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SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

19th Reinhardsbrunn Symposium:

"Modern fungicides and anti-fungal compounds"



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ABSTRACT

Dr Suzanne McKay, Senior Research Officer, Crop Sciences, SARDI, attended the 19th Reinhardsbrunn symposium: "Modern fungicides and anti-fungal compounds", held in Friedrichroda, Germany, April 7-11, 2019. The majority of the symposium focussed on aspects of fungicide resistance, including monitoring, risk assessment, molecular mechanisms, management and regulation. Other topics focussed on biorational fungicides/biocontrol, new technologies and applications, and new fungicides. Attendance at the symposium was preceded by a visit to the field headquarters of BASF at Limburgerhof, Germany. Here, Dr McKay spent time in the Fungicide Resistance research groups' laboratories, led by Dr Gerd Stammler, discussing issues concerning fungicide resistance and observing their resistance testing methods for several plant-pathogen systems including grapevine powdery mildew.

EXECUTIVE SUMMARY

The Reinhardsbrunn symposium: "Modern fungicides and anti-fungal compounds" is held every three years in Friedrichroda, Germany. The symposium brings together scientists from academia, world-leading research institutes and industry all working in the area of fungicides.

The 19th symposium, held from 7 to 11th of April, 2019, attracted over 160 delegates from 23 countries, including four delegates from Australia. Eight of the 11 sessions were dedicated to aspects of fungicide resistance, and the other three covered biorational fungicides/biocontrol, new technologies and applications, and new fungicides. Dr Suzanne McKay, Senior Research Officer, Crop Sciences, SARDI, presented results of her research into fungicide resistance in powdery mildew of grapevines, part of the Wine Australia funded project SAR1702-1.2. Although much of the content of the symposium around the topic of resistance was about cereal pathogens, in particular *Zymoseptoria tritici* (Septoria leaf blotch of wheat), *Pyrenophora teres* (barley net blotch), and *Phakopsora pachyrhizi* (Asian soybean rust), the findings and outcomes are translatable to other pathosystems. Many European countries, USA and Brazil have resistance detection and monitoring research programs, frequently in collaboration with chemical companies. Most monitoