Fungicide resistance

Effective management practices for healthy vines and budgets

Simone Madden-Grey investigates the potential of new and un-utilised techniques to provide greater efficiencies and cost savings when managing grapevine disease pressures.

Botrytis effected grapes

Botrytis, powdery mildew and downy mildew cost the wine industry more than $200 million dollars each year according to Wine Australia estimates. Those costs combined with the rising cost of vineyard operations mean a robust fungal management programme is essential to vineyard longevity, both in terms of vine health and financial management.

Tests capable of rapid in-situ testing for fungicide resistance have potential for significant chemical, time and labour savings. Identifying and quantifying site-specific pathogen populations would help growers design a well-informed spray programme that maximises spray efficacy. Chemical inputs may also be reduced by eliminating those sprays that are determined to be less effective as indicated by resistant populations.

Speaking at the Australian Wine Industry Technology Conference 2022 (AWITC), Dr Francisco Lopez-Ruiz, Associate Professor at Curtin University, described the development of one such test as part of a project funded by Wine Australia and led by the South Australian Research and Development Institute (SARDI), the research arm of the Department of Primary Industries and Regions (PIRSA). Laying the groundwork for his presentation, Dr Lopez-Ruiz emphasised the absence of causality in the relationship between fungicide use and resistance. Instead, he said the emergence of resistance was due to the selection of resistant pathogens carrying mutations that already exist in nature.

The aim of the Wine Australia project was to adapt a qPCR (quantitative polymerase chain reaction) technique in order to detect botrytis fungicide resistance. The technique was originally designed and created by Dr Lopez-Ruiz and the team at Curtin University for the detection of powdery mildew fungicide resistance in wheat. The testing methodology they designed identified and quantified fungal pathogen populations in-situ and provided results within the hour, rather than the standard number of days for laboratory tests. Although the qPCR test itself has been used in a laboratory setting for a number of years, designing a test suitably robust for in-field testing in agriculture was a breakthrough.

The team adapted existing PCR testing methods for in-field testing by designing different chemistry so as to quantify fungi possessing genetic changes that correlated with fungicide resistance.

Field sample collected. Photo: Lincoln Harper, Centre for Crop and Disease Management, Curtin University

The PCR test, which in recent years has become familiar to us all, was developed in the 1980s. Small segments of genetic material are amplified, or copied, to amounts sufficient for molecular and genetic analysis. It works by separating the two strands of the DNA helix and replicating them using primers and a polymerase enzyme. Primers are short nucleic acid sequences designed to recognise and bind to the section of DNA that is of interest. A polymerase enzyme then copies the DNA sequence, which results in a DNA helix consisting of one old and one new strand of DNA. In this way DNA can be amplified quickly and to amounts sufficient for analysis. The team at Curtin University developed a particular DNA extraction technique capable of producing DNA in under one minute and at a cost of approximately 2 – 3 cents per sample. They also screened a

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range of DNA polymerase enzymes until they found one that could withstand the conditions of in-field testing and provide robust results.

At this stage the in-situ qPCR test functions as a research resource for a variety of crops. The team at Curtin University work with the grower on-site to identify the most useful sites for testing, after which samples are taken and results are analysed on-site within an hour. Dr Lopez-Ruiz says understanding nature will increase efficiency in managing fungal problems and in this way the qPCR test is able emphasise particular points in fungicide management for optimal outcomes.

Research projects

Research projects that identify and isolate the fungicide resistance that has been selected underpin a broader application of the qPCR test. Data from these projects is used in designing chemistry for each test to successfully select a particular target. Once fungal pathogen populations are then quantified, says Dr Lopez-Ruiz, a link is established between crop management and results. The final stage is sharing the data with industry in order to drive practical outcomes.

Future plans include adapting the test to quantify powdery mildew resistance in grapes. Work to modify the primer chemistry required to correctly identify the specific powdery mildew fungicide resistance mutations has begun, which will be followed by testing and validation for in-field application. At this stage, the qPCR test for botrytis fungicide resistance detection is currently in use as a monitoring tool for wine industry research. SARDI/PIRSA Project Lead, Dr Mark Sosnowski, confirmed that plans are underway for future development of the qPCR test to include adaptation for use in high-throughput and in-field testing in order to make it more accessible to the industry.

Integral to the management of fungal pathogen populations is a resilient vineyard, from which in-field testing could offer increased agility in managing spray programmes. At Charles Sturt University, Professor Geoff Gurr is running a biodiversity project aimed at building vineyard ecosystems that deliver services beyond pest management to include pathogen suppression.

The two year project, *Functional biodiversity solutions for Australian vineyards: harnessing groundcovers, vineyard surrounds and native plants to deliver key ecosystem services*, is funded by Wine Australia in collaboration with See Saw Wines and Angullong Vineyard. It is due to conclude in June this year and data from the first season has been able to demonstrate a link between ground cover, beneficial insect populations and pathogen suppression. Professor Gurr says the project has generated measurable evidence that increased populations of insects and spiders suppressed light brown apple moth (LBAM) populations, thereby reducing incidence and severity of botrytis bunch rot. The project successfully identified ground covers, which through the provision of suitable nectar sources, not only increased the longevity of beneficial wasps (a key enemy of the LBAM), but also extended the period of time over which the wasps parasitised LBAM eggs.

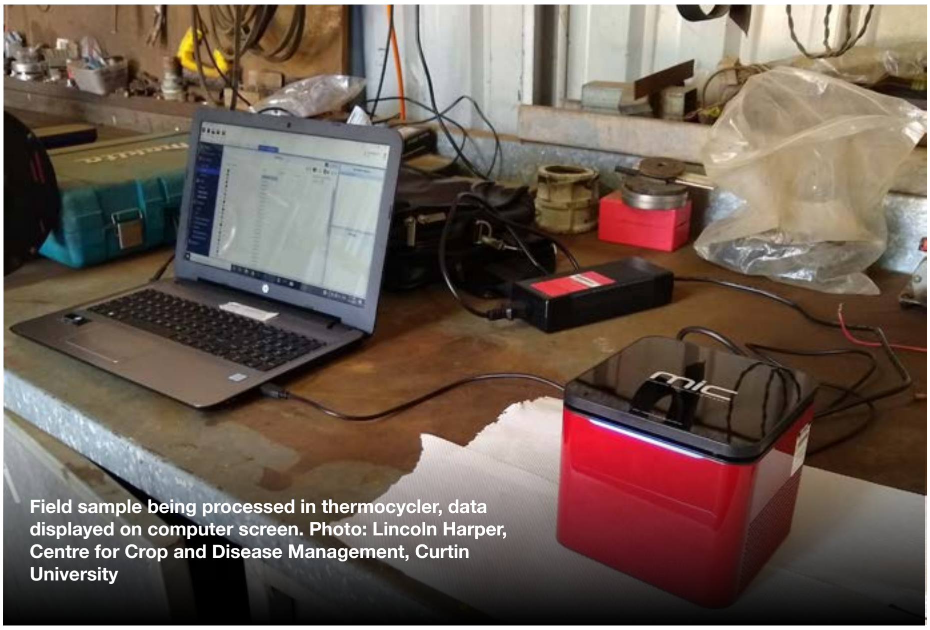
The identification of suitable plant species was fast tracked by an extended period of lab work. Plants were tested in tiny flight cages containing parasitoid wasps in order to evaluate the suitability of nectar produced and subsequent use later in the project. Laboratory results concluded that eucalyptus species and myoporum yielded greater benefits in terms of nectar provision for LBAM parasitoid wasps than other species such as kunzea and acacia.

In the field, the project trialled midrow and undervine groundcover. Three different treatments were applied to each trial plot, in addition to the experimental control where standard vegetation existed as the baseline. The treatments were;

- Alyssum, native seed mix, seed mix including clover and plantain - undervine
- Buckwheat, native seed mix, seed mix including peas, brassica, radish – mid-row

The first part of the project has addressed the practicalities of working with groundcover, such as ease of establishing plant species and identification of measurable benefits in terms of height, weed competition, suppression of plant diseases and suppression of insect pests. The aim of the second season is to establish a library of plant species identified by the vineyard ecoservice offered, in addition to generating industry recommendations.

Following the completion of the project



Field sample being processed in thermocycler, data displayed on computer screen. Photo: Lincoln Harper, Centre for Crop and Disease Management, Curtin University

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in 2023 a final report will be issued and learnings will be shared on the Wine Australia website. Dr Mary Retallack of Retallack Vineyards Pty Ltd, which runs the national EcoVineyard programme, also confirmed that aspects of the project will be included in extension materials for growers over the next three years.

Far from being mutually exclusive, these two approaches to managing fungicide resistance have the potential for use as complementary techniques. The tool box from which to monitor and manage the nuances of site-specific pathogen populations in the vineyard is augmented by the addition of in-field testing alongside a suite of ecoservices as provided by targeted biodiversity in the vineyard.

Simone Madden-Grey is a writer based in Melbourne, Australia writing about the people, places and stories she has discovered on her travels. Her portfolio can be found at happywinewoman.com including articles on climate and sustainability, new tech and the latest in scientific research supporting the wine industry in Australia and her home country, New Zealand.

Further information

Functional biodiversity solutions for Australian vineyards: harnessing groundcovers, vineyard surrounds and native plants to deliver key ecosystem services. https://sway.office.com/EWpG5dAN9LikpIrN

Managing fungicide resistance in Australia viticulture. www.wineaustralia.com/research/projects/managing-fungicide-resistance-in-austral

Interviews

Gurr, Geoffrey Professor, Professor of Applied Ecology, School of Agricultural & Wine Sciences, Charles Sturt University, Orange, NSW

Lopez-Ruiz, Francisco Dr, Associate Professor, School of Molecular and Life Sciences (MLS), Faculty of Science and Engineering, Curtin University, Perth WA

Retallack, Mary Dr, Viticulturist, Agroecologist, Managing Director, Retallack Viticulture Pty Ltd National EcoVineyards Program Manager

Sosnowski, Mark Dr, Sub-Program Leader, Horticulture Pathology Crop Sciences, Plant Health & Biosecurity, South Australian Research and Development Institute – SARDI

References

'Fungicide resistance on the research radar, R&D News, June 2022', Wine Australia. www.wineaustralia.com/news/articles/fungicide-resistance-on-the-research-radar

Understanding fungicide resistance in powdery mildew, downy mildew and botrytis, Final Report to Australian Grape and Wine Authority. Research Organisation, SARDI. Project number SAR 1204, January 2017. Principal Investigator: Barbara Hall (SARDI). Chief Investigators: Dr Suzanne McKay (SARDI), Dr Fran Lopez (Curtin University), Lincoln Harper (Curtin University), Dr Sandra Savocchia (NWGIC), Dr Anthony Borneman (AWRI), Andrew Taylor (DAFWA)

Rapid in situ quantification of the strobilurin resistance mutation G143A in the wheat pathogen Blumeria graminis f. sp. Tritici. Kejal N. Dodhia (Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Bentley, Perth, WA). Belinda A. Cox (Faculty of Science and Engineering, Curtin University, Perth, WA), Richard P. Oliver (Curtin University, Perth, WA) & Francisco J. Lopez-Ruiz (Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Bentley, Perth, WA). February 2021.

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