BS19001: Developing knowledge and management of strawberry Red Leaf Disorder

Michelle Paynter: Research Scientist, Joanna Kristoffersen: Research Scientist, Red leaf Disorder Research Team
Queensland Department of Agriculture and Fisheries

Red Leaf Disorder (RL) of strawberry, characterised by interveinal reddening of the older leaves, is an emerging disorder occurring in commercial strawberry fields in Queensland since 2014, and a major cause of fruit yield loss. This report describes what progress has been made in identifying the causal agent, how the disorder is affecting commercial fruit growers, and ongoing research planned.

Progress report

A research project conducted by the Department of Agriculture and Fisheries (DAF) and the University of Queensland (UQ), co-funded through Hort Innovation from the Strawberry Fund was initiated in 2020 to investigate potential causal agents of Red Leaf (RL).

Molecular investigations undertaken in this project using plants displaying varying degrees of RL symptoms in comparison to RL-free plants did not identify any clear pathogens that were specific to only RL symptomatic plants. However, several organisms, particularly *Phytoplasma*, are of particular interest.

RNA sequencing

To identify potential pathogens, collaborative partner Professor Peer Schenk from UQ, used Ribonucleic Acid (RNA) based meta sequencing data to detect any eukaryotic, bacterial, or viral organisms in strawberry plant samples.

Phytoplasma (and related *Acholeplasma*) was the only genus that was found across all samples tested. *Phytoplasma* is a cell wall-free bacterium that often resides in vascular tissue and causes virus-like symptoms.

Plasmopara, a genus of the oomycetes, was also dominant across all samples. *Plasmopara* species are plant pathogens known for downy mildew disease.

Many narna- and mitoviruses that infect *Plasmopara* were also found. This could suggest that either *Plasmopara* or the narna- and mitoviruses transmitted by *Plasmopara* could be involved. Narnaviruses have single-stranded RNA genomes and are associated with a fungi host. Mitoviruses are simple RNA viruses that replicate in host mitochondria and are also associated with fungi.

The *Pleosporales* family was detected across all samples, and *Xanthomonas* bacteria (which cause bacterial spots of leaves, stems and fruit) including X. *arboricola* was present in all samples except one. It is believed that a pathogen from Acholeplasmataceae (includes *Phytoplasma* and *Acholeplasma*) shows the greatest promise as the causal agent of RL from these analyses.

It may be that these species, along with the viruses they contain, contribute to the RL symptoms in the presence of other biotic or abiotic constraints. Further molecular analysis is needed to verify the ongoing relationship between the potential targets and RL in subsequent seasons.

Transcriptome analyses of plants demonstrating various stages of RL symptoms compared to RL-free controls need to be undertaken to identify plant response genes or pathways that are differentially regulated based on disorder state.

Microscopy analysis and PCR

In conjunction with research undertaken by UQ, DAF analysed strawberry plants under a transmission electron microscope (TEM) and by polymerase chain reaction (PCR), comparing plants with symptoms of RL and asymptomatic controls.

Additional RNA sequence data were incorporated from previous DAF project work to enable analysis across the largest available dataset and across multiple seasons (2018, 2019, 2020).

It was determined that the most informative RNA sequencing data analysis would 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 RL symptoms but not in symptom-free plants. The emphasis was placed on identifying potential pathogens that were present in all samples.

A range of virus particles or virus-like particles were observed in TEM analyses of strawberry leaf samples analysed: full and empty 21 nm isometric/spherical particles, and 70 nm isometric particles. Possible fragments of closterovirus-like particles were detected in a small number of samples.

Additionally, 27 nm isometric particles were present in a range of but not all samples and may have an association with RL 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.

Farm survey – season 2021

Nine strawberry fruiting farms in the SEQ region were selected to collect data on RL incidence and severity in the 2021 season. Other properties in relation to the plants, such as plugs vs bare-rooted, soil type, bed height and spacing, and fumigation were examined.

We also looked at the effects of using 2-year-old plastic to determine if there could be a build-up of any potential pathogens. Farms were visited every four weeks from May to October. Due to numerous factors late in the 2021 season, several farms removed some of the cultivars we were surveying early, limiting our data collection for visiting September and October data collections.

Cultivars surveyed included: Red Rhapsody, Scarlet Rose-ASBP, Parisienne Kiss, Aussiegem, Sundrench, Splendor and Confidence.

Several methods (Generalised linear mixed models, conditional inference trees), were used for the statistical analysis of the RL data collected in 2021. Time, Farm, Propagator, as well as the interaction between Cultivar and Farm were significant predictors

of RL in all (or most) of the models. That is, there were significant differences in RL among farms and where plants were sourced from.

