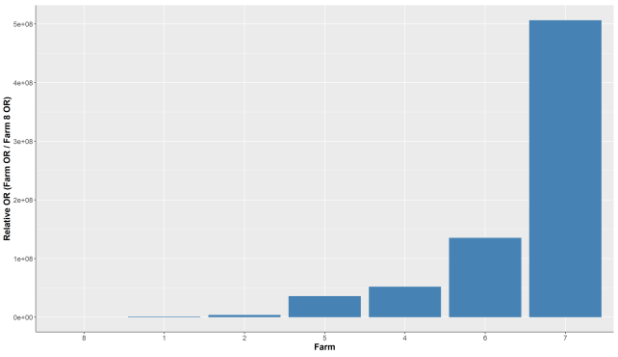

Figure 8 Farm relative ORs obtained in the best fitting model for the proportion of plants with Red Leaf Disorder in the dataset for Farm Visits in 2020.

Overall, in the SEQ region, there was a lower incidence of RLD in the 2020 fruiting season compared to 2019. There was also a delay in RLD symptoms appearing on plant leaves, with a low level of symptoms observed in May, peaking in August and then a decline in September. This delay in visual symptoms could possibly be due to the late plantings of 2020 due to availability of runners/plugs or issues unknown. There was, however, a higher occurrence of other reddening and purpling leaf issues observed, which does not concur with our visual diagnostic factors for RLD. These were attributed to nutritional and/or other factors.

There was an uneven distribution of the cultivars across farms. For example, out of the seven cultivars considered, four occurred only in a single farm (Aussie gem, Festival, Fortuna, and Parisienne Kiss) and one in two farms (Sundrench) (Appendix 2 & 3). Only Red Rhapsody was well represented across farms (present in five out of the six farms). Similarly, out of the six farms, two only contained a single cultivar ('Farm 4' and 'Farm 5') and another two contained two cultivars ('Farm 8' and 'Farm 7'). This uneven distribution of the cultivars across farms hinders a clear separation of the cultivar and farm effects (Table 3). That is, it is more difficult to estimate if a high/low RLD incidence is due to a particular Cultivar and/or Farm. For example, at a first glimpse Farm 1 seems to have a significant problem with RLD. However, the statistical models suggest that much of this poor performance is due to the cultivars that are found in the farm, which includes the two highest RLD % cultivars Parisienne Kiss and Festival. Unfortunately, these two cultivars are not found in any other farms, which would have helped separate the cultivar and farm effects. In the models, Farm 1 performed better than expected based on the only available comparison for this farm. A higher incidence of RLD in Sundrench was observed in Farm 2 than in Farm 1. A greater overlap between cultivars among farms (i.e., a more 'crossed design') is recommended for future monitoring seasons.

Table 3 Number of data points (row surveys x times checked per cultivar/farm combination.

Farm #	Red Rhapsody	Aussie gem	Festival	Fortuna	Parisienne Kiss	Scarlet Rose	Sundrench	Cultivar/Farm
8	42	0	0	0	0	36	0	2
1	0	0	45	40	50	0	40	4
2	78	90	0	0	0	72	78	4
4	140	0	0	0	0	0	0	1
5	72	0	0	0	0	0	0	1
6	150	0	0	0	0	0	0	1
7	25	0	0	0	0	40	0	2
Farms/Cultivar	6	1	1	1	1	3	2	

Plugs vs bare rooted plants

There was very little RLD surveyed from Farm 3 (substrate farm), and plants were generally healthy all season. However, in May 2020 RLD was observed in plants with reduced water supply. On inspection it was noted the dripper supplying these plants were either blocked or kinked, therefore water supply was limited, causing plant stress. On the same day of observing/recording RLD, the dripper issues were fixed. At the next RLD inspection date (Approximately one month after) the plants had lost the mature leaves with RLD and no new symptomatic leaves developed throughout the season. For the duration of the survey, RLD was observed (less than 0.2%) in bare rooted plants (Figure 9).

The ORs and Relative ORs for bare-rooted plants were slightly larger than for plug plants. These indicate that plug plants would be less likely to develop RLD. The data available from the 2020 monitoring does not show enough evidence for any of the two main effects (Planting Type (Plugs vs Bare) and Time) being significant. However, the dataset was very limited, with only 4 data points available per planting type and time combination. More data would be required to properly assess the significance of the main terms. Similarly, more data would help to determine whether the observed RLD decrease over time is a true process and how common it is. In the 2020 data the RLD decrease was driven by the initial values observed for plugs. There initial values consisted of only four observations that were quite variable, including a very large observation (the largest value in the whole data set, 6 plants with RLD). Finally, a larger dataset would have likely allowed to fit and run models including the interaction between Planting Type and Time. These models could not be run with the 2020 data due to limited data. Therefore, the collection of more data is encouraged in future monitoring seasons.

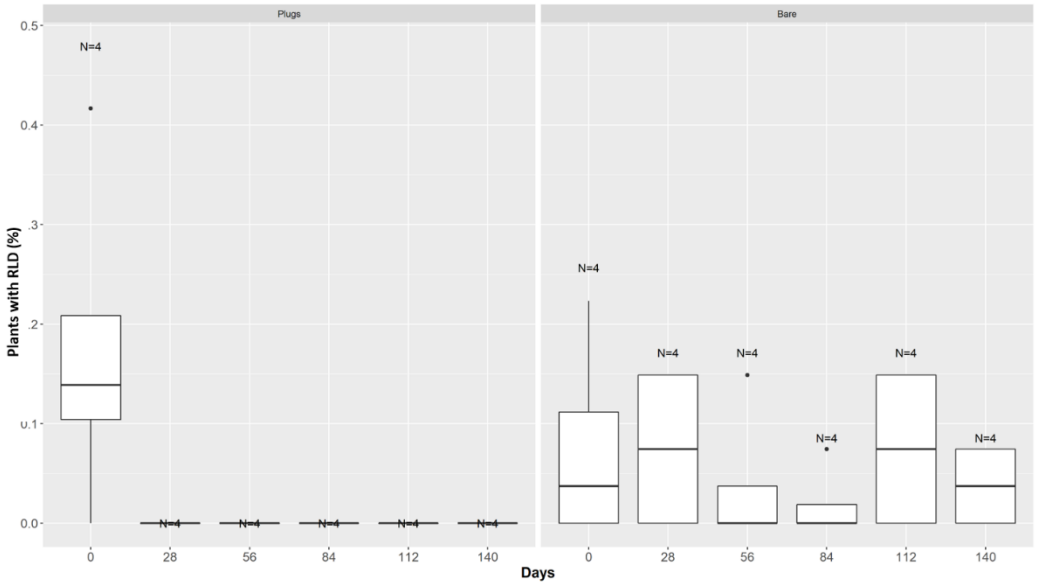

Figure 9 Distribution of the percentage of plants with Red Leaf Disorder per day for each per planting method (plugs vs bare).

Red Leaf Disorder, yield and root health data

In this trial, RLD rating (severity) increased progressively over the subsequent weeks of the trial (Figure 10); however, towards the end some plants recovered to some extent. This concurred with observations on the eight farms from SEQ region. All plants showed some degree of reddening, with six mortalities. The RLD profiles over time varied largely among plants. Although in general RLD increases over time, there are multiple cases where RLD decreases over time (plant 2), increases and then decreases (e.g., plants 23 and 38), or remains mostly constant (e.g., plant 15).

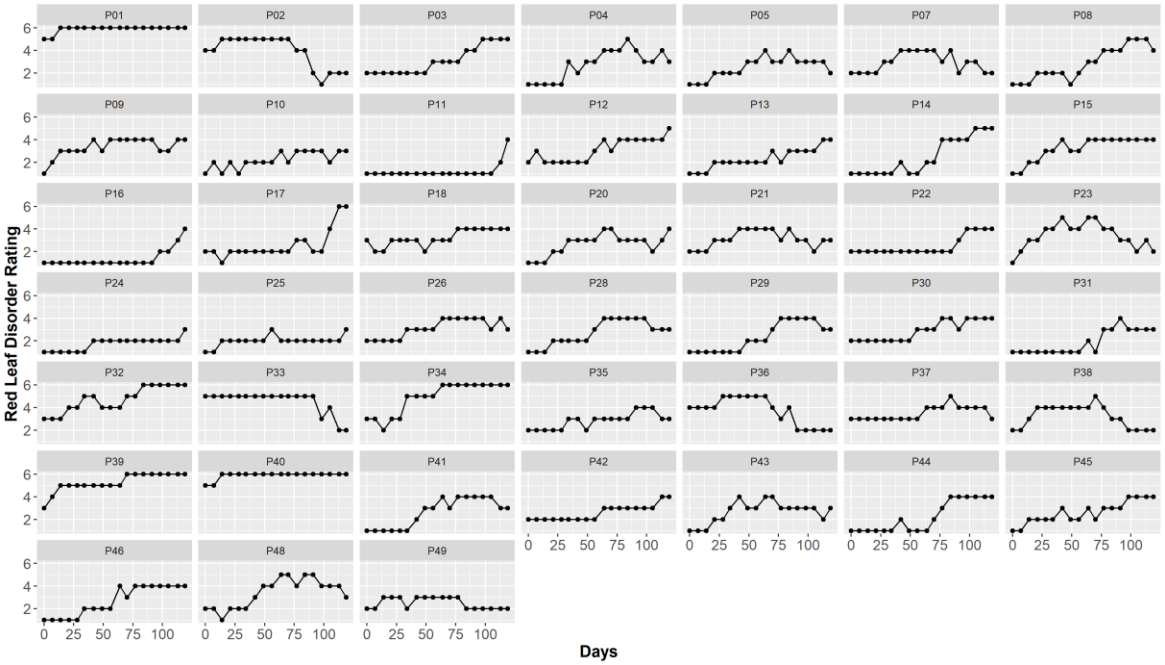

Figure 10 Relationship between Red Leaf Disorder rating and Time for plants of cv. Red Rhapsody over the 2020 fruiting season. 1= no RLD and 6 = plant dead.

Root browning and Health

Plants without visible leaf symptoms of RLD (the control group) had longer roots and less browning. Root browning (%) was significantly related to severity of RLD (P= 4.64 x 10^{-8}). Differences in overall root health score were also apparent between the treatment groups (plants with and without visible RLD symptoms). As RLD incidence increased (ratings increased) there was a positive correlation with the root health ratings. That is, as RLD severity increased overall root health decreased (Figure 11). There was more variation in the treatment group (with RLD) and more root browning (Figure 12). Root browning (% of browning roots) and root rating are highly correlated (Spearman-rank rho = 0.8686 and Pearson's r = 0.0607. An increase of 1% in root browning produces an ~9.3% increase in the Odds Ratio of increasing the RLD severity ranking (95% confidence interval: ~4.9% - ~11.8%).

Figure 13 shows the differences between the length of green leaves (including petioles) on plants with and without RLD. Plant without RLD symptoms had longer and more uniform length leaves, all over 30 cm, compared to those of the treatment group which ranged from 18-28 cm. The crown number predictor is also correlated to the two root predictors but to a lower extent (Crown number and root rating: rho = 0.5871 and r = 0.6066, and crown number and root browning (%): rho = 0.5364 and r = 0.6068. Crown number was not significant (p-value = 0.22) to RLD, however, there were only 19 samples and 1 control plant, so it is recommended to explore this possible relationship further in future trials.

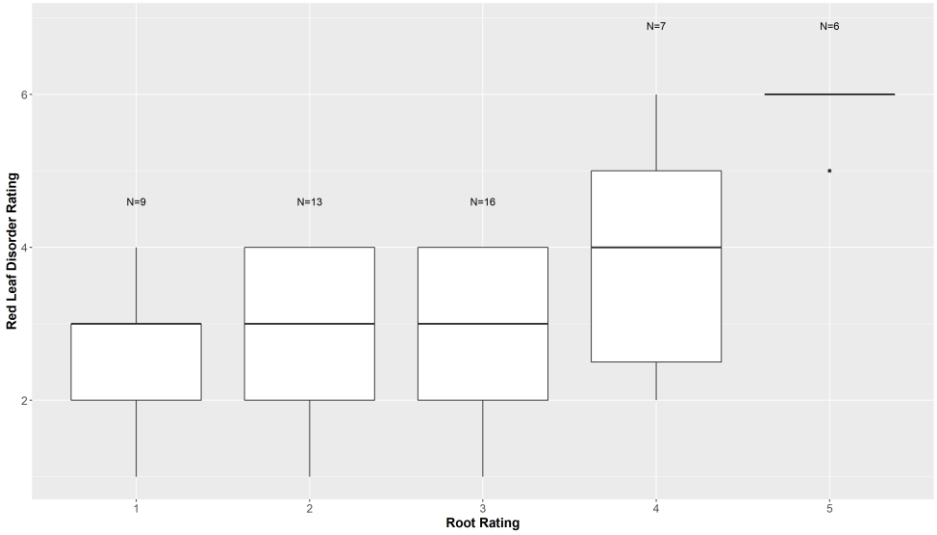

Figure 11 Relationship between Red Leaf Disorder and Root rating. RLD rating where 1= no RLD and 6 = plant dead. Root rating where 1 = healthy/long roots and 5 = roots unhealthy/short.

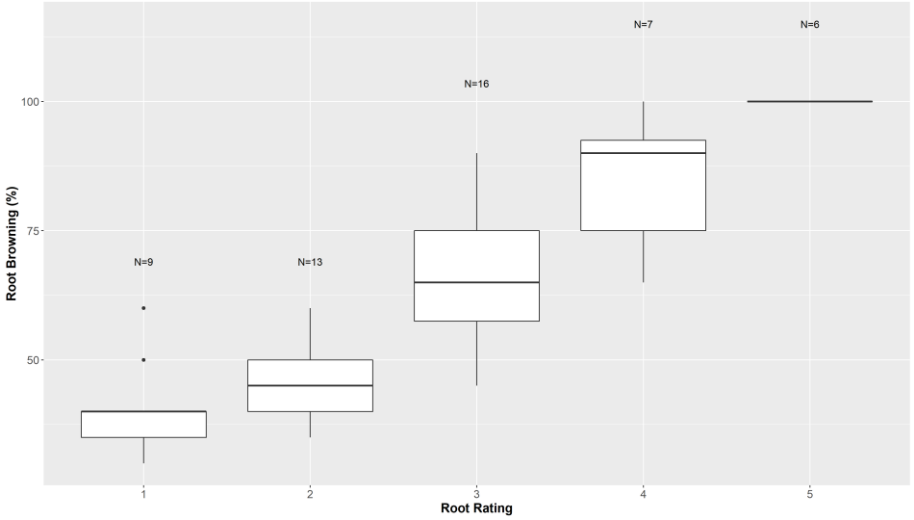

Figure 12 Relationship between root browning (%) and root health rating. Root rating where 1 = healthy/long roots and 5 = roots unhealthy/short.

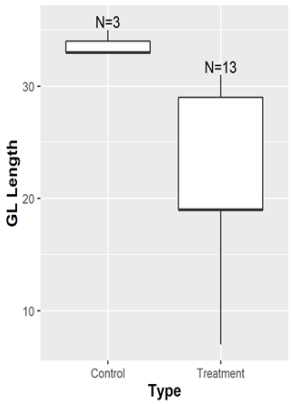

Figure 13 Green leaf (GL) length, for control group (plants without Red Leaf Disorder) and treatment group (plants with RLD).

Total fruit weight (yield)

There was an obvious progressive decline in yield as reddening developed, i.e., the number and weight of 1st grade fruit progressively deceased as RLD severity increased. Figure 14 shows the average weight per fruit for each RLD rating, illustrating how the average size of fruit decreased as RLD rating (RLD severity) increased. Figure 15 shows the average yield per plant of 1st grade fruit for each rating of RLD severity. As plants became severely affected by RLD, they produced fewer and smaller fruit, and thus more waste.

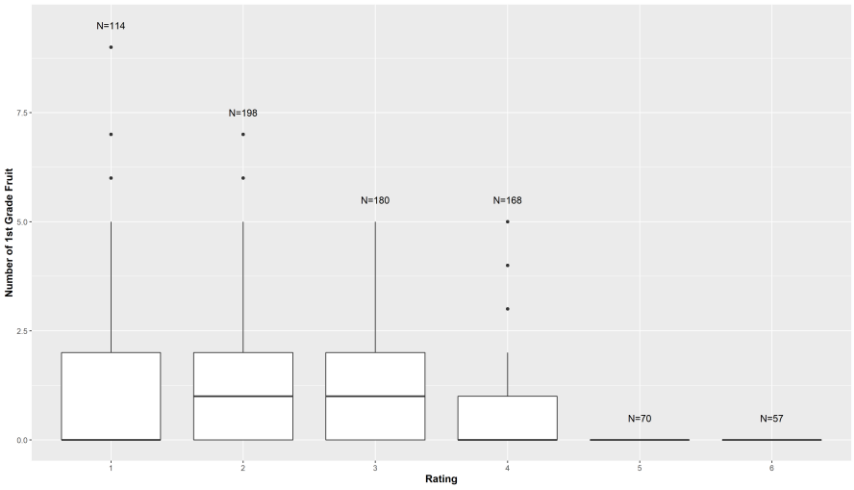

Figure 14 Average fruit weight per Red Leaf Disorder rating, where 1= no RLD and 6= plant dead.

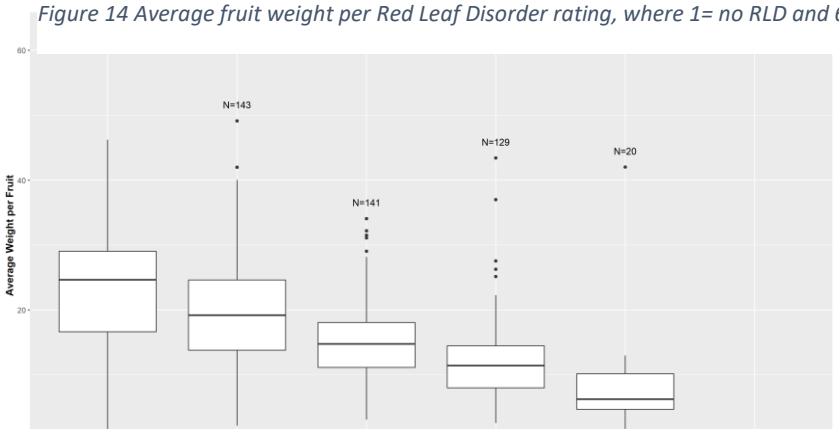

Figure 16 Average 1st grade fruit number per Red Leaf Disorder rating, where 1= no RLD and 6= plant dead.

RLD rating, time, their interaction, and root browning (%) all showed statistically significant relationships with total fruit weight and class 1 fruit weight. All RLD rating other than rating 4 were associated with a decrease in total fruit weight compared with asymptomatic plants (rating 1) (Figures 16 & 17). The effect is particularly pronounced for the highest rating. Root browning (%) was also observed to have a negative effect on total fruit weight. Each 1% increase in root browning was associated with a decrease in the total weight of fruit by ~0.23 grams on average.

Total fruit weight (yield) shows seasonal fluctuations for most RLD severity ratings (Figure 17). There are significant interactions between RLD rating and time, which modify this behaviour (Figure 18). Some of the interactions were difficult to estimate from the available data (e.g., there are few or no plants with a RLD rating of 1 in last days of the trial and with a rating of 6 in the final days of the trial).

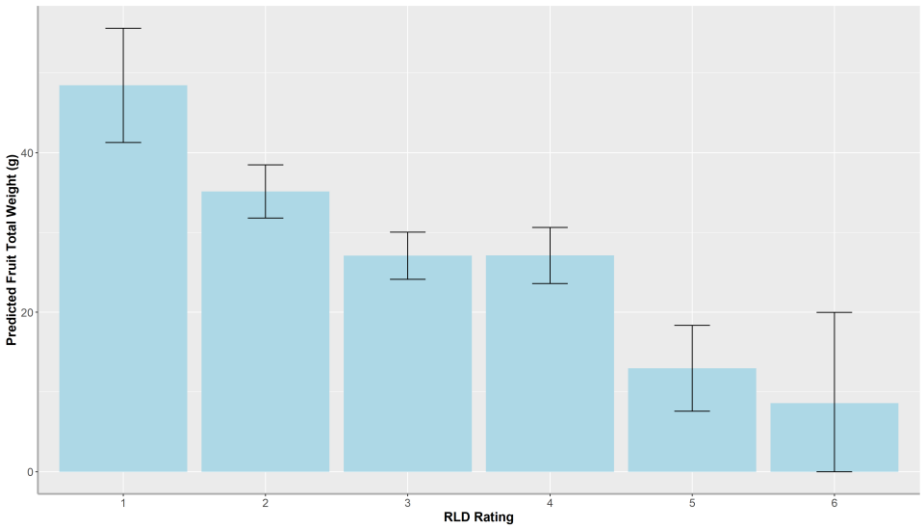

Figure16 Predicted total fruit weight (yield) per plant for each Red Leaf Disorder rating ±LSD. Predictions are in grams and were obtained from models fitted using the untransformed total fruit weights as the response. Predictions are in grams back-transformed from a square-root based scale and were obtained from models fitted using the square root transformation of the total fruit weights as the response.

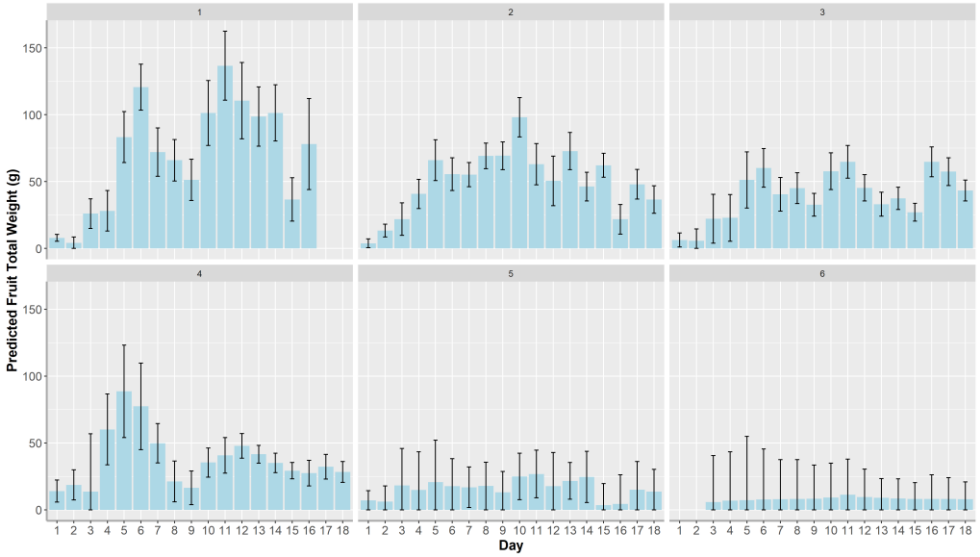

Figure 17 Predicted total fruit weight (yield) per plant for each Red Leaf Disorder rating and day (time) ±LSD. Predictions are in grams and were obtained from models fitted using the untransformed total fruit weights as the response.

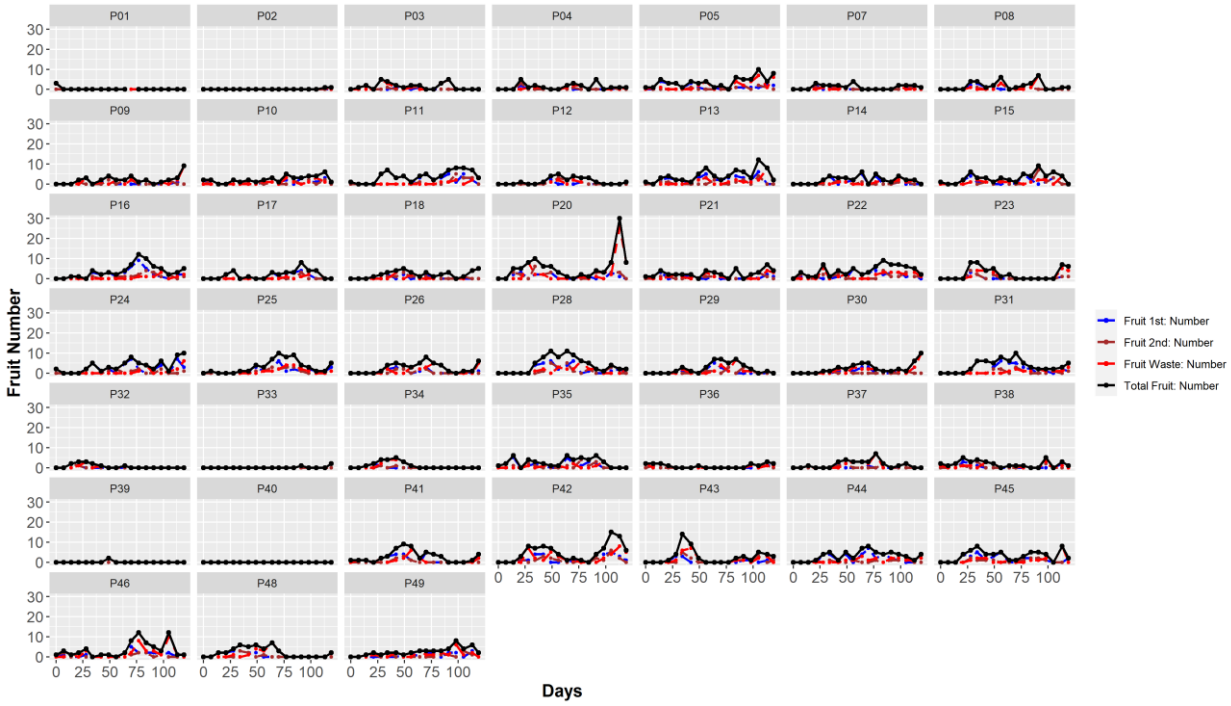

Figure 18 Relationship between Fruit Number and Time (i.e., Evolution of Fruit Number).

Fruit weight of class 1 (1st grade) was negatively associated with RLD severity (Figure 19). Plants with a RLD rating of 6 were observed to have average class 1 fruit weight 16 grams lower than plants with a rating of 1. Root browning (%) was also observed to be negatively associated with class 1 fruit weight. A 1% increase in root browning was seen to decrease the class 1 weight of fruit by ~0.15 grams on average.

Time, on the other hand, increases class 1 fruit weight, except in the Day 2. The effect is particularly large in days 6, 10, 11, and 12 (i.e., it also looks bimodal, with to production peaks). A significant interaction was observed between RLD rating and time. Some of the interactions where difficult to estimate from the available data (e.g., there are few or no plants with a RLD rating of 1 in last days of the trial and with a rating of 6 in the final days of the trial). RLD rating and Day have statistically significant relationships with class 3 (waste) Fruit weight. However, their interaction, and root browning (%) do not have a statistically significant relationship with class 3 fruit weight.

RLD Rating, day, their interaction, and root browning (%) all have statistically significant relationships with class 1 (1st grade) fruit weight, total fruit weight, and class 3 (waste) fruit weight. RLD rating and day have statistically significant relationships with class 2 fruit weight, but no their interaction or root browning (%).

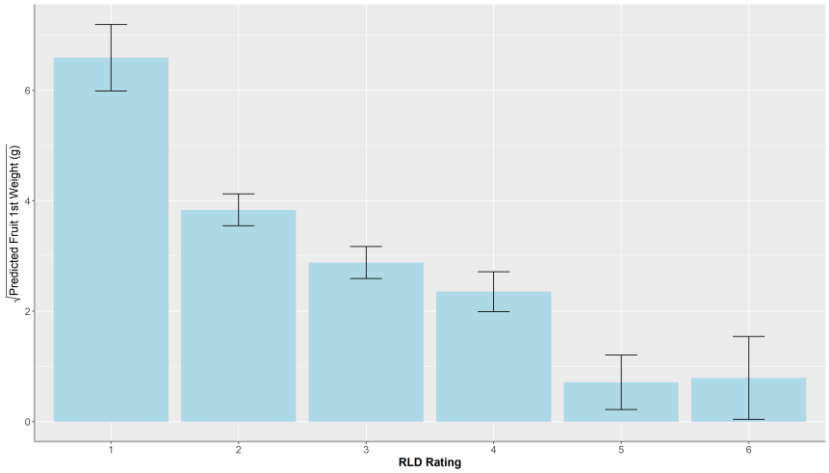

Figure 19 Predicted total fruit weight per Red Leaf Disorder Rating. Predictions are in grams and were obtained from models fitted using the untransformed total fruit weights as the response

Red Leaf Fungal isolations

Plants with RLD symptoms presented various internal crown symptoms when cut longitudinally. These included nil symptoms, vascular discolouration, vascular rot, crown rot (from very mild to severe) and speckling (associated with nitrogen toxicity). Most of the isolations taken from 'red' areas of the leaf did not recover any fungal growth. In a few cases, *Colletotrichum* sp., *Neopestalotiopsis* sp., *Altenaria* sp. and a small number of unknown fungal species grew, but not consistently. There was similarly no consistent fungal pathogen recovered for crown and root isolations from both healthy plants and plants with RLD symptoms. Rather, several genera of pathogens were observed on Potato Dextrose Agar media, such as *Phytophthora, Colletotrichum, Fusarium, Macrophomina, Altenaria, Pythium, Phomophsis, Neopestalotiopsis, Rhizoctonia* and many others (unknown). In some samples, there were no recovery of any fungal growth observed. In very few cases, there were also bacteria-like growth on the PDA plates. All fungal and bacteria-like cultures were not formally identified molecularly. For both leaf and crown isolations, identification of the recovered pathogens was mainly by morphological characteristics.

Pathology testing of plants with and without RLD symptoms have produced inconclusive results to support the notion that a fungal pathogen may or may not be the causal agent for RLD.

Biological indexing

No phenotypic symptoms of RLD infection were observed on all indicator plants after 6-12 weeks grafted with the breeding accessions and varieties tested.

Microscopy analysis and PCR (DAF)

A range of virus particles or virus-like particles were observed in the strawberry leaf sample analysed: full and empty 21 nm isometric/spherical particles, and 70 nm isometric particles (Figure 20). In a small number of samples, possible fragments of closterovirus-like particles were detected (Table 4). Additionally, nucleorhabdovirus particles with dimensions of 47 x 330 nm were detected in one symptomatic sample (Figure 21), and many phage particles (viruses of bacteria) were observed in the root sample, likely originating from rhizosphere bacteria.

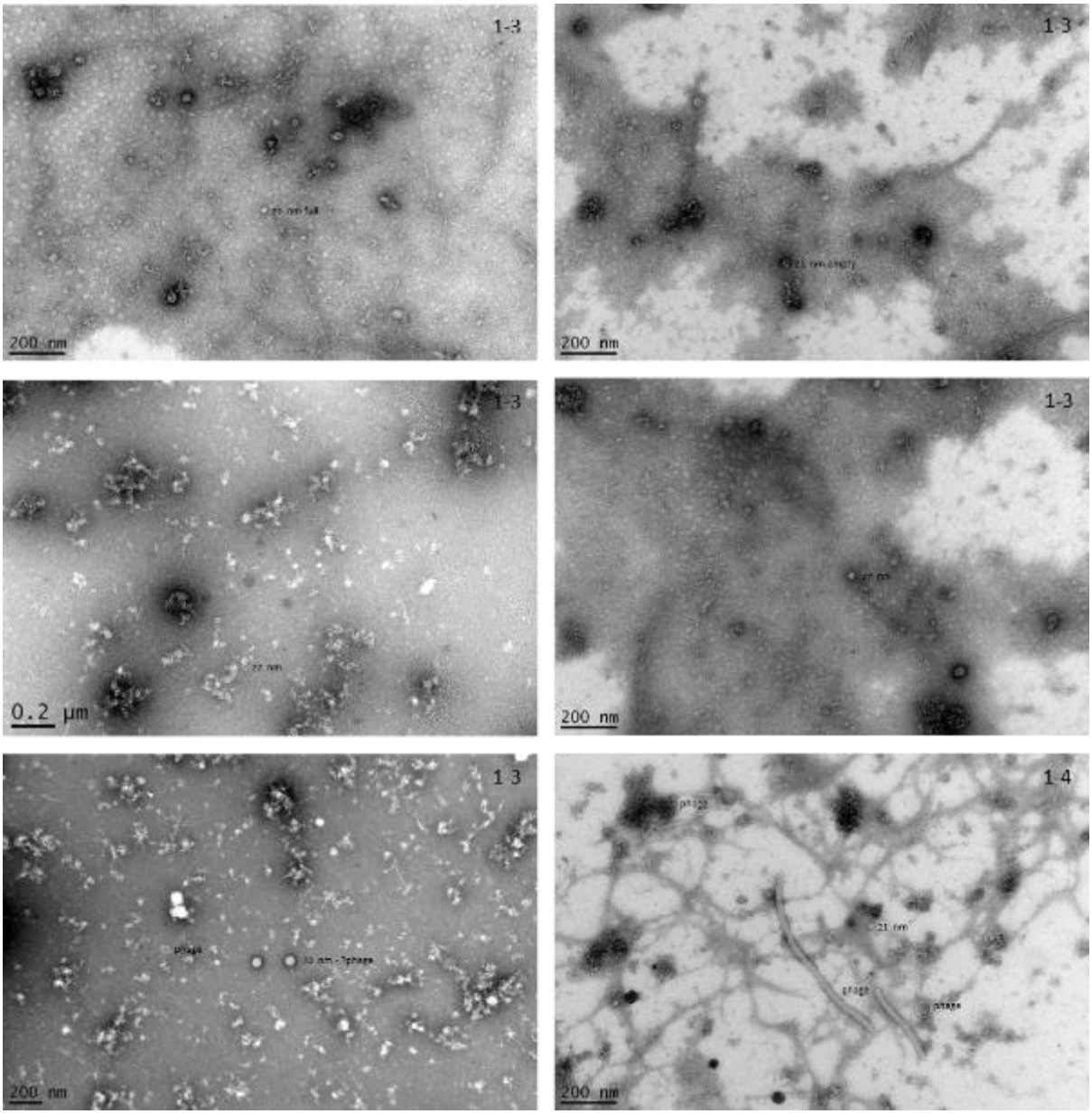

Figure 20 Variety of virus or virus-like particles detected in strawberry samples.

Only the empty 21 nm isometric particles were in moderately high concentration, all other viruses were in low concentration, possibly close to the limit of detection by electron microscopy. After viewing the first batch of samples, it was thought the 21 nm isometric particles were in higher concentration in the symptomatic samples and that they may have belonged to the genus *Nanovirus*, however generic PCR assays for these viruses were negative. The 27 nm isometric particles were present in a range of but not all samples, however, may have an association with red leaf symptoms. Identification of these viruses and verification of the closterovirus-like particles requires use of specific laboratory assays as a range of isometric viruses are known in strawberry. These viruses may also represent novel viruses, for which there are currently no specific tests.

A similar red leaf disorder called strawberry decline occurred western coast of North America, beginning in the early 2000s, with the symptoms correlating with the presence of multiple viruses (Tzanetakis and Martin, 2008). Current advice from the University of California dated 2018 (https://www2.ipm.ucanr.edu/agriculture/strawberry/Virus-Decline-of-Strawberry/) suggests this disease remains a problem for their industry.

Table 4 Virus detections for samples checked under the TEM.

Sample	Symptoms	21 nm empty	21 nm full	27 nm empty	27 nm full	70nm	Comments
1-1	NA*						24nm hexagonal isometric particles
1-2	no	yes			yes		
1-3	yes	yes	yes	yes	yes	yes	Nucleorhabdovirus particles
1-4	yes		yes				Lots of phage, Maybe closterovirus particles
1-5	no	yes				yes	
1-6	yes	yes					
1-8	yes						
1-9	no	yes					
1-10	yes	yes			yes		
1-11	no	yes					
1-12	yes	yes			yes		
2-2	no	yes	yes			yes	
2-3	yes			yes		yes	
2-4	no		yes				Maybe closterovirus particles
2-5	yes		yes		yes		
2-6	no	yes					Maybe closterovirus particles
2-7	yes		yes				

* Ageratum sp not strawberry sample.

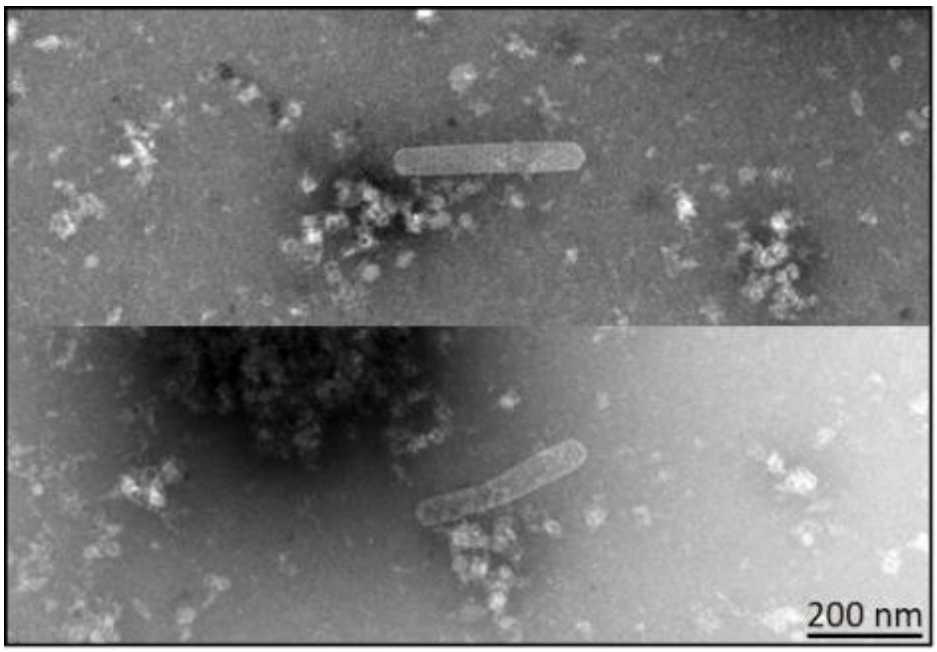

Figure21 Nucleorhabdovirus particles detected in the Parisienne Kiss sample with red leaf symptoms

Rhabdovirus particles (47 x 330 nm) were also detected in sample 1-3 (Figure 21). Two rhabdoviruses are currently recognised by the ICTV: strawberry crinkle cytorhabdovirus and strawberry cytorhabdovirus 1 (Walker et al. 2020). A third cytorhabdovirus has recently also been reported (Ding et al., 2019; Fránová et al., 2019). A fourth strawberry rhabdovirus is known, strawberry latent C nucleorhabdovirus (Yoshikawa et al. 1986), however no genome is available, and it is therefore not recognised by ICTV (Tzanetakis and Martin 2013). The virus does not cause symptoms in single infections in modern strawberry cultivars but does have a synergistic effect in disease severity when found in complexes with other aphid-borne viruses (Millee, 1960).

Cytorhabdoviruses have an expected size of 60–75 nm in diameter and 200–350 nm long. Nucleorhaboviruses have an expected size of 45–100 nm in diameter and 130–300 nm long. The size of the observed particles indicate that they belong to the nucleorhabdovirus group. Amplification using generic primers and sequencing is required to confirm this identification.

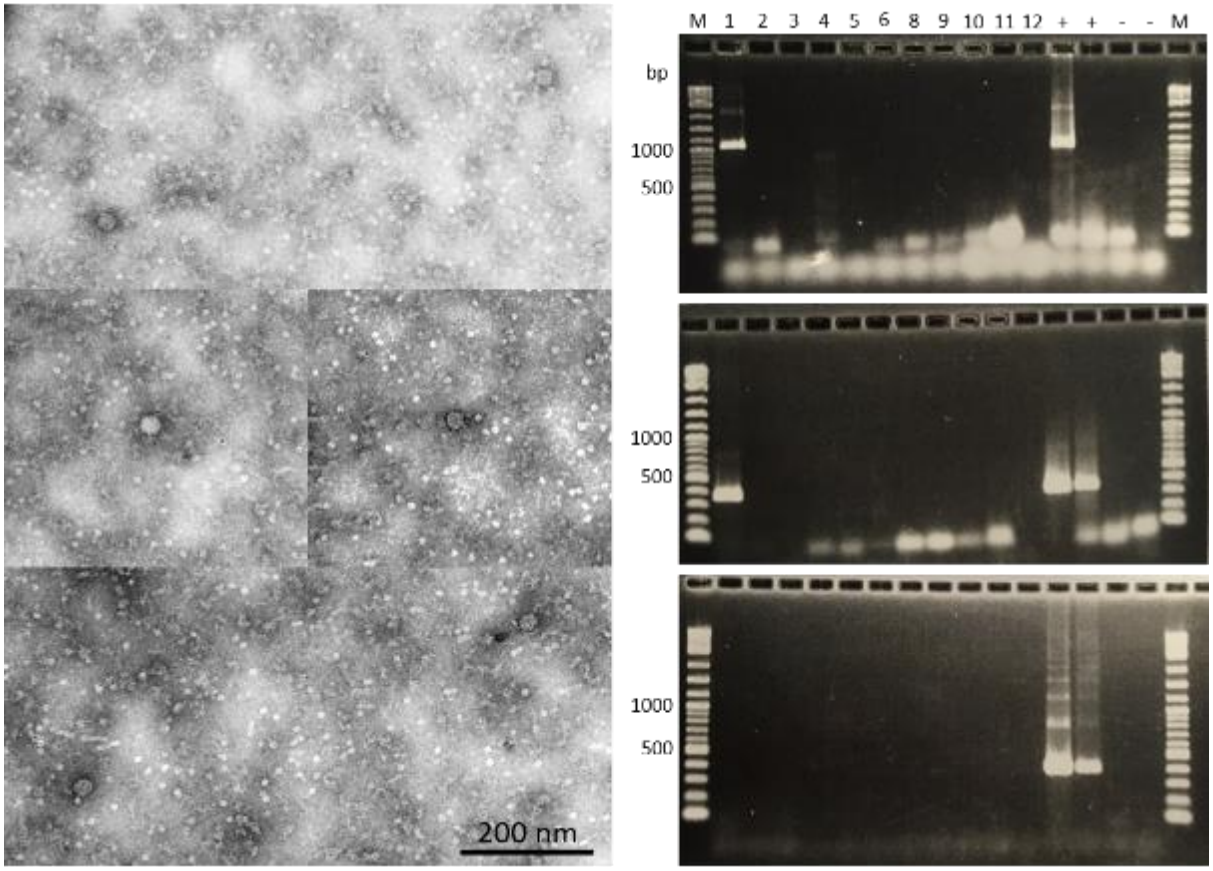

Figure 22 Composite micrograph image of 24 nm hexagonal isometric particles from Ageratum sp with red leaves growing near strawberry red leaf disorder plants. B. Luteoviridae generic RT-PCR assays PLF/PLR, Pol3628F/Pol3982F and Pol3870/AS3 (top to bottom).

The sample of *Ageratum* sp with red leaves collected from beside the glasshouse in which red leaf strawberry plants were grown had many 24 nm hexagonal isometric particles (Figure 22) which are suggestive of the *Luteoviridae* family. This sample, and sample 2-1 from the second batch were positive by RT-PCR, whereas the strawberry samples run alongside were negative. Sequences of the amplicons from the PLF/PLR and Pol3628F/Pol3982F primer pairs (covering the 3' end of the RdRp and 5' end of the CP/MP region of the genome) generated a 996nt combined fragment that was only 83% identical to the GenBank sequences for cucurbit aphid-borne yellows virus (CABYV).

Translated protein matches were:

- The partial RdRp (last 146aa) is at best 90.4% identical to the closest GenBank match (CABYV).
- The first 140aa of the coat protein are 76% identical to CABYV (but there appears to be an issue with the first 20aa of the sequence) and melon aphid-borne yellows virus.

- The first 110aa of the movement protein are at best 81% identical to CABYV.
- These sequences are similar but not identical to viruses previously detected from Queensland and have been reported to Biosecurity Queensland.

RNA sequencing

When applying criterion one (From methods mentioned above), Phytoplasma (and related Acholeplasma) was the only genus that was found across all samples (Appendix 5). Phytoplasma is a cell wall-free bacterium that often resides in vascular tissue and causes virus-like symptoms. By far, the highest amount of Phytoplasma was found in sample 3.

Plasmopara, a genus of the oomycetes, was dominant across all samples, except those from sample 4. In addition, many viruses were found that infect Plasmopara in all samples, that could suggest that either Plasmopara or the narna- and mitoviruses transmitted by Plasmopara could be candidates. A virus was observed from the sequences that is like above “narnavirus/mitovirus/pepper chlorotic spot”; at least one of these seems to be associated with Plasmopara. Reads for these viruses were detected even in samples where Plasmopara was not detected (Sample 4), possibly something from “Orthornavirae”. There were also several reads from the Pleosporales family across all samples, and additionally Xanthomonas bacteria were detected in all samples, including X. arboricola in all samples except sample 3 and 4.

There were clusters of microorganisms observed that were associated with the 2018/2019 DAF samples that are very different to those of the 2020 samples. For example, Spiroplasma, Cladosporium, Gelatoporia, and Alternaria were only found in the original samples. Spiroplasma was not detected in great numbers in newly sequenced samples, while previous DAF sequencing data revealed high levels of reads from a Spiroplasma namely, Spiroplasma kunkelii. New sequencing does not have this high level of support, however there is a higher degree of support for a pathogen from Acholeplasmataceae (includes Phytoplasma and Acholeplasma).

Using criterion (2), searching for microbes more present or only present in symptomatic samples, did not yield any noteworthy consistent pattern.

Communication and engagement with industry stakeholders

The project incorporated current communication programs in the strawberry industry to deliver results of the project to producers through industry seminars, field days, articles, and newsletters. The project contributes to Outcome 3: Greater skills, capacity, and knowledge in the industry, of the 2017-2021 Strategic Plan. Presentations were given at the growers meetings (QSGA), two articles published in industry publications (Australian Berry Journal, summer 2020 & winter 2021), and general communication channels (email/phone calls) with industry members was used.

A project overview was published in the first issue of the Australian Berry Journal following project commencement (Appendix 6). This allowed the wider industry to learn that research was underway in key areas, in a larger collaborative project with multi-disciplinary organisations. General information and project updates were shared throughout the project in later issues of the journal and via grower meeting presentations. Project staff have continued to enhance communication between primary and secondary audiences as per the scope of the M& E plan. This has involved updates on key activities, achievements, and outputs relevant to red leaf disorder research.

Some key outcomes have included:

- An increase in grower confidence in identifying and in some cases improved management of RLD
- Advisors and consultants are more confident in providing RLD information and advice to strawberry growers
- New areas of information have been discovered, that can potentially be extended through a PhD investigation
- Increased information sharing and integration amongst growers and advisors
- better understanding of incidence, spread, and economic impact on commercial strawberry producers through farm surveys, monitoring, and yield study investigations
- Publications and presentations have allowed information readily available to key stakeholders

DAF worked closely with the industry development officer for Queensland Strawberry Growers Association (QSGA) throughout the project and will maintain this connection throughout phase 2 of the project. DAF

project co-leaders continue to engage with industry and provide updates by attending on farm site visits throughout the remainder of the 2021 fruiting season.

As the project moves into Phase 2, we can further improve the knowledge of RLD epidemiology, potentially confirming a candidate responsible for the cause of the disorder, and further analysing the economic impacts on commercial strawberry producers that can be shared directly with industry and stakeholders.

Figure 23 (L-R): Michelle Paynter, Joanna Kristoffersen and Roger Broadley examining Red Leaf Disorder affected plants in a commercial fruit farm.

Figure 24 (L-R): Michelle Paynter, Peer Schenk, Reuben Brown, and Jodi Neal. Bottom (L-R): Kapah Alu, Joanna Kristoffersen and Apollo Gomez.

Outputs

During this project, team members have delivered and presented on the project background, activities and progress at industry meetings and events both nationally and internationally.

- Interacted with multiple strawberry producers, multiple government representative, researchers, and industry affiliates nationally and internationally
- Presented at three strawberry meetings
- Authored three articles for the national industry magazine (Australian Berries Journal) distributed to more than 650 growers and industry associates per edition
- Authored one peer-reviewed manuscript (Refer to Referred Scientific Publication section)

Grower and industry affiliates meetings and, presentations

- Presented 'Red Leaf Disorder of Strawberry-New project update' Paynter & Kristoffersen, at the 'Queensland Strawberry Growers' Association meeting (QSGA), Caboolture RSL, (August 2020)
- Presented 'Overview of the new Red Leaf Disorder project', David Innes, at the 'Queensland Strawberry Growers' Association meeting (QSGA), Caboolture RSL, (August 2020)
- Presented 'Red Leaf Disorder of Strawberry' at DAF SFGI Presentations (Virtual) (August 2020)
- Presented 'Red Leaf Disorder, Qld farms 2020 survey" to Strawberry industry reps, Caboolture RSL, (February 2021)

Conferences

- 9th International strawberry symposium 'Red Leaf Disorder of Strawberry plants In Australia' (Virtual conference, 3rd May 2021)

Articles

- Kristoffersen, J., Paynter, M., Constable, F., Gomez, A., Neal, J. and Hung, T. 2021. 'Red Leaf': A new disorder in Australian strawberry plants. Acta Horticulturae, 1309. pp. 773-780. ISSN 05677572 (ISSN)
  Kristoffersen, J., Paynter, M., 2020. Investigation into the cause of Red Leaf Disorder in strawberry plants: an update (Internal report). Department of Agriculture and Fisheries, Nambour
  Red Leaf Disorder, Australian Berry Journal, Winter edition 3
- Paynter & Kristoffersen, 2020. Developing knowledge and management of strawberry red leaf disorder – Hort Innovation project: BS19001, Australian Berry Journal, Summer edition 5
- Paynter, M., Kristoffersen, J., Betts, M., Buck, C. 2021. Red leaf disorder 2020 farm survey update, Australian Berry Journal, Winter edition
- Paynter, M. and Kristoffersen, J. 2020. Developing knowledge and management of strawberry red leaf disorder – Hort Innovation project: BS19001, Australian Berry Journal, Summer edition 5

Outcomes

The outcomes produced in this project have been beneficial to the Australian strawberry industry and have built foundations for ongoing research into Phase 2. A better understanding of incidence, spread and economic impact on commercial strawberry producers has been developed. Although we have not confirmed the cause of RLD, knowledge acquired, and potential leads discovered in this phase of the project will assist Phase 2. This research has narrowed the search for the cause of RLD. The information gaps discovered can provide potential areas of research for a PhD investigation. Recommendations have been included to aid future research priorities.

Industry engagement was important to this project and was maintained by communicating project findings and developments through various communication channels. These included QSGA meetings, industry newsletters, on farm interactions, phone calls, emails and more widely in Berry journal publications. Throughout the project, advisors and consultants have been updated and can now be more confident in providing RLD information and research updates to strawberry growers. An increase in grower confidence in identifying and monitoring red leaf disorder was achieved.

RLD will continue to threaten the viability of the local industry and potentially have serious implications for the national industry if not identified and managed.

Monitoring and evaluation

This project aimed to address gaps in developing knowledge and a better understanding of incidence, spread and economic impact of strawberry red leaf disorder, for the benefit of the Australian strawberry producers and potentially biosecurity agencies.

The project was successfully delivered following the Program logic below (Figure 25) and by:

- Conducting detailed DNA tests of strawberry plant samples (collected from each major growing region) coupled with pathology and microscopy studies and linked with strawberry disease management and productivity
- Searching broadly for a potential RLD cause
- Contributing to RLD identification and potentially assisting a short-term potential management guide if the cause was identified
- Establishing a network and communication channel as part of a 'Communities of Practice' through presenting updates and findings at grower and industry expert meetings
- Developing a pathway for a potential RLD PhD study if a causal agent was identified

Relevant SIP outcome(s)	”Greater skills, capacity and knowledge in the industry”			
End of project outcomes	Knowledge of the cause(s) of RLD and the development of cost-effective control strategies adopted by industry			
Intermediate outcomes	A more complete understanding of the cause(s) of RLD through filling of knowledge gaps	Clarity on the mechanism(s) involved in RLD symptom expression	Clarity on the fruit yield impact for RLD associated plants (pilot study)	
Outputs	Effect of RLD on productivity and cost benefit analysis of controls developed	Contribute to RLD identification and short-term management guide	Efficacy of current and alternative control strategies of RLD tested	Confirmation of the status of potential RLD alternative host plants
	Update RLD research to relevant industry websites	Article submitted to the Australian Berry Journal	Peer-reviewed research publications submitted	Presentations at grower and industry expert meetings
Activities	PCR and HTS analysis of existing sequence data from RLD symptomatic and asymptomatic plants.	Symptomatic plants from farms examined for viral, bacterial, fungal pathogens and insect pests.	Next generation sequencing (NGS) of DNA and RNA from the plants’ holobiont (leaves including colonising microbes and herbivores).	Gene expression profiling of leaves from healthy vs diseased, and RLD-free plants to determine the genes induced or repressed in development of RLD.
	Survey commercial farms to determine the impact of RLD on crop yield and quality.	Develop priorities for ongoing RLD research (Phase 2) i.e., develop PhD pathways		
Foundational outputs	Establishment of data sharing and storage protocols from previous and/or related RLD projects			
Foundational activities	A program logic in a monitoring and evaluation plan	A project risk register that includes how risks will be managed	A stakeholder engagement/ communication plan	Partnerships

Figure 25 Logic model for Phase 1 BS19001

This project was delivered through a close collaboration between DAF and UQ and is divided into two phases, 1 and 2. Phase 1 was to prepare and form the basis of a proposed collaborative DAF-led four-year study (Phase 2).

Phase 1 (6 months) expanded on existing discovery-driven next generation sequencing analyses undertaken by DAF, UQ and AgriBio to identify potential pests and diseases common to plants with RLD symptoms. In addition to these DNA analyses, we included an expansion in the number of farms and severity of RLD sampled.

Understanding how symptomatic plants are responding to RLD may help identify potential causes of the disorder. This phase of the project complemented DAF's existing multi-disciplinary research activities investigating epidemiology, potential causative agents, nutrition, and the overall industry impact of RLD.

Outcomes from Phase 1 included information back to growers on potential causes of RLD and was planned to form the basis of a proposed collaborative DAF-led four-year study (Phase 2). It is proposed that Phase 2 will be underpinned by a PhD project, to fully research the causal mechanisms and provide management options for the industry.

At the beginning of this project, Hort Innovation and the project team set four key evaluation questions below:

Table 5 Project key evaluation questions

Key evaluation questions	Relevant?	Project-specific questions
Effectiveness		
1. To what extent has the project achieved its expected outcomes?	Yes	Has the project developed new knowledge that provides value to industry?
Relevance		
2. How relevant was the project to the needs of intended beneficiaries?	Yes	Did the project provide useful biological and epidemiological information?
Process appropriateness		
3. How well have intended beneficiaries been engaged in the project?	Yes	Have regular project updates been provided through linkage with the industry communication project?
4. To what extent were engagement processes appropriate to the target audience/s of the project?	Yes	Were project outcomes provided in a readily accessible form to stakeholders? How effective was engagement with the strawberry industry? Was the information presented in a way that was useful to growers?
Efficiency		
5. What efforts did the project make to improve efficiency?	Yes	What has the project achieved to assist growers to identify or potentially manage RLD? To what extent has the project identified scientific or knowledge gaps that require future prioritisation and investment?

1. To what extent has the project achieved its expected outcomes?

This project has delivered outputs (page 26) and outcomes (page 27) above expectations in the program logic. This results from the practicality and applied nature of the research, combined with the strong engagement the team members had with growers and industry members. This project has had great success in educating growers on the symptoms of red leaf disorder and assisted growers to monitoring effectively.

2. How relevant was the project to the needs of intended beneficiaries?

The project provided useful biological and epidemiological information relevant to strawberry producers in South East Queensland. Yield data and physiological changes to the strawberry RLD affected plants were studied. Extensive molecular biological tests were carried out to determine potential candidates to study further. Unfortunately, the causal agent for Red Leaf Disorder has not yet been identified, which makes it difficult to provide control and mitigation strategies to prevent it. Comprehensive outcomes from: Farm surveys, yield and root health data analysis, fungal isolations, biological indexing, microscopy analysis, RNA sequencing, and through extensive communication with industry stakeholders, has provided useful biological

and epidemiological information.

3. How well have intended beneficiaries been engaged in the project?

Internal regular team meetings have been held throughout the project to ensure communication has been abundant in all key research areas to improve outputs. External publications and presentations were produced for the Queensland Strawberry Growers Association meetings outlining project scope, project team and general updates throughout the season (as per Outputs section).

4. To what extent were engagement processes appropriate to the target audience/s of the project?

The strawberry industry was heavily involved in our project and assisted with on-site farm visits and sample collections that have been beneficial to the project. The strawberry industry is highly engaged and helpful to assist in the research to the cause of Red Leaf Disorder. All growers visited for this project, allowed us on their properties to collect data and samples, and have subsequently allowed further access to continue future RLD research work.

The information has been provided to growers in various styles, including Berry journal articles, PowerPoint presentations at growers' meetings, phone calls and face to face communication. Upon completion of this final report, we are prepared to provide a project update of what was found to better understand red leaf disorder in strawberry plants at an industry event.

5. What efforts did the project make to improve efficiency?

A better understanding of RLD has been achieved through this project. As no cause has been confirmed, this project has not necessarily assisted growers to manage the disorder but has allowed them to be more aware of the incidence. Growers are now more aware of identifying RLD and can be more useful in their observation of key information that may assist our research.

The project has developed new knowledge and potential leads that will benefit the strawberry industry; however, future prioritisation for further funding for phase two of this project should be considered. Potential leads that were found in this project require further research as well as to keep a broad search open to potential candidates. Once a strong candidate is recognised, effective management strategies will need to be researched.

This research study has delivered data and useful information, enabling future research to build upon existing foundations. This will allow strawberry growers and industry to get closer to knowing the cause of RLD and prepares for Phase 2 of the project.

Recommendations

RLD research to date and the search to find the causal agent still holds several gaps in knowledge of the complex mechanisms behind the disorder and how best to manage RLD on farm sites. The project successfully concentrated on key areas to investigate and can recommend the following:

Continue progress towards identifying causal agent of RLD by maintaining a comprehensive approach across multi-disciplinary academic working groups.

The results of farm surveys have contributed to our knowledge of occurrence and spread of RLD in fields. Continuing data collation at previously visited commercial strawberry farms is vital to identify statistical trends, and to data mine potential changes that show correlations with spikes in RLD occurrence. A comprehensive approach looking at all factors involved in the strawberry growing process should be investigated further (e.g., soil type, climatic events, nutritional regimes, farm management etc.).

Preliminary trials undertaken by DAF suggest strongly that nutrition plays an important role in RLD expression. Plants in a trial at Nambour, stressed from poor nutrition and showing RLD symptoms lost the reddening when they were subsequently fertilized. Further work is required to determine nutritional effects on RLD.

Further molecular testing should be investigated. While DNA and RNA analysis have identified preliminary leads, further research in this area should be pursued.

Eventually, if a causal agent is identified; and if it is found to be pathogenic; it is recommended that research be conducted to develop a broad set of control options. This is required to sustain the strawberry industry into the future, to provide effective disease controls for all industry sectors (conventional, organic, nursery), and to build a greater level of precision and flexibility in management. Areas of research with the greatest benefit to growers include:

- A reliable diagnostic tool
- Non-chemical treatments for reducing the amount of the pathogen before planting (if it is a pathogen)
- Investigating RLD tolerant varieties
- Establishing a farm biosecurity plan to protect against RLD within farms. Training of farm staff in hygiene practices for use in daily operations.

Studies overseas have indicated several alternative treatments that reduce pathogens in the soil to varying degrees, including: biofumigation with brassica crops, anaerobic soil disinfestation, high soil temperature by microwave or steam treatment, repeated removal of plant debris for the depletion of pathogen inoculum over the medium to long term, and crop rotation. The suitability and effectiveness of these techniques in local strawberry production systems has yet to be proven. There has also been preliminary work done on strawberry plants in Victoria, Australia, and efficacy demonstrated on microwave treatment.

Non-chemical treatments give industry the opportunity to reduce chemical usage and provide control options for organic growers. Non-chemical treatments may complement, offset, or replace the need for chemical fumigants for controlling RLD.

Although we have not confirmed any causal agent of RLD, potential leads discovered in this phase of the project may assist Phase 2. As the project moves into Phase 2, we will be improving the knowledge of RLD epidemiology, working closer towards confirming a potential candidate for the cause of the disorder, and further analysing the economic impacts on commercial strawberry producers that can be shared directly with industry and stakeholders.

Refereed scientific publications

Chapter in a book or Paper in conference proceedings

Kristoffersen, J., Paynter, M., Constable, F., Gomez, A., Neal, J. and Hung, T. 2021, 'Red Leaf': A new disorder in Australian strawberry plants. Acta Horticulturae, 1309. pp. 773-780. ISSN 05677572 (ISSN)

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Fránová J., Přibylová, J., and Koloniuk, I. 2019 Molecular and Biological Characterization of a New Strawberry Cytorhabdovirus. Viruses. 11(11): 982. https://doi.org/10.3390/v11110982

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Wood, D. E., Salzberg, S. L., 2014. Kraken: Ultrafast metagenomic sequence classification using exact alignments. Genome Biol; 15: R46.

Xinlun Ding, X., Chen, D., Du, Z., Zhang, J., and Wu, Z. 2019. The complete genome sequence of a novel cytorhabdovirus identified in strawberry (Fragaria ananassa Duch.). Arch Virol. 164(12):3127-3131 https://doi.org/10.1007/s00705-019-04390-y.

Yoshikawa, N., Inouye, T., and Converse, R. H. 1986. Two Types of Rhabdovirus in Strawberry. Japanese Journal of Phytopathology 52(3): 437-444. https://doi.org/10.3186/jjphytopath.52.437.

Intellectual property, commercialisation and confidentiality

No commercial IP will be generated in this project and there are no restrictions on the use of any pre-existing IP noting that both DAF and UQ bring background IP into this project.

This project will generate best-practice information to manage RLD in the Australian strawberry fruit industry.

Isolates of any identified pathogens that have been collected by DAF and UQ from strawberry and other host plants will be made available for use in the research within this project. These isolates are owned by DAF and UQ.

Project data and information products (e.g. factsheets, bulletins, photographs) generated within the project will be jointly owned and shared by DAF, UQ and Hort Innovation, as tenants in common.

Acknowledgements

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We thank the strawberry Industry Development Officers, particularly in Queensland, for their enthusiasm in facilitating events and generally fostering the delivery of information from this project directly to strawberry growers.

We are forever grateful of the patience and cooperation of the multiple growers who allow us to perform on farm recording of disorder incidence and occurrence throughout the season. These engagements were essential for ensuring we are building on our knowledge of the epidemiology of Red Leaf Disorder in South East Queensland.

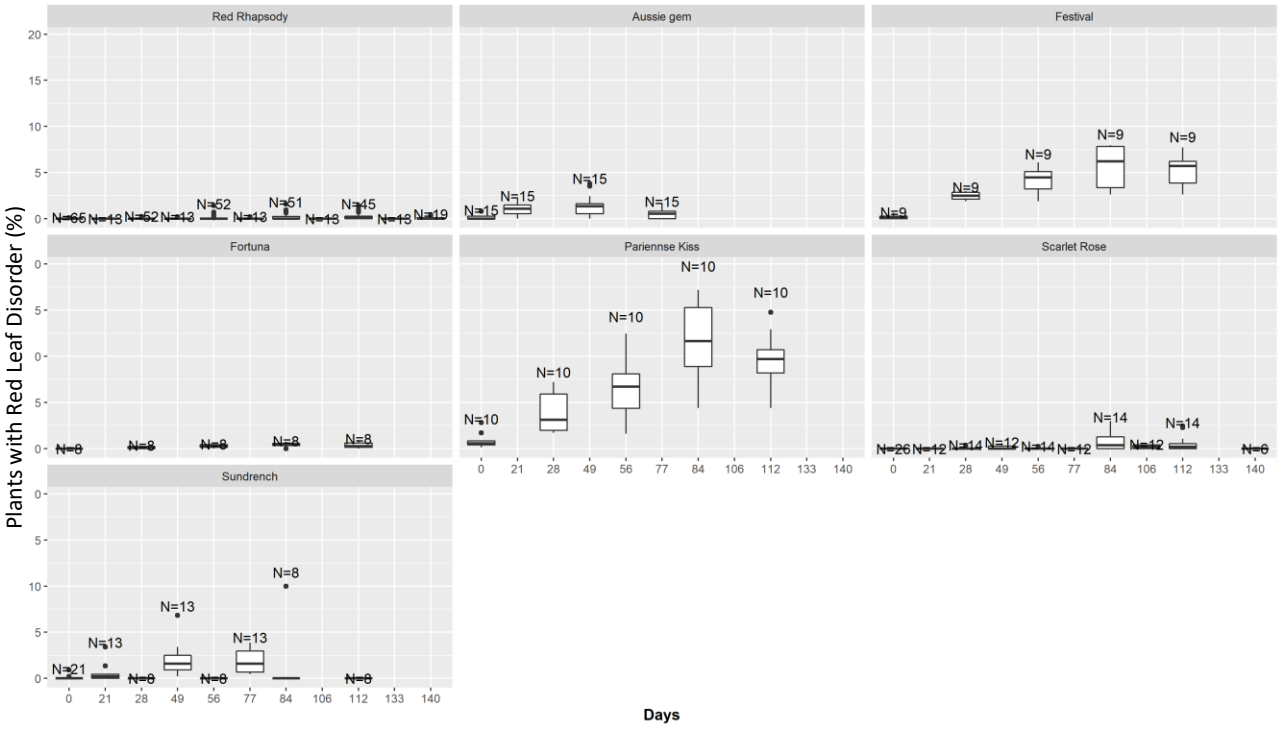
The Queensland Government, through the Department of Agriculture and Fisheries have co funded the research.

Appendices

Appendix 1. Coefficients, exponentiated coefficient, odd ratios, and relative odd ratios obtained in the best fitting model for the proportion of plants with RLD in the dataset for Farms visits in 2020.

Term	Coef	Coef.Exp	OR	OR.Rel.Int
(Intercept)	-28.99109955	2.57E-13	2.57E-13	1
CultivarAussie gem	7.659073208	2119.79191	5.44E-10	2119.79191
CultivarFestival	9.426149156	12408.6508	3.18E-09	12408.6508
CultivarFortuna	5.859897185	350.688086	9.00E-11	350.688086
CultivarPariennse Kiss	10.21027792	27181.1208	6.98E-09	27181.12061
CultivarScarlet Rose	13.24575267	565662.376	1.45E-07	565662.2944
CultivarSundrench	6.205560343	495.496526	1.27E-10	495.496526
Farm_Code1	13.95320405	1147623.75	2.95E-07	1147623.408
Farm_Code2	15.16148348	3841922.6	9.86E-07	3841918.812
Farm_Code4	17.76574633	51947613.2	1.33E-05	51946920.62
Farm_Code5	17.39676392	35918531.7	9.22E-06	35918200.65
Farm_Code6	18.72291615	135287967	3.47E-05	135283270.1
Farm_Code7	20.04317796	506572474	0.00012999	506506624.5
Days	0.087527162	1.09147191	2.80E-13	1.091471911
I(Days^2)	-0.000426391	0.9995737	2.57E-13	0.9995737
CultivarScarlet Rose:Farm_Code2	-13.08767624	2.07E-06	5.31E-19	2.07E-06
CultivarScarlet Rose:Farm_Code7	-16.36556786	7.81E-08	2.00E-20	7.81E-08
CultivarAussie gem:Days	-0.001818482	0.99818317	2.56E-13	0.99818317
CultivarFestival:Days	-0.025075097	0.97523667	2.50E-13	0.975236672
CultivarFortuna:Days	-0.008864053	0.99117512	2.54E-13	0.991175117
CultivarParisienne Kiss:Days	-0.028373464	0.97202528	2.49E-13	0.972025283
CultivarScarlet Rose:Days	0.064060418	1.06615681	2.74E-13	1.066156811
CultivarSundrench:Days	0.026226639	1.02657358	2.63E-13	1.026573584
CultivarAussie gem:I(Days^2)	-0.000558222	0.99944193	2.56E-13	0.999441933
CultivarFestival:I(Days^2)	7.74E-05	1.00007742	2.57E-13	1.000077417
CultivarFortuna:I(Days^2)	-1.39E-05	0.99998606	2.57E-13	0.999986062
CultivarParisienne Kiss:I(Days^2)	0.000105811	1.00010582	2.57E-13	1.000105817
CultivarScarlet Rose:I(Days^2)	-0.000366587	0.99963348	2.57E-13	0.99963348

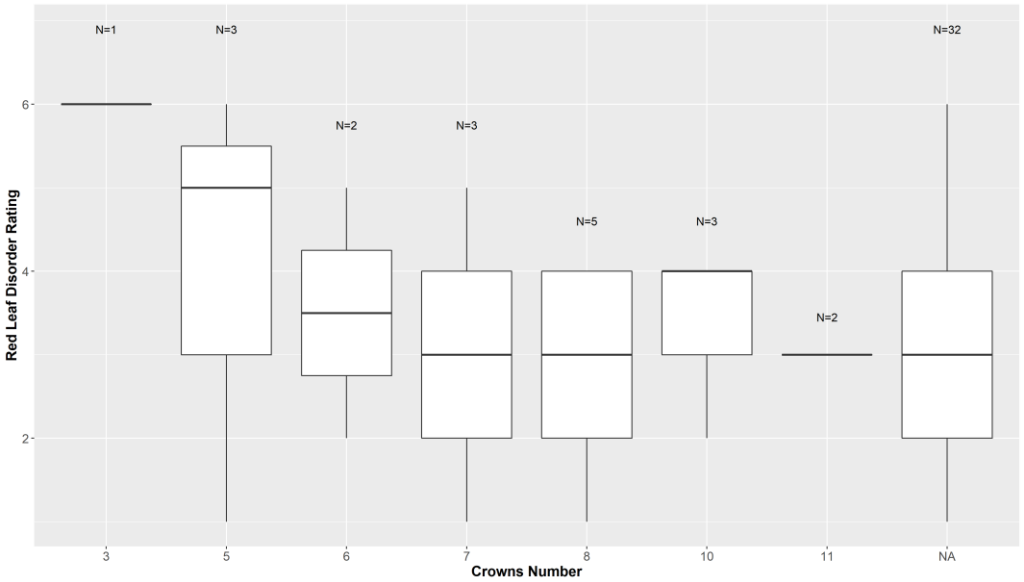
Appendix 2. Distribution of the percentage of plants with RLD per day (time) for each farm



Appendix 3. Coefficients, exponentiated coefficient, odd ratios, and relative odd ratios obtained in the best fitting model for the proportion of plants with RLD in the dataset for Planting method (Plugs vs Bare) in 2020.

Term	Coef	Coef.Exp	OR	OR.Rel.Int
(Intercept)	-7.332443279	0.000653974	0.000653546	1
TypeBare	0.600116976	1.822331957	0.001190339	1.821353104
Days	-0.021919165	0.978319314	0.000639386	0.978333176

Appendix 4. Relationship between RLD and Crown number



Appendix *5.* The following tables provide summaries of the percentage of abundance for different taxa for each sample. Note the boxes highlighted in yellow show matches for putative causative agents for RLD as they were abundant in all samples tested

Kraken – Genus Level

Name	Mean	Dieter-healthy	Dieter_Infected	NEWDAF_1	NEWDAF_5	OLDDAF_Healthy	OLDDAF_Infected	PB4	SW_s
Nostoc	19.01	18.43%	27.15%	7.204%	27.31%	2.295%	2.48%	31.48%	24.65%
Pasteurella	16.722	13.64%	13.91%	4.165%	14.81%	29.68%	21.77%	18.17%	15.27%
Fischerella	15.006	13.7%	20.78%	5.864%	24%	0.06934%	1.105%	25.86%	19.31%
Pseudomonas	9.0073	2.406%	1.548%	39.11%	6.254%	4.654%	24.77%	0.7828%	1.063%
Acinetobacter	5.4477	1.054%	0.4296%	0.2538%	0.4126%	26.17%	18.14%	0.6453%	1.171%
Geminocystis	2.9564	4.846%	2.362%	1.012%	2.218%	0.0627%	0.2039%	4.511%	6.295%
Halomonas	1.9984	0.05871%	0.0535%	0.04787%	0.05314%	10.21%	7.509%	0.01394%	0.02191%
Rivularia	1.9466	2.775%	3.841%	0.5949%	0.6811%	0.004771%	0.0824%	2.297%	5.109%
Methylobacterium	1.2678	4.715%	0.9525%	3.814%	1.214%	0.002117%	0.03823%	0.227%	0.2883%
Tolypothrix	1.2347	2.454%	0.7359%	0.3533%	0.4834%	0.002208%	0.05565%	1.829%	3.161%
Sphingomonas	1.126	3.351%	1.058%	3.353%	1.832%	0.01587%	0.05898%	0.2522%	0.1202%
Moorea	1.1185	2.193%	0.9263%	0.3067%	0.4531%	0.04185%	0.08732%	1.439%	2.844%
Pantoea	0.99515	0.8583%	0.2287%	1.695%	0.9041%	0.003025%	0.5419%	0.01655%	0.03174%
Campylobacter	0.91486	0.02428%	0.02367%	0.03988%	0.04967%	4.824%	3.14%	0.01461%	0.1001%
Mycoplasma	0.89233	0.03392%	0.07815%	0.1036%	0.1267%	4.544%	2.887%	0.02824%	0.1735%
Calothrix	0.83226	1.423%	0.8414%	0.3179%	0.5029%	0.006499%	0.05863%	1.131%	1.777%
Microbacterium	0.74938	1.906%	1.791%	1.652%	1.044%	0.01471%	0.1166%	0.06637%	0.08749%
Bacillus	0.70438	0.5212%	2.566%	0.2234%	0.7793%	0.1031%	0.09199%	1.227%	0.3846%
Streptomyces	0.70418	1.575%	0.5051%	0.5773%	0.4081%	1.349%	1.558%	0.06752%	0.2354%
Clostridium	0.69087	0.47%	0.6113%	0.2337%	0.7315%	0.8769%	0.9749%	0.7807%	0.7496%
Halothece	0.68881	0.01913%	0.04348%	0.09796%	0.1332%	3.383%	2.326%	0.02188%	0.1212%
Synechococcus	0.66647	0.2936%	0.3112%	0.1027%	0.1787%	2.377%	1.538%	0.3295%	0.5718%
Xanthomonas	0.63559	1.055%	1.069%	1.982%	0.5912%	0.3298%	0.5356%	0.01267%	0.1117%
Euhalothece	0.55707	0.941%	1.236%	0.1396%	0.1867%	0.005248%	0.04401%	0.6298%	1.25%
Curtobacterium	0.54277	2.132%	0.4537%	1.41%	0.7414%	0.0009142%	0.0222%	0.02442%	0.009507%
Crocosphaera	0.53226	0.9483%	0.8637%	0.1917%	0.2071%	0.008325%	0.03902%	0.5087%	1.466%
Neptunomonas	0.51436	0.4246%	1.253%	0.147%	0.2818%	0.0008777%	0.009953%	0.9652%	1.018%
Saccharomonospora	0.3961	0.0167%	0.02373%	0.03292%	0.03964%	2.003%	1.392%	0.00594%	0.03593%
Variovorax	0.38633	0.9465%	0.6799%	1.397%	0.2704%	0.0334%	0.07837%	0.01764%	0.02352%
Blattabacterium	0.33862	0.3053%	0.6684%	0.191%	0.3941%	0.002229%	0.02536%	0.2236%	0.9265%
Candidatus Phytoplasma	0.32823	0.0002943%	0.0001728%		0.0001239%	0.00212%	0.001363%	2.95%	
Rickettsia	0.31389	0.2256%	0.05246%	0.3762%	0.3197%	0.01719%	0.09036%	0.1256%	1.312%
Erwinia	0.28	0.02251%	0.01486%	2.009%	0.3089%	0.00109%	0.006001%	0.001333%	0.003061%
Rhizobium	0.26064	0.2962%	0.3599%	1.1%	0.463%	0.01109%	0.06228%	0.008727%	0.03351%
Nocardioides	0.25909	0.5154%	0.4634%	1.024%	0.2138%	0.02135%	0.02975%	0.01885%	0.02433%
Kozakia	0.22778	0.2096%	0.3996%	0.07407%	0.09252%	0.0003584%	0.005549%	0.3996%	0.6036%
Sphingobium	0.22503	0.3991%	0.3978%	0.4987%	0.2414%	0.02605%	0.09744%	0.05933%	0.2414%
Rhodococcus	0.22352	0.5667%	0.1657%	0.699%	0.5446%	0.001558%	0.02134%	0.003273%	0.005317%
Methylorubrum	0.2203	0.6338%	0.2302%	0.859%	0.201%	0.0001397%	0.006647%	0.01806%	0.01853%
Rathayibacter	0.21678	0.975%	0.2305%	0.5046%	0.2197%	0.0002278%	0.004516%	0.00897%	0.002578%
Serratia	0.2129	0.02568%	0.01538%	0.2679%	0.02428%	0.1172%	1.409%	0.006424%	0.03561%
Fibrobacter	0.20106	0.0002207%		0.0001583%	0.0003716%	1.154%	0.6543%	0.00006061%	0.0003223%
Conchiformibius	0.19427	0.00007357%	0.00005759%			1.053%	0.6951%	0.0001212%	
Novosphingobium	0.18591	0.2053%	0.363%	0.666%	0.3276%	0.02074%	0.07558%	0.007455%	0.004673%
Arthrospira	0.17419	0.2006%	0.3062%	0.2092%	0.2213%	0.001133%	0.014%	0.1496%	0.3442%
Aeromicrobium	0.15349	0.3455%	0.2081%	0.5118%	0.2611%	0.009442%	0.03673%	0.002727%	0.003867%
Chondrocystis	0.1506	0.2195%	0.2735%	0.08514%	0.1302%	0.002336%	0.01256%	0.1566%	0.3244%
Agrobacterium	0.14868	0.1905%	0.2123%	0.5372%	0.2942%	0.001163%	0.02517%	0.01012%	0.05897%
Stenotrophomonas	0.14206	0.1101%	0.296%	0.3329%	0.4547%	0.01426%	0.04018%	0.006364%	0.01837%
Parachlamydia	0.14041	0.09785%	0.09848%	0.1761%	0.1226%	0.001336%	0.02616%	0.04297%	0.6539%

Kraken bacteria species level

Name	Mean	Dieter-healthy	Dieter_Infected	NEWDAF_1	NEWDAF_5	OLDDAF_Healthy	OLDDAF_Infected	PB4	SW_s
Nostoc sp. ATCC 53789	19.588	20.3%	27.39%	8.982%	27.95%	0.05257%	1.156%	32.73%	25.84%
Pasteurella multocida	18.474	15.44%	14.74%	5.462%	15.82%	31.75%	26.08%	19.35%	16.88%
Fischerella sp. NIES-4106	15.657	14.8%	21.05%	7.32%	24.54%	0.04662%	1.229%	26.32%	20.25%
Acinetobacter baumannii	5.5763	0.1101%	0.1119%	0.07555%	0.1214%	27.99%	21.66%	0.02543%	0.06825%
Halomonas sp. JS92-SW72	2.2345	0.04499%	0.04235%	0.03155%	0.04591%	10.92%	8.989%	0.01059%	0.0196%
Rivularia sp. PCC 7116	2.1376	3.142%	4.07%	0.7802%	0.7275%	0.005105%	0.0987%	2.446%	5.65%
Tolypothrix sp. PCC 7910	1.3629	2.779%	0.7797%	0.4634%	0.5164%	0.002362%	0.06666%	1.948%	3.496%
Moorea producens	1.2342	2.484%	0.9815%	0.4022%	0.4839%	0.04478%	0.1046%	1.532%	3.145%
Campylobacter geochelonis	0.9969	0.006082%	0.01672%	0.01847%	0.02474%	5.139%	3.736%	0.005422%	0.01889%
Enterobacteriaceae endosymbiont of Donacia cincticornis	0.91703	0.002583%	0.0122%	0.02148%	0.02381%	4.652%	3.505%	0.003485%	0.0253%
Halothece sp. PCC 7418	0.77343	0.02166%	0.04607%	0.1265%	0.1422%	3.62%	2.787%	0.0233%	0.134%
Pseudomonas fluorescens	0.73766	0.2194%	0.09599%	3.834%	0.3215%	0.07125%	1.979%	0.01736%	0.07627%
Fischerella sp. NIES-3754	0.70647	0.6348%	0.9269%	0.3571%	1.079%	0.01989%	0.06815%	1.187%	0.9944%
Clostridium botulinum	0.69094	0.4965%	0.5962%	0.2206%	0.6733%	0.8307%	1.077%	0.8087%	0.7055%
Bacillus velezensis	0.66968	0.5014%	2.566%	0.2285%	0.7466%	0.002083%	0.006073%	1.214%	0.3386%
Nostoc linckia	0.61878	0.3629%	1.241%	0.4003%	1.105%	0.002164%	0.02485%	0.6272%	1.161%
Euhalothece natronophila	0.61138	1.066%	1.31%	0.1831%	0.1994%	0.005615%	0.05271%	0.6707%	1.383%
Crocosphaera subtropica	0.58734	1.074%	0.9151%	0.2513%	0.2212%	0.008907%	0.04673%	0.5418%	1.621%
Neptunomonas concharum	0.56034	0.4808%	1.327%	0.1926%	0.301%	0.0009034%	0.01178%	1.028%	1.126%
Pseudomonas graminis	0.48149	0.04507%	0.05913%	3.092%	0.8558%	0.0009911%	0.1789%	0.09365%	0.002138%
Synechococcus sp. PCC 7336	0.46675	0.01208%	0.02764%	0.01193%	0.02381%	2.402%	1.701%	0.006519%	0.0123%
Geminocystis sp. NIES-3708	0.44873	0.4037%	0.5406%	0.271%	0.7239%	0.01199%	0.07764%	0.5896%	0.5259%
Saccharomonospora marina	0.44067	0.007665%	0.01831%	0.03259%	0.03929%	2.142%	1.666%	0.006003%	0.03813%
Blattabacterium cuenoti	0.37283	0.3455%	0.708%	0.2502%	0.4206%	0.001628%	0.02971%	0.2377%	1.024%
Pseudomonas azotoformans	0.36773	0.008498%	0.007506%	1.119%	0.03612%	0.2733%	1.855%	0.002775%	0.00499%
Candidatus Phytoplasma australiense	0.3485	0.0003333%	0.000122%			0.000013%	0.000006092%	3.136%	
Pantoea vagans	0.34523	0.1252%	0.09385%	0.3396%	0.149%	0.0002795%	0.2989%	0.007422%	0.004811%
Pseudomonas rhizosphaerae	0.32575	0.2724%	0.05645%	2.192%	0.3732%	0.001095%	0.03194%	0.002259%	0.001604%
Pseudomonas tolaasii	0.31799	0.03941%	0.02874%	0.3802%	0.05887%	0.6238%	1.522%	0.0133%	0.167%
Streptomyces lividans	0.30059	0.03816%	0.02911%	0.05687%	0.04181%	1.156%	1.213%	0.0122%	0.1283%
Pantoea agglomerans	0.26888	0.1081%	0.0166%	0.538%	0.2377%	0.0002795%	0.07383%	0.003808%	0.0196%
Kozakia baliensis	0.24946	0.2374%	0.4234%	0.09714%	0.09883%	0.0003835%	0.006646%	0.4256%	0.6675%
Sphingomonas sp. HMP9	0.24488	0.5122%	0.1267%	0.8688%	0.6117%	0.00009424%	0.0005178%	0.0455%	0.001782%
Sphingomonas sp. PAMC26645	0.24152	0.3508%	0.08805%	1.142%	0.5273%	0.00008124%	0.0003716%	0.0355%	0.005881%
Fibrobacter succinogenes	0.22446	0.00025%		0.0002076%	0.0003969%	1.235%	0.7837%	0.00006454%	0.0003564%
Conchiformibius steedae	0.21776	0.00008332%	0.00006102%			1.127%	0.8325%	0.0001291%	
Pseudomonas oryzihabitans	0.21442	0.6206%	0.2776%	0.5395%	0.3004%	0.0003835%	0.01063%	0.06422%	0.1048%
Arthrospira platensis	0.19473	0.2272%	0.3245%	0.2744%	0.2364%	0.001212%	0.01677%	0.1594%	0.3806%
Pseudomonas rhodesiae	0.19355	0.004666%	0.003783%	1.482%	0.01693%	0.006015%	0.2209%	0.001097%	0.002317%
Curtobacterium flaccumfaciens	0.192	0.2639%	0.04058%	0.9654%	0.3673%	0.00013%	0.004721%	0.002969%	0.001247%
Rickettsia canadensis	0.17608	0.1206%	0.01538%	0.2587%	0.1531%	0.002479%	0.0581%	0.05034%	0.7702%
Pseudomonas sp. S09G 359	0.16874	0.003416%	0.002563%	1.111%	0.02739%	0.009408%	0.3615%	0.0005163%	0.00196%
Chondrocystis sp. NIES-4102	0.16627	0.2485%	0.2898%	0.1117%	0.139%	0.002499%	0.01504%	0.1668%	0.3587%
Pseudomonas synxantha	0.16411	0.007332%	0.006834%	0.8878%	0.02791%	0.02435%	0.5163%	0.001936%	0.003029%
Curtobacterium sp. BH-2-1-1	0.16287	0.9721%	0.05364%	0.2892%	0.1285%	0.0001105%	0.005952%	0.003679%	0.002851%
Synechococcus sp. PCC 7117	0.16215	0.2029%	0.1905%	0.06258%	0.07911%	0.0005037%	0.008157%	0.2696%	0.4284%
Parachlamydia acanthamoebae	0.15881	0.1108%	0.01043%	0.2309%	0.131%	0.00143%	0.03134%	0.04576%	0.7231%
Pseudomonas sp. HLS-6	0.15732	0.1079%	0.1417%	0.4052%	0.2815%	0.0008871%	0.03678%	0.1381%	0.2026%
Rickettsia felis	0.14552	0.1143%	0.01007%	0.1973%	0.1232%	0.0006402%	0.02209%	0.06764%	0.6242%
Methylorubrum extorquens	0.14484	0.38%	0.1373%	0.6612%	0.1001%	0.00005524%	0.003734%	0.009101%	0.006059%

Kraken eukaryote

Name	Mean	Dieter-healthy	Dieter_Infected	NEWDAF_1	NEWDAF_5	OLDDAF_Healthy	OLDDAF_Infected	PB4	SW_s
Guillardia theta	35.505	47.52%	58.31%	18.04%	26.26%	1.697%	5.479%	60.76%	42.71%
Thalassiosira pseudonana	28.281	28.08%	27.54%	24.48%	20.9%	39.79%	34.28%	22.31%	30.98%
Toxoplasma gondii	6.9634	1.441%	0.4428%	4.066%	2.212%	28.58%	24.05%	0.3118%	1.147%
Bigelowiella natans	4.7656	5.307%	4.768%	2.377%	4.423%	5.714%	4.743%	5.349%	3.797%
Babesia bigemina	2.5885	2.206%	0.6716%	6.556%	5.78%	0.6493%	2.155%	1.022%	3.871%
Cryptomonas paramecium	2.2641	2.711%	3.107%	2.241%	2.687%	3.254%	3.345%	1.28%	1.108%
Neospora caninum	2.1523	1.779%	0.3247%	5.123%	5.889%	0.04905%	1.955%	0.7796%	3.063%
Theileria orientalis	1.7607	0.6431%	0.3764%	3.17%	5.821%	0.2604%	2.064%	0.9731%	1.808%
Babesia microti	1.6895	0.5657%	0.6274%	7.212%	4.03%	0.1371%	0.4413%	0.6183%	0.7741%
Phaeodactylum tricornutum	1.6525	0.9605%	0.4207%	2.786%	2.822%	1.325%	2.06%	1.484%	1.944%
Cryptosporidium parvum	1.6279	0.2076%	0.1624%	0.7604%	0.9227%	7.183%	5.15%	0.03226%	0.1921%
Besnoitia besnoiti	1.2465	1.799%	0.1107%	5.451%	2.646%	0.07003%	0.3179%	0.08065%	0.2373%
Theileria equi	1.1829	0.5983%	0.1181%	3.306%	2.89%	0.3266%	0.9037%	0.371%	1.379%
Trypanosoma brucei	1.0132	0.9972%	0.2952%	3.242%	1.669%	0.03453%	1.196%	0.328%	0.9776%
Babesia bovis	1.0055	0.4151%	0.1329%	1.721%	1.045%	0.8474%	1.001%	1.22%	1.701%
Hemiselmis andersenii	0.94349	0.07326%	0.0369%	0.1681%	0.3935%	4.658%	3.145%	0.005376%	0.0113%
Dictyostelium discoideum	0.84893	0.6756%	0.3764%	1.657%	2.144%	0.4908%	1.285%	0.1774%	0.6329%
Theileria parva	0.82113	0.05698%	0.0369%	0.1921%	0.09498%	4.057%	2.772%	0.03763%	0.06781%
Plasmodium relictum	0.70794	1.042%	0.6126%	0.9125%	0.692%	0.2288%	0.4624%	1.812%	0.3447%
Plasmodium chabaudi	0.68571	0.4477%	0.1181%	1.513%	1.737%	0.1459%	0.6448%	0.2796%	1.153%
Leishmania braziliensis	0.49232	0.5454%	0.02952%	1.769%	0.9362%	0.003872%	0.2191%	0.1774%	0.4916%
Plasmodium malariae	0.37724	0.2686%	0.1476%	0.5523%	0.7327%	0.04163%	0.7869%	0.1935%	0.3729%
Plasmodium vivax	0.34059	0.3256%	0.3395%	0.3682%	0.7598%	0.163%	0.3967%	0.1183%	0.5425%
Plasmodium coatneyi	0.23061	0.289%	0.6126%	0.3602%	0.5156%	0.03227%	0.07225%	0.08602%	0.09606%
Plasmodium gaboni	0.13938	0.1791%	0.1033%	0.2802%	0.2171%	0.01226%	0.2216%	0.04839%	0.1695%
Plasmodium knowlesi	0.13021	0.1384%		0.1841%	0.6242%	0.01388%	0.0885%	0.03763%	0.06216%
Leishmania donovani	0.10765	0.1587%	0.007381%	0.4643%	0.2849%		0.001806%		
Leishmania major	0.10173	0.118%	0.05166%	0.2882%	0.19%	0.009358%	0.1294%	0.02688%	0.07911%
Leishmania mexicana	0.099948	0.1709%	0.05904%	0.2802%	0.2035%	0.002582%	0.1162%	0.02151%	0.0226%
Plasmodium yoelii	0.068146	0.00814%		0.1521%	0.08141%	0.151%	0.1698%		0.05086%
Theileria annulata	0.065928	0.1262%	0.007381%	0.1441%	0.1493%	0.01194%	0.04816%	0.02151%	0.08476%
Plasmodium cynomolgi	0.042415	0.05291%		0.1121%	0.04071%	0.03421%	0.03432%	0.005376%	0.07911%
Leishmania panamensis	0.041082	0.01628%		0.01601%	0.09498%	0.003872%	0.1421%	0.01075%	0.02825%
Plasmodium falciparum	0.019889	0.03256%	0.02952%	0.008004%	0.04071%	0.004518%	0.04154%	0.01075%	0.005651%
Plasmodium reichenowi	0.014953	0.01221%	0.02214%	0.02401%	0.02714%	0.002904%	0.0295%	0.005376%	0.0113%
Plasmodium berghei	0.012584	0.01221%		0.02401%	0.04071%	0.002259%	0.01144%	0.005376%	
Plasmodium sp. gorilla clade G2	0.0073933	0.00407%			0.01357%	0.01097%	0.03793%		
Leishmania infantum	0.0031676	0.02035%					0.002408%		

Kraken tenericutes genus

Name	Mean	Dieter-healthy	Dieter_Infected	NEWDAF_1	NEWDAF_5	OLDDAF_Healthy	OLDDAF_Infected	PB4	SW_s
Mycoplasma	83.262	85.21%	92.44%	92.18%	93.77%	99.78%	99.6%	0.9464%	91.74%
Candidatus Phytoplasma	11.111	0.7394%	0.2044%		0.09166%	0.04655%	0.04703%	98.87%	
Acholeplasma	2.1741	4.251%	0.1362%	5.211%	3.208%	0.007069%	0.05861%	0.04062%	4.514%
Mycoplasmopsis	2.0825	5.73%	6.131%	0.6338%	1.558%	0.06435%	0.1049%	0.1137%	1.704%
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Mesoplasma	0.043478	0.1848%			0.09166%	0.0001334%	0.002106%		
Entomoplasma	0.039544			0.2113%		0.02994%	0.02948%		0.08518%
Ureaplasma	0.020743		0.06812%			0.01707%	0.01632%		0.08518%
Candidatus Hepatoplasma	0.0084056					0.03968%	0.03597%		
Candidatus Izimaplasma	0.00080511					0.002934%	0.002281%	0.002031%	

Kraken tenericutes species

Name	Mean	Dieter-healthy	Dieter_Infected	NEWDAF_1	NEWDAF_5	OLDDAF_Healthy	OLDDAF_Infected	PB4	SW_s
Mycoplasma anserisalpingitidis	40.597	58.63%	69.97%	52.38%	58.49%	0.2784%	9.457%	0.6334%	60.68%
Candidatus Phytoplasma australiense	11.12	0.8677%	0.213%			0.03375%	0.01548%	38.95%	
Mycoplasma capricolum	8.1714	0.2169%	0.213%		0.2358%	42.5%	29.95%		0.1795%
Mycoplasma anseris	5.439	11.28%	7.881%	7.66%	5.189%	0.3122%	2.476%	0.04073%	7.181%
Mycoplasma cloacale	4.5977	5.857%	1.704%	4.141%	4.481%	0.05062%	1.192%	0.04684%	4.847%
Acholeplasma axanthum	4.3796	3.037%	0.1065%	14.29%	7.783%	0.1181%	2.476%	0.03666%	7.361%
Mycoplasmopsis columboralis	3.0628	5.64%	8.733%	1.035%	2.358%	0.1097%	2.167%	0.1018%	1.975%
Tenericutes bacterium MO-XQ	2.4309	0.4338%	0.426%	7.039%	7.547%	0.05906%	1.563%	0.00611%	4.309%
Mycoplasmopsis californica	1.8586	0.6508%	0.426%	0.8282%	0.7075%	8.007%	5.386%	0.004073%	0.7181%
Mycoplasma bovirhinis	1.5103					8.269%	5.324%		
Mycoplasma sp. NEAQ87857	1.3969					7.13%	5.015%		0.1795%
Mycoplasma hominis	1.3856	1.952%	1.491%	1.863%	1.651%	0.0675%	0.7739%	0.004073%	4.668%
Mycoplasma iowae	1.3303			0.207%		6.488%	5.278%		
Mycoplasma sp. Phocoena C-264-GEN	1.3041					6.691%	5.046%		
Candidatus Hepatoplasma crinochetorum	0.91033					5.02%	3.173%		
Mycoplasma nasistruthionis	0.80515	1.518%	0.213%	1.449%	1.651%	0.008437%	0.4488%	0.002037%	0.7181%
Entomoplasma luminosum	0.69667					3.763%	2.507%		
Acholeplasma brassicae	0.65487	1.518%		0.4141%		0.1097%	1.873%	0.004073%	1.975%
Candidatus Phytoplasma ziziphi	0.60226					2.97%	2.43%	0.02037%	
Mycoplasma anatis	0.53331	0.4338%	4.366%						
Mycoplasma bovis	0.4901	1.085%	0.213%	1.035%	0.2358%	0.02531%	0.6655%	0.00611%	0.8977%
Mycoplasmopsis gallinacea	0.48376	0.4338%	0.3195%		0.9434%	0.008437%	1.254%	0.002037%	0.8977%
Ureaplasma urealyticum	0.41444		0.1065%			2.067%	1.377%		0.1795%
Spiroplasma citri	0.36395	0.4338%	0.3195%	0.4141%	0.9434%	0.008437%	0.2322%	0.002037%	0.1795%
Mycoplasma alkalescens	0.311			0.207%		1.493%	1.099%		

Kraken Xantomonadales

Name	Mean	Dieter_Infected	Dieter-healthy	NEWDAF_1	NEWDAF_5	OLDDAF_Healthy	OLDDAF_Infected	PB4	SW_s
Xanthomonas euvesicatoria	22451	326	294	451	199	107208	92649	112	557
Luteibacter pinisoli	1818.6	584	121	2084	277	187	13107	4	
Pseudoxanthomonas spadix	900.89	1739	651	4257	258	163	1028		3
Xanthomonas translucens	853.11	2423	1620	2775	321	22	505	2	3
Xanthomonas sp. GW	705.44	1213	1697	2799	204	17	413		3
Stenotrophomonas rhizophila	657.33	1983	185	1363	1671	12	653	11	15
Luteibacter rhizovicinus	627.56	425	164	2444	536	91	1977	3	3
Xanthomonas sp. SI	618.67	1196	1713	2041	238	18	358		
Xanthomonas sp. SS	618.33	1171	1507	2254	261	19	351		2
Xanthomonas hyacinthi	539.78	2475	792	1182	153	3	243	1	6
Xanthomonas arboricola	489.67	1027	1210	1274	833	7	35		
Stenotrophomonas maltophilia	457.22	1119	321	748	290	676	849	54	29
Xanthomonas theicola	351.22	1994	357	581	107	9	107	2	3
Xanthomonas campestris	280.44	427	218	1129	255	22	172	64	103
Xanthomonas sacchari	261.67	968	315	549	368	7	138	5	1

Kraken virus

Name	Mean	Dieter-healthy	Dieter_Infected	NEWDAF_1	NEWDAF_5	OLDDAF_Healthy	OLDDAF_Infected	PB4	SW_s
Synechococcus virus SIOM18	33.907	55.7%	40.83%	9.339%	0.4711%	3.087%	16.75%	61.92%	69.76%
Pepper chlorotic spot orthotospovirus	10.967	0.02261%	0.03752%	0.03585%	0.002176%	80.46%	18.04%	0.02621%	0.09066%
Prochlorococcus virus PSSP7	8.5449	5.676%	24.65%	2.294%	0.2927%	0.6205%	3.699%	20.44%	8.432%
Staphylococcus virus Andhra	8.424	2.533%	3.715%	2.258%	0.07616%	13.03%	46.07%	1.232%	5.122%
Alternaria alternata chrysovirus	8.249	5.608%	0.07505%	68.18%	0.07398%		0.2176%		0.04533%
Stenotrophomonas phage Mendera	5.3317	8.232%	11.07%	2.904%	0.1969%	0.605%	5.534%	6.63%	6.8%
Grapevine associated narnavirus-1	4.1008	0.8367%	0.1126%	0.4123%	23.72%				0.04533%
Sclerotinia sclerotiorum mitovirus 3	3.3862	0.6106%	0.03752%	0.1434%	25.58%				0.136%
Botrytis cinerea mitovirus 3	2.7529	1.832%	0.1501%	0.1613%	18.39%				0.09066%
Botrytis cinerea mitovirus 1	2.6073	0.4749%		0.1255%	18.12%				
Yersinia virus AP10	2.1894	2.261%	5.816%	3.424%	0.1262%			3.407%	3.218%
Botrytis cinerea mitovirus 4	1.031	0.04523%		0.0717%	8.855%				
Alternaria arborescens mitovirus 1	0.62782	3.709%		0.3585%	0.0729%		0.005579%	0.05241%	
Hubei picorna-like virus 64	0.43467	3.912%							
Tobacco virtovirus 1	0.32337			2.133%					
Prochlorococcus phage P-TIM68	0.29362	0.2261%	1.351%	0.1792%	0.006528%		0.06695%	0.3407%	0.2267%
Klebsiella phage Soft	0.27008	0.1809%	0.7505%	0.3585%	0.008704%			0.5241%	0.3626%
Synechococcus virus ST4	0.26347	0.1809%	0.2251%	0.1434%	0.01632%	0.1551%	0.3069%	0.3407%	0.6346%
Klebsiella phage KPN N137	0.2351	0.1809%	0.4503%	0.3406%	0.01958%			0.6027%	0.3173%
Sclerotinia sclerotiorum hypovirus 2	0.19808				1.66%				
Cronobacter phage CR5	0.19464	0.2035%	0.4878%	0.2509%	0.01197%			0.4455%	0.04533%
Sclerotinia sclerotiorum hypovirus 1	0.18901			0.03585%	1.129%				0.04533%
Enterococcus virus LY0322	0.18312	0.06784%	0.07505%			0.6516%	0.7922%		
Thika virus	0.17284	0.3166%					1.239%		
Paramecium bursaria Chlorella virus 1	0.16054		0.03752%	0.08962%	0.00544%	0.07757%	0.8648%		0.2267%

Kaiju bacteria genus

Name	Mean	Dieter healthy	Dieter infected	New Daf 1	New Daf 5	OLDDAF_healthy-taxa	OLDDAF_Infected-taxa	PB	SW
Frankia	11.531	9.864%	10.19%	12.18%	17.48%	0.08702%	1.672%	12.76%	23.37%
Paenarthrobacter	8.2216	14.32%	8.87%	2.777%	8.983%	0.07696%	0.9347%	16.89%	5.243%
Klebsiella	5.1771			3.496%	4.772%	17.48%	14.49%		6.356%
Staphylococcus	4.8729	9.19%	5.242%	3.394%	6.918%			8.182%	4.714%
Acinetobacter	3.3221			6.438%	5.744%	4.514%	5.486%		7.717%
Pasteurella	3.0609			0.07621%	0.2394%	15.21%	11.82%		0.2021%
Photobacterium	3.054	7.546%	5.634%					7.659%	
Escherichia	2.8642			5.799%	5.703%	3.339%	4.079%		6.858%
Pseudomonas	2.7919			13%	2.971%	1.381%	6.006%		1.769%
Vibrio	2.0958			2.733%	3.427%	5.219%	4.584%		2.899%
Microbacterium	2.0108	1.867%	1.768%	0.8309%	0.6766%	7.103%	5.468%	0.113%	0.06864%
Labilibacter	1.9433	2.756%	3.134%	1.077%	1.407%			3.376%	2.377%
Crocinitomix	1.7852	0.04249%	0.02958%	0.05833%	0.02444%	8.559%	7.312%	0.01302%	0.01368%
Acinetobacter	1.7717	2.219%	4.158%					4.093%	
Escherichia	1.7449	2.343%	5.258%					3.594%	
Mesorhizobium	1.5947	1.152%	1.679%	1.957%	1.733%		0.3613%	1.27%	4.267%
Vibrio	1.4154	3.19%	2.428%					3.437%	
Hymenobacter	1.3811	2.363%	2.457%	0.4178%	0.4727%	0.005628%	0.08591%	2.194%	2.07%
Mycobacteroides	1.3468	2.789%	1.273%	0.4887%	1.057%	0.03622%	0.1072%	2.69%	1.309%
Klebsiella	1.2316	2.241%	2.767%					2.689%	
Pseudomonas	1.1717	3.012%	2.355%					3.274%	
Halomonas	1.171	3.308%	1.715%					3.07%	
Enterococcus	1.1441	0.5928%	1.459%	1.561%	1.976%			1.321%	2.275%
Photobacterium	1.1258			1.852%	3.56%	0.01651%	0.1677%		4.536%
Taylorella	1.0315	0.005671%	0.005359%	0.001625%		5.226%	4.045%		
Sphingomonas	1.0309	1.337%	3.699%	2.346%	0.959%		0.0575%	0.4277%	0.32%
Thermogemmatispora	1.0082	0.05268%	0.009791%	0.08957%	0.1007%	4.954%	3.715%	0.01326%	0.07398%
Bacillus	0.93114	0.8852%	0.7591%	1.331%	2.139%			1.007%	1.187%
Steroidobacter	0.89934			0.01336%	0.02123%	4.42%	3.628%		0.01146%
Flectobacillus	0.79475	0.006821%	0.008142%	0.01282%	0.01061%	4.105%	2.964%	0.009554%	0.03148%
Enterobacter	0.78835			3.107%	2.829%	0.00866%	0.6007%		0.5498%
Candidatus Phytoplasma	0.74111							6.67%	
Soehngenia	0.71244	0.188%	0.7477%	0.9252%	0.7817%	0.007485%	0.2615%	0.5694%	1.687%
Salmonella	0.65525			1.31%	2.094%	0.07683%	0.2834%		2.133%
Methylobacterium	0.6552	0.5556%	2.235%	2.119%	0.6058%		0.01136%	0.136%	0.08165%
Halomonas	0.64316			1.301%	2.191%	0.2968%	0.5646%		1.435%
Nocardioides	0.60233	0.8347%	0.8722%	1.002%	0.3463%	1.084%	0.7766%	0.1876%	0.1155%
Muricauda	0.57897	0.806%	0.6358%	0.3708%	0.3951%	0.4228%	0.3447%	0.8503%	0.4944%
Streptomyces	0.57726	0.7576%	0.958%	0.5545%	0.6409%	0.3866%	0.7295%	0.3892%	0.5471%
Cuspidothrix	0.54679	1.309%	0.9259%	0.1135%	0.1567%	0.000898%	0.02777%	0.9234%	0.6547%
Salmonella	0.50231	0.7905%	0.7743%					1.274%	
Microcystis	0.48009	0.02901%	0.03195%	0.02339%	0.02374%	2.34%	1.768%	0.02926%	0.03905%
Yangia	0.46436	0.06953%	0.5873%	0.8715%	0.9199%		0.1359%	0.3354%	0.589%
Arthrobacter	0.45491	0.3888%	0.4599%	0.5816%	0.5075%	0.04668%	0.1042%	0.2661%	0.6694%
Rhizobium	0.42847	0.9843%	0.5869%	0.7545%	0.5043%		0.07652%	0.4391%	0.2732%
Desulfovibrio	0.41129	0.8766%	0.3339%	0.1732%	0.4342%			0.7917%	0.3743%
Novosphingobium	0.40258	0.7903%	0.637%	1.28%	0.6307%		0.2233%	0.03296%	0.01257%
Scytonema	0.35844	0.01035%	0.005565%	0.009661%	0.1387%	1.717%	1.314%	0.006807%	0.01057%
Enterococcus	0.35556					1.033%	2.167%		
Blautia	0.347					1.862%	1.261%		

Kaiju bacteria species

Name	Mean	Dieter healthy	Dieter infected	New Daf 1	New Daf 5	OLDDAF_healthy-taxa	OLDDAF_Infected-taxa	PB	SW
Paenarthrobacter nicotinovorans	7.1701	12.5%	7.523%	2.478%	7.839%	0.07291%	0.8769%	14.77%	4.521%
Candidatus Frankia datiscae	5.7541	6.463%	4.939%	6.114%	9.281%	0.04765%	0.8199%	6.487%	10.4%
Klebsiella pneumoniae	4.6187			2.879%	3.913%	16.46%	13.39%		4.926%
Staphylococcus aureus	4.2097	7.966%	4.383%	3.004%	6.008%			7.107%	3.998%
Candidatus Frankia meridionalis	4.0351	1.906%	3.501%	4.529%	5.457%	0.0335%	0.7076%	4.339%	9.273%
Pasteurella multocida	2.8835			0.06799%	0.2089%	14.41%	11.09%		0.1743%
Marinilabiliaceae bacterium JC017	2.6793	4.807%	3.249%	1.413%	3.55%			4.94%	2.192%
Acinetobacter baumannii	2.6416			4.777%	4.111%	4.271%	4.988%		5.627%
Escherichia coli	2.5616			5.178%	4.977%	3.163%	3.823%		5.913%
Photobacterium alginatilyticum	2.5214	6.169%	4.523%					6.378%	
Labilibacter sediminis	1.6895	2.406%	2.659%	0.9616%	1.228%			2.951%	2.05%
Crocinitomix sp. SM1701	1.6819	0.03623%	0.02483%	0.05194%	0.02097%	8.108%	6.86%	0.01117%	0.01151%
Escherichia coli	1.5107	2.041%	4.46%					3.14%	
Nitriliruptoraceae bacterium ZYF776	1.3559	0.9356%	1.975%	1.803%	1.845%	0.01401%	0.4415%	1.381%	2.587%
Mesorhizobium sp.	1.3412	0.9429%	1.355%	1.667%	1.46%		0.2649%	1.094%	3.609%
Microbacterium sp. HM58-2	1.3286	0.05216%	0.04118%	0.01774%	0.04791%	6.728%	5.024%	0.02975%	0.01257%
Hymenobacter sp.	1.1898	2.057%	2.035%	0.3654%	0.405%	0.004867%	0.07759%	1.913%	1.78%
Mycobacteroides abscessus	1.1732	2.433%	1.078%	0.4346%	0.9217%	0.03431%	0.1001%	2.35%	1.128%
Acinetobacter baumannii	1.16	1.289%	2.976%					2.691%	
Klebsiella pneumoniae	1.0066	1.901%	2.148%					2.222%	
Taylorella asinigenitalis	0.97299	0.00495%	0.004547%	0.001452%		4.95%	3.796%		
Thermogemmatispora aurantia	0.9479	0.0457%	0.007695%	0.07993%	0.08777%	4.693%	3.485%	0.01148%	0.0636%
Halomonas heilongjiangensis	0.92967	2.596%	1.28%					2.481%	
Photobacterium alginatilyticum	0.89878			1.475%	2.791%	0.01243%	0.1406%		3.67%
Vibrio viridaestus	0.85948			0.2735%	0.7964%	3.429%	2.734%		0.5024%
Steroidobacter sp. SA29-B	0.84646			0.00734%	0.01438%	4.187%	3.403%		0.006427%
Enterococcus faecium	0.78677	0.3116%	0.878%	1.156%	1.231%			0.9497%	1.75%
Clostridia bacterium k32	0.74439	0.6494%	0.5962%	0.7761%	1.391%			0.9198%	1.277%
Flectobacillus sp. BAB-3569	0.74427	0.005381%	0.002798%	0.005888%	0.005486%	3.889%	2.78%	0.003758%	0.002974%
Enterobacter hormaechei	0.69101			2.749%	2.44%	0.007408%	0.5511%		0.4716%
Pseudomonas aeruginosa	0.68432	1.83%	0.9149%					2.125%	
Pseudomonas syringae	0.63812			3.814%	0.5417%	0.2556%	0.8721%		0.2597%
Candidatus Phytoplasma australiense	0.63644							5.728%	
Soehngenia saccharolytica	0.62262	0.1641%	0.6344%	0.8265%	0.6823%	0.00709%	0.2454%	0.4978%	1.455%
Salmonella enterica	0.56318			1.14%	1.797%	0.07253%	0.2591%		1.8%
Bacillus paralicheniformis	0.53016	0.4237%	0.2147%	0.8397%	1.436%			0.4979%	0.655%
uncultured Chloroflexi bacterium HF0200_09I09	0.49469	0.02733%	0.005334%	0.04186%	0.0629%	2.335%	1.886%	0.008561%	0.03962%
Sphingobacteriaceae bacterium	0.49065	0.7006%	1.007%	0.2413%	0.302%	0.001997%	0.03991%	0.6961%	0.6924%
Cuspidothrix issaczevii	0.47476	1.142%	0.7849%	0.1014%	0.1368%	0.0008506%	0.02605%	0.807%	0.5643%
Halomonas heilongjiangensis	0.44853			1.055%	1.769%	0.004761%	0.106%		1.102%
Microcystis aeruginosa	0.44692	0.01887%	0.02177%	0.01895%	0.01414%	2.217%	1.659%	0.01545%	0.03194%
Muricauda alvinocaridis	0.43199	0.701%	0.5342%	0.3246%	0.3414%	0.002514%	0.04597%	0.7413%	0.419%
bacterium	0.42172	0.8614%	0.537%	0.4068%	0.6531%	0.00192%	0.04753%	0.6108%	0.358%
Salmonella enterica	0.41203	0.6035%	0.6168%					1.058%	
Vibrio parahaemolyticus	0.41172			1.214%	1%	0.06618%	0.3143%		1.111%
Vibrio viridaestus	0.41059	1.368%	0.3616%					1.095%	
Yangia sp. PrR004	0.40626	0.06041%	0.4976%	0.7784%	0.803%		0.1275%	0.2931%	0.508%
Marinifilaceae bacterium T3-2 S1-C	0.40284	0.7186%	0.8331%	0.1975%	0.1848%			0.5678%	0.5567%
Acinetobacter indicus	0.36316	0.6242%	0.5171%					0.8431%	
Desulfovibrio sp. JC022	0.35368	0.7595%	0.276%	0.1462%	0.3713%			0.69%	0.3154%

Kaiju eukaryote genus

Name	Mean	Dieter healthy	Dieter infected	New Daf 1	New Daf 5	OLDDAF_healthy-taxa	OLDDAF_Infected-taxa	PB	SW
Gelatoporia subvermispora	7.3078					38.11%	27.66%		
Microspora stagnorum	4.6812	4.773%	3.672%	4.688%	7.147%		1.093%	6.182%	7.963%
Chlorella sp. ArM0029B	4.6644					24.71%	17.27%		
Plasmopara halstedii	4.1915	0.6513%	0.3304%	0.2305%	0.298%	20.69%	15.33%	0.193%	
Callipsygma wilsonis	2.4066	7.587%	2.862%	0.4764%	0.5794%	0.00159%	0.07062%	4.514%	2.19%
Golovinomyces cichoracearum	2.2076	7.232%	3.171%				0.1653%	4.892%	2.022%
Marsupiomonas sp. NIES 1824	2.1047	4.269%	1.301%	0.7551%	2.108%	0.02431%	0.2475%	4.305%	0.977%
Nephroselmis olivacea	1.7318	2.826%	1.508%	1.076%	2.146%	0.02135%	0.4272%	2.799%	2.084%
Micromonas commoda	1.6604	1.201%	1.78%	2.042%	2.117%	0.03044%	0.8139%	1.806%	2.922%
Chloropicon primus	1.5697	1.143%	1.912%	1.949%	2.063%	0.02886%	0.7682%	1.702%	2.867%
Symbiochloris handae	1.4553	5.322%						3.984%	
Micromonas pusilla	1.4532	0.757%	1.151%	1.27%	1.317%	1.818%	1.794%	1.127%	1.954%
Nephroselmis astigmatica	1.3688	2.406%	0.8092%	0.7515%	1.452%	0.003408%	0.05169%	3.157%	0.7355%
Coccomyxa subellipsoidea	1.2514		2.701%	2.652%	2.585%				3.325%
Chlamydomonas eustigma	1.1662	0.8462%	1.446%	1.443%	1.384%		0.6192%	1.31%	1.928%
Neochlorosarcina sempervirens	1.1223	2.478%	1.866%	0.2709%	0.2696%		0.07563%	2.029%	1.287%
Saccharomycodes ludwigii	1.0662	0.5067%	0.3673%	0.2961%	0.107%	3.826%	3.904%	0.1152%	0.2249%
Rhodotorula graminis	1.0574	0.5801%	2.545%	3.269%	2.14%		0.0298%	0.2062%	
Trebouxia sp. A1-2	1.0514		2.066%	2.25%	2.161%				2.986%
Botrytis cinerea	1.0194			0.03416%	9.14%				
Raphidocelis subcapitata	0.99383	0.6397%	1.044%	1.378%	1.213%		0.5261%	0.9287%	1.596%
Bathycoccus prasinos	0.95931	0.6793%	1.005%	1.153%	1.259%	0.01699%	0.4505%	1.103%	1.593%
Auxenochlorella protothecoides	0.90656					4.507%	3.652%		
Picocystis salinarum	0.89825	3.666%	0.5158%	0.1844%	0.7039%	0.00116%	0.01887%	1.711%	0.5046%
Gayralia oxysperma	0.84988	2.396%	1.194%	0.1715%	0.2141%	0.02084%	0.1%	1.51%	0.8345%

Kaiju eukaryote species

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Gelatoporia subvermispora	7.3078					38.11%	27.66%		
Microspora stagnorum	4.6812	4.773%	3.672%	4.688%	7.147%		1.093%	6.182%	7.963%
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Chlamydomonas eustigma	1.1662	0.8462%	1.446%	1.443%	1.384%		0.6192%	1.31%	1.928%
Neochlorosarcina sempervirens	1.1223	2.478%	1.866%	0.2709%	0.2696%		0.07563%	2.029%	1.287%
Saccharomycodes ludwigii	1.0662	0.5067%	0.3673%	0.2961%	0.107%	3.826%	3.904%	0.1152%	0.2249%
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Gayralia oxysperma	0.84988	2.396%	1.194%	0.1715%	0.2141%	0.02084%	0.1%	1.51%	0.8345%

Kaiju eukaryota ascomyota species

Name	Mean	Dieter healthy	Dieter infected	New Daf 1	New Daf 5	OLDDAF_healthy-taxa	OLDDAF_Infected-taxa	PB	SW
Golovinomyces cichoracearum	20.675	63.08%	13.67%				2.437%	53.01%	25.94%
Saccharomycodes ludwigii	17.946	4.418%	1.584%	0.9428%	0.4946%	89.48%	57.55%	1.248%	2.885%
Botrytis cinerea	4.7054			0.1088%	42.24%				
Golovinomyces cichoracearum	1.6481			3.868%	10.75%	0.2149%			
Alternaria arborescens	1.5816	0.2725%	2.172%	9.956%	1.726%			0.09603%	0.01194%
Cryphonectria parasitica	0.83359			7.476%		0.01212%		0.01423%	
Neopestalotiopsis sp. 37M	0.679		5.073%				1.038%		
Botrytis cinerea	0.6352	0.01404%	0.9222%				0.002819%		0.01672%
Cadophora sp. DSE1049	0.63488			4.32%	1.308%	0.08588%			
Sphaerosporella brunnea	0.61087	0.08426%	0.2209%	0.3177%	0.6434%	0.01746%	0.4132%	0.4268%	0.1481%
Valsa mali	0.56533			2.492%		0.476%		2.12%	
Coniella lustricola	0.55178			4.896%		0.002465%		0.06758%	
Epicoccum nigrum	0.51631	0.3736%	3.67%	0.2582%	0.2417%			0.0818%	0.0215%
Yarrowia lipolytica	0.50711	0.1741%	0.2393%	0.2057%	0.419%	0.04397%	0.4473%	0.6651%	1.985%
Candida albicans	0.43098	0.4382%	0.3049%	0.3902%	0.476%	0.0152%	0.4583%	0.7362%	0.7046%
Sugiyamaella lignohabitans	0.42411	0.3427%	0.3484%	0.2008%	0.2851%	0.0152%	0.2563%	0.6011%	1.233%
Hortaea werneckii	0.41498	0.132%	1.698%	1.049%	0.1128%			0.2916%	0.4514%
Lachnellula subtilissima	0.40138			0.01894%	0.007438%	3.586%			
Nadsonia fulvescens	0.3784	0.2303%	0.3216%	0.2165%	0.2678%	0.01952%	0.3796%	0.5406%	1.068%
Eremothecium cymbalariae	0.37665	0.1264%	0.09518%	0.1137%	0.1574%	0.001233%	0.1069%	0.4588%	0.6712%
Dissoconium aciculare	0.35098	0.1461%	1.991%	0.3193%	0.1041%			0.4908%	0.1075%
Ascobolus immersus	0.3501	0.1404%	0.1876%	0.2192%	0.2479%	0.01808%	0.257%	0.3486%	1.139%
Schizosaccharomyces pombe	0.34399	0.2331%	0.2005%	0.1905%	0.2678%	0.01603%	0.3418%	0.3201%	0.9363%
Polytolypa hystricis	0.32436			0.2712%				1.167%	1.481%
Cadophora sp. DSE1049	0.29595	0.2556%	1.528%				0.5152%	0.03912%	0.08121%

Kaiju eukaryota basidiomycota species

Name	Mean	Dieter healthy	Dieter infected	New Daf 1	New Daf 5	OLDDAF_healthy-taxa	OLDDAF_Infected-taxa	PB	SW
Gelatoporia subvermispora	20.629					95%	90.66%		
Rhodotorula graminis	14.392	10.38%	25.36%	37.6%	31.78%		0.09767%	6.623%	
Rhizoctonia solani	4.2972	36.7%		1.975%					
Rhodotorula diobovata	3.947	2.915%	7.478%	8.903%	9.308%		0.03379%	2.204%	
Sanghuangporus sanghuang	2.7442		1.262%		3.789%			6.665%	8.334%
Rhizoctonia solani	1.195		1.117%		1.092%			4.356%	1.728%
Cylindrobasidium torrendii	1.1094		0.7516%		1.43%			4.735%	1.093%
Saitozyma podzolica	1.0497		3.021%	0.565%	1.116%	0.001401%	0.04283%	2.025%	1.089%
Cryptococcus cf. gattii	0.94512		0.02776%	0.005865%	0.03188%	4.323%	4.111%		
Phellinidium pouzarii	0.89809		0.6278%		1.056%			2.088%	1.816%
Mixia osmundae	0.88006	0.5415%	1.204%	0.9873%	1.247%	0.004796%	0.05093%	1.287%	1.103%
Puccinia triticina	0.86479	0.53%	0.3609%	0.5298%	0.9005%	0.001752%	0.04617%	2.869%	1.334%
Naematelia encephala	0.78716		2.441%	0.5845%	1.044%	0.001511%	0.06785%	0.7699%	0.8987%
Coprinopsis marcescibilis	0.77748		0.679%		0.9323%			2.563%	1.756%
Leucosporidium creatinivorum	0.76164	0.3169%	0.5851%	4.665%	0.4741%		0.01927%	0.3269%	
Ceratobasidium theobromae	0.7365	6.216%		0.4125%					
Jaminaea rosea	0.71714			0.9443%				2.521%	
Puccinia striiformis	0.64618	0.4263%	0.7644%	1.398%	0.6614%	0.1736%	0.2129%	0.9703%	0.8663%
Apiotrichum porosum	0.64497		1.475%	0.5298%	0.8048%	0.0009635%	0.04758%	1.329%	0.63%
Malassezia vespertilionis	0.63256	0.2881%	0.3203%	0.3793%	0.6933%	0.001664%	0.06769%	1.339%	1.807%
Phanerochaete carnosa	0.63019		0.521%		0.7969%			1.339%	2.205%
Melampsora larici-populina	0.60692	0.3802%	0.4121%	0.6393%	0.6415%	0.05117%	0.1091%	1.192%	1.339%
Sanghuangporus sanghuang	0.582	3.75%		1.488%					
Obba rivulosa	0.5729		0.3566%		0.8646%			0.8859%	1.765%
Laccaria bicolor	0.54062		0.3053%		0.7013%			1.023%	1.302%

Kaiju eukaryota mucoromycota species

Name	Mean	Dieter healthy	Dieter infected	New Daf 1	New Daf 5	OLDDAF_healthy-taxa	OLDDAF_Infected-taxa	PB	SW
Rhizophagus irregularis	27.524	36.16%	11.8%	16.57%	15.99%	71.47%	52.16%	18.68%	9.765%
Rhizophagus clarus	7.821	3.618%	4.571%	3.694%	4.433%	22.55%	16.19%	3.744%	4.252%
Gigaspora rosea	6.5875	5.24%	7.696%	8.092%	7.687%	0.1981%	2.822%	11.25%	9.458%
Diversispora epigaea	5.8218	4.206%	11.61%	11.21%	7.888%	2.141%	3.622%	2.45%	4.123%
Jimgerdemannia flammicorona	5.7015	6.414%	6.874%	6.026%	6.924%	0.0842%	1.8%	5.684%	9.813%
Hesseltinella vesiculosa	4.7706	3.665%	6.839%	5.056%	5.491%	0.07553%	2.116%	6.821%	5.246%
Syncephalastrum racemosum	4.1281	4.041%	5.601%	4.962%	4.942%	0.06893%	2.122%	4.449%	5.634%
Glomus cerebriforme	3.9527	5.686%	3.518%	3.201%	4.366%	1.579%	2.369%	4.292%	5.723%
Phycomyces blakesleeanus	3.1148	2.491%	3.9%	3.905%	4.366%	0.246%	1.58%	2.881%	4.333%
Mortierella elongata	2.9773	3.195%	3.715%	3.733%	4.031%	0.08957%	1.616%	3.587%	3.84%
Absidia glauca	2.8674	2.89%	3.009%	3.467%	2.933%	0.06563%	0.9702%	3.842%	5.012%
Bifiguratus adelaidae	2.7926	2.491%	3.437%	3.6%	3.589%	0.03962%	1.263%	3.195%	3.613%
Choanephora cucurbitarum	2.449	3.548%	2.789%	3.788%	2.638%	0.07058%	1.703%	1.646%	2.886%
Lobosporangium transversale	2.3277	2.138%	2.453%	2.81%	3.857%	0.08874%	1.32%	2.626%	2.668%
Mortierella verticillata	2.1096	1.739%	2.951%	2.63%	2.451%	0.04953%	1.443%	2.274%	2.918%
Rhizopus delemar	1.9339	1.316%	2.928%	2.223%	1.848%	0.03054%	0.7547%	2.92%	2.837%
Rhizopus stolonifer	1.9242	1.48%	1.852%	2.817%	2.464%	0.02765%	0.8785%	2.117%	3.355%
Endogone sp. FLAS-F59071	1.8613	2.068%	2.592%	1.98%	2.759%	0.03632%	0.9508%	2.568%	2.32%
Absidia repens	1.7653	1.433%	2.569%	2.082%	2.598%	0.04499%	0.963%	2.058%	2.425%
Mucor circinelloides	1.5436	1.809%	1.921%	1.917%	2.009%	0.01321%	0.4625%	1.254%	2.401%
Mucor lusitanicus	1.4074	0.8459%	1.227%	0.7122%	1.018%	0.01899%	0.1518%	7.193%	0.3961%
Lichtheimia ramosa	1.3225	1.057%	1.759%	1.44%	1.634%	0.02353%	0.6079%	1.568%	2.166%
Parasitella parasitica	1.1252	0.5639%	1.562%	1.534%	1.42%	0.04045%	0.6115%	1.137%	1.56%
Rhizopus microsporus	0.74503	0.5169%	0.8448%	1.276%	1.085%	0.009906%	0.285%	0.4704%	0.9943%
Lichtheimia corymbifera	0.65897	0.6109%	1.099%	0.6652%	0.6964%	0.02559%	0.3659%	0.98%	1.148%

Kaiju eukaryota oomycota species

Name	Mean	Dieter healthy	Dieter infected	New Daf 1	New Daf 5	OLDDAF_healthy-taxa	OLDDAF_Infected-taxa	PB	SW
Plasmopara halstedii	32.548	41.28%	17.55%	12.34%	15.24%	99.18%	94.87%	12.77%	
Thraustotheca clavata	4.7661	7.125%	9.127%	9.545%	8.504%	0.0141%	0.4151%	8.165%	
Aphanomyces stellatus	3.5445	3.491%	6.339%	6.015%	6.09%	0.01082%	0.3993%	9.555%	
Aphanomyces euteiches	3.4373	4.594%	4.894%	6.26%	7.338%	0.6371%	0.8934%	6.319%	
Pythium oligandrum	3.3378	4.063%	6.236%	6.351%	5.733%	0.01065%	0.3505%	7.296%	
Pythium brassicum	2.8513	4.614%	4.928%	6.069%	5.87%	0.008588%	0.263%	3.909%	
Globisporangium splendens	2.731	3.369%	5.417%	5.787%	4.54%	0.006525%	0.2691%	5.19%	
Achlya hypogyna	2.7265	3.022%	5.895%	4.359%	4.512%	0.007241%	0.4669%	6.276%	
Pythium insidiosum	2.6657	2.715%	5.576%	5.014%	4.663%	0.00762%	0.2608%	5.755%	
Nothophytophthora sp. Chile5	2.5084	4.859%	4.108%	3.03%	4.252%	0.007999%	0.2388%	6.08%	
Aphanomyces astaci	2.352	3.226%	4.268%	5.032%	4.595%	0.01255%	0.2121%	3.822%	
Phytophthora pseudotsugae	2.0761	1.49%	4.95%	3.13%	3.772%	0.009304%	0.2958%	5.038%	
Albugo candida	2.0028	2.572%	2.014%	6.433%	4.746%	0.004841%	0.2352%	2.02%	
Phytophthora infestans	1.5086	1.572%	2.834%	3.303%	3.182%	0.003242%	0.1862%	2.497%	
Bremia lactucae	1.4543	1.817%	2.504%	3.185%	3.251%	0.004126%	0.1344%	2.193%	
Albugo laibachii	1.1893	1.184%	1.696%	2.684%	2.592%	0.005431%	0.1103%	2.432%	
Peronospora effusa	0.97372	1.143%	2.162%	2.038%	1.372%	0.02244%	0.137%	1.889%	
Phytophthora kernoviae	0.8758	1%	1.741%	1.765%	1.55%	0.001642%	0.06555%	1.759%	
Aphanomyces invadans	0.79586	0.8779%	1.252%	1.711%	1.783%	0.003284%	0.1236%	1.412%	
Saprolegnia diclina	0.53997	0.735%	1.07%	1.092%	1.138%	0.002358%	0.1058%	0.7166%	
Phytophthora megakarya	0.50766	0.9392%	1.172%	0.5551%	1.015%	0.001852%	0.06063%	0.8252%	
Phytophthora palmivora	0.49573	0.6329%	1.184%	1.046%	0.8641%	0.02656%	0.07823%	0.6298%	
Phytophthora fragariae	0.48468	0.8167%	0.8535%	0.9827%	0.8641%	0.0007157%	0.01916%	0.8252%	
Phytophthora cactorum	0.39864	0.5717%	0.4552%	0.5187%	0.6172%	0.007241%	0.02771%	1.39%	
Saprolegnia parasitica	0.21655	0.49%	0.478%	0.3458%	0.3703%	0.0007157%	0.02526%	0.2389%	

Kaiju tenericutes genus level

Name	MAD	Dieter healthy	Dieter infected	New Daf 1	New Daf 5	OLDDAF_healthy-taxa	OLDDAF_Infected-taxa	PB	SW
Mycoplasma	26.657	98.6%	94.37%	63.47%	76.39%				92.55%
Candidatus Phytoplasma	6.6554	1.295%	4.489%	33.92%	20.64%				6.349%
Acholeplasma	0	0.0175%		0.1044%	0.2372%				0.03239%
Spiroplasma	0.58103	0.06999%	0.3919%	0.8351%	1.779%				0.5507%
Candidatus Izimaplasma	0.42223	0.0175%	0.7481%	1.461%	0.7117%	0.000869%	0.05536%	0.006712%	0.2915%
Ureaplasma	0			0.1044%					
Mesoplasma	0			0.1044%	0.2372%				0.1296%
Spiroplasma	0					98.86%	97.13%		
Mycoplasma	0					0.7097%	1.629%		
Ureaplasma	0					0.05069%	0.03739%		
Candidatus Phytoplasma	0					0.3766%	1.12%		
Acholeplasma	0					0.0005793%	0.0151%		
Mesoplasma	0					0.004055%	0.01294%		
Entomoplasma	0						0.001438%		
Anaeroplasma	0						0.000719%		
Candidatus Phytoplasma	0							93.71%	
Mycoplasma	0							6.265%	
Ureaplasma	0							0.001678%	
Spiroplasma	0							0.0151%	
Mesoplasma	0							0.001678%	
Entomoplasma	0								0.03239%
Anaeroplasma	0								0.06479%
Candidatus Hepatoplasma	0								

Kaiju virus

Name	Mean	Dieter healthy	Dieter infected	New Daf 1	New Daf 5	OLDDAF_healthy-taxa	OLDDAF_Infected-taxa	PB	SW
uncultured marine virus	20.041	5.852%	12.12%	1.883%	0.76%	8.617%	18.58%	75.02%	32.43%
Puma lentivirus	6.2239	0.01712%	0.07314%	0.01355%	0.003343%	91.59%	4.216%		
Rhizoctonia cerealis mitovirus	5.1517	46.15%						0.1951%	
Grapevine associated narnavirus-1	4.288	0.01284%	0.6765%	0.149%	21.5%	0.1134%	5.317%		0.07305%
Macrophomina phaseolina mitovirus 3	4.0574	21.15%				3.968%	11.35%	0.04878%	
Mitovirus sp.	3.6279	3.163%	0.768%	2.736%	0.8625%	6.576%	15.14%	1.512%	1.242%
Sclerotinia sclerotiorum mitovirus 3	3.3471	0.01284%	0.4754%	0.06095%	25.3%		0.02077%		0.3652%
Okra enation leaf curl virus	2.8155	0.03425%	1.042%	0.9956%	0.06686%	3.628%	3.655%	1.463%	11.91%
European chub iridovirus	2.6737	0.06849%	1.445%	0.8805%	0.07466%	2.268%	3.51%	2.293%	11.61%
Alternaria alternata chrysovirus	2.6504	0.004281%	3.456%	19.93%	0.05906%		0.2906%		0.07305%
Botrytis cinerea mitovirus 3	2.4413	0.3467%	1.371%	0.06095%	16.3%				0.1461%
Plasmopara viticola associated mitovirus 21	1.4464	0.7876%	1.207%	0.3928%	0.07578%	0.4535%	0.7477%	1.902%	7.085%
Botrytis cinerea mitovirus 4	0.93349		0.03657%	0.6028%	7.538%				
Plasmopara viticola associated ourmia-like virus 7	0.90743		0.01828%	0.006773%	0.006686%		0.03115%		
Ambrosia artemisiifolia mitovirus 1	0.9021	0.2697%	0.4023%	0.1829%	0.02786%	0.4535%	1.225%	0.9268%	4.529%
Rhizoctonia solani mitovirus 13	0.86602			0.006773%		0.1134%	7.674%		
Cladosporium cladosporioides negative-stranded RNA virus 1	0.83504	0.008561%	2.505%	0.04741%	0.2485%	0.3401%	0.8307%		0.07305%
Botrytis cinerea mitovirus 1	0.81571		0.1828%	0.01355%	5.659%				
Plasmopara viticola associated ourmia-like virus 3	0.77328		0.6583%	5.818%	0.003343%		0.07269%		
Rhizoctonia solani mitovirus 4	0.74327	6.434%	0.01828%			0.2268%	0.01038%		
Penicillium sumatrense ourmia-like virus 1	0.69166		0.7497%	2.452%	0.006686%	0.2268%	0.02077%		
Rhizoctonia solani mitovirus 18	0.64915	5.822%							
Leucostoma persoonii mitovirus 1	0.62603	0.3125%	0.4571%	0.1355%	0.03009%	1.134%	1.132%	0.9756%	1.315%
Rhizoctonia solani mitovirus 12	0.57722			5.195%					
Hyperionvirus sp.	0.56972	0.06421%	0.4571%	0.5215%	0.0312%	0.3401%	1.537%	0.09756%	1.753%

Developing knowledge & management of strawberry red leaf disorder – Hort Innovation project: BS19001

Michelle Paynter & Joanna Kristoffersen, Queensland Department of Agriculture and Fisheries

Red Leaf Disorder (RLD), characterised by reddish/maroon discolouration of strawberry leaves, has become more noticeable since its first sightings in 2014 in cultivar 'Fortuna'. It is now present in several commercial cultivars grown during Queensland's winter production.

Photo credit: Michelle Paynter, DAF

The cause of RLD is unknown. In 2019, over 40% of the growers surveyed estimated up to 20% of plants were affected by RLD. These plants typically display reduced vigour and yield. The implications for industry may potentially be significant if the cause of this disorder is not identified and managed.

Currently, there is no definitive scientific evidence of the cause of RLD in strawberry, making diagnosis based on symptoms alone challenging. Investigations to date covering a wide range of potential causes of RLD by the Department of Agriculture and Fisheries (DAF), University of Queensland (UQ) and AgriBio have found no obvious single causal agent, suggesting that the disorder and mechanisms behind its transmission may be quite complex. Thus, there is currently no standard for strawberry producers and advisors to accurately identify and manage plants with RLD. Running in conjunction with the ongoing DAF investigation, a new Hort Innovation funded project (BS19001), was established in August 2020, to identify and better understand possible causes of RLD in strawberry via expanded genetic studies.

The major focus of the project will be to:

- Identify the cause of RLD conducting detailed DNA analyses of strawberry plant samples coupled with pathology and microscopy studies
- Improve knowledge on RLD identification and collate a potential management guide to provide growers with tools for identification and management of RLD
- Establish a network and communication channel as part of a 'Communities of Practice'
- Present project updates at industry meetings/events
- Develop a pathway for a potential RLD PhD study

RLD team: Top (L-R): Michelle Paynter, Peer Schenk, Reuben Brown and Jodi Neal. Bottom (L-R): Kapah Alu, Joanna Kristoffersen and Apollo Gomez.
Photo credit: Christopher Menzel, DAF

Through a close collaboration between DAF and UQ, this project is divided into two phases. Phase 1 (6 months) will expand on existing discovery-driven next generation sequencing analyses undertaken by DAF, UQ and AgriBio to identify potential pests and diseases common to plants with RLD symptoms. In addition to DNA analyses, which will include an expansion in the number of farms and severity of RLD sampled, gene expression studies will be undertaken. Understanding how symptomatic plants are responding to RLD may help identify potential causes of the disorder. This phase of the project will complement DAF's existing multi-disciplinary research activities investigating epidemiology (i.e. incidence and spread), potential causative agents, nutrition and economic impact of strawberry RLD. Outcomes from Phase 1 will include information back to growers on potential causes of RLD and be used as the basis of a proposed collaborative DAF/UQ four-year study (Phase 2). It is proposed that Phase 2 will be underpinned by a PhD project, to fully research the causal mechanisms and provide management options for the industry.

The team, led by Joanna Kristoffersen and Michelle Paynter, consists of a large cross-organisational and multi-disciplinary team from DAF (Dr David Innes, Apollo Gomez, and Dr Jodi Neal), UQ (Professor Peer Schenk, Reuben Brown, Kapah Alu) and AgriBio (Dr Fiona Constable and David Lovelock).

Each have extensive experience including plant pathology, bioinformatics, data analysis and information management of genomic, metagenomic, pangenomic and transcriptomic data for diverse applications including diagnostics and discovery.

Queensland Government
Hort Innovation
Strategic levy investment
STRAWBERRY FUND