RL increased with time, but it did so in a curvilinear fashion (Figure 1). RL (%) peaked in observations #4 and #5 (August, September). RL % increased sometime between observations #4 and #5 followed by a decrease. Unfortunately, there was not as much data (i.e., quite a few missing plants) for observations #5 and especially #6 due to early removal of plants.

Analyses of the data from the farms surveyed in 2021, show Parisienne Kiss, Aussiegem, and Sundrench had higher RL (%), while Confidence, Scarlet Rose-ASBP, and Splendor had lower RL (%) (Figure 2).

Red Rhapsody had very high variability in RL (%). This may be in part because this cultivar is planted the most in Queensland, produced the most data, and is possibly grown in more variable conditions than the other cultivars. Cultivars and farms differed in their RL rankings and relative values.

Among the cultivars, Sundrench displayed consistently high RL and Splendor consistently low RL.

All the farms surveyed in 2021 had RL present on their properties, with observations of RL in plants from both runners and plugs.

Of interest was a big increase of RL observed in 2021 from one farm growing plants in above ground Coco peat substrate.

This farm showed an average of up to 0.007% plants per row showing RL in August 2020, increasing to 3.79% in August 2021. This is suggestive but not conclusive that RL is not necessarily soil transmissible, or only limited to field systems.

Our results show that there was more RL in the farms we surveyed in 2021 than the previous year. From the 11 farms we surveyed in the years 2020 and 2021, seven of these were surveyed both years, the remaining four farms surveyed once in these two years.

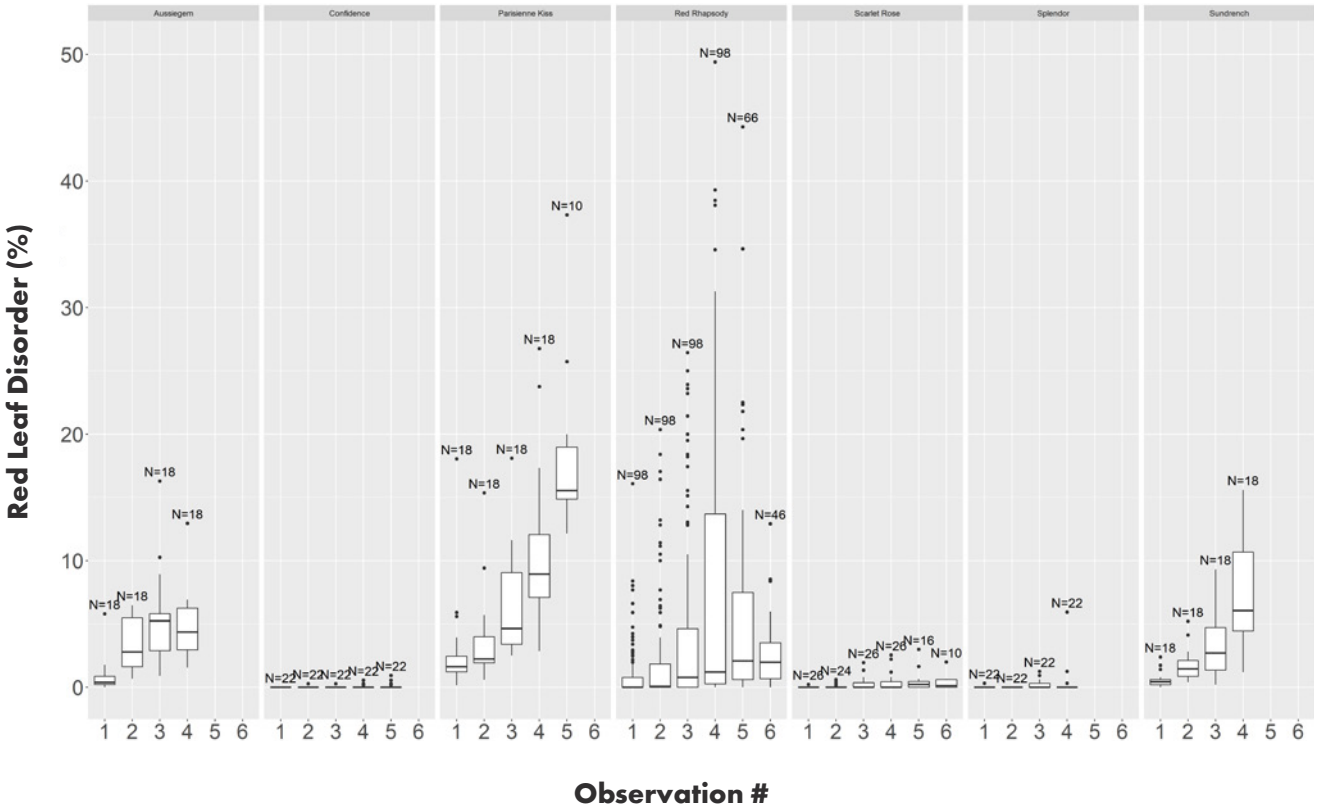

Figure 1. RL% vs strawberry Cultivar by Observation for 2021 farm survey

One-off farm survey

One-off farm visits on the Sunshine Coast were also conducted towards the end of the 2021 fruiting season. There were overwhelmingly high levels of RL in some of these farms (see Figure 3 for highest and lowest RL %), especially from cultivars Festival reaching 35.5%, Petaluma 42%, Grenada 61%, and Red Rhapsody 68.5%. Our ongoing aim is to survey as many cultivars as we can to capture a bigger picture of how RL is affecting the industry, and to build our knowledge of possible tolerance genes to RL.

Where are we going from here?

We are building a better understanding of the spread and economic impact RL has on the commercial Queensland strawberry industry; however this disorder is complex and there is still work to do. Research undertaken by DAF continues. Our intent is to further improve the knowledge of RL 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.

This is a complex issue and not easy to combat in today's environment.

Eventually, if a causal agent is identified; and if it is found to be pathogenic, it is recommended that research to 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
- Treatments for reducing the amount of the pathogen (if it is a pathogen)
- Investigating RL tolerant varieties
- Establishing a farm biosecurity plan to protect against RL within farms (if pathogenic)

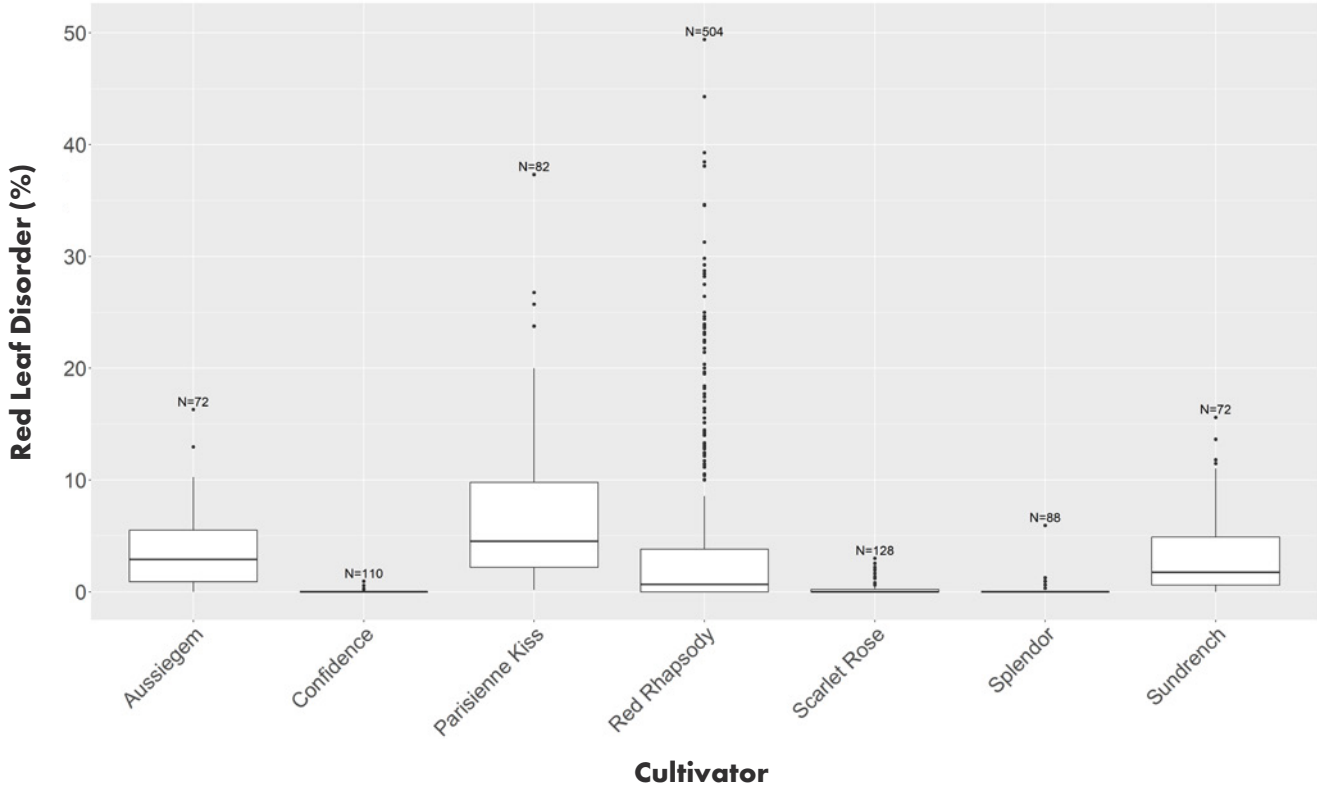

Figure 1. RL% vs strawberry Cultivar by Observation for 2021 farm survey

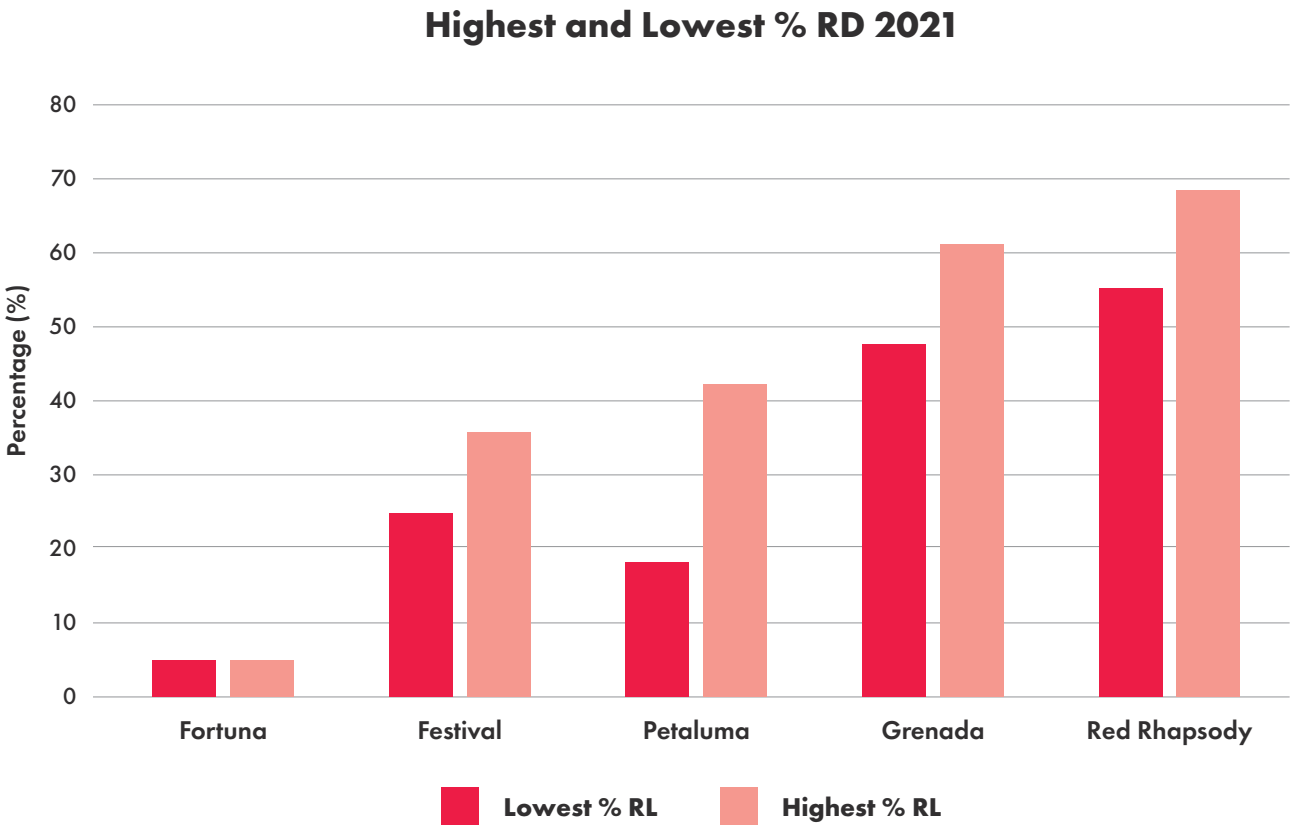

Figure 3. Highest and lowest percentages for strawberry plants showing RL. One off farm visit, 2021.

If you grow different cultivars than the ones mentioned in this article and would be happy to have the RL team visit once a month through the winter season, we would love to hear from you.

Acknowledgements

BS19001: Developing knowledge and management of strawberry red leaf disorder has been funded by Hort Innovation using the strawberry research and development levy, with co-contributions from the Queensland Government through its Department of Agriculture and Fisheries and funds from the Australian Government.

A special thank you to the Queensland strawberry production growers that allowed extensive data collection on their properties throughout the season as well as the Queensland Strawberry Growers Association for ongoing support. All funding support for this work is from the Queensland Government's: Department of Agriculture and Fisheries.

Disclaimer: the above information is sourced from trials conducted on strawberry in Australia. This is the best available information on Queensland local conditions at these sites. The information provided here may not be applicable for all regions and varieties. The Queensland Department of Agriculture and Fisheries, and Horticulture Innovation Australia provide the above information as a guide only and take no responsibility for data accuracy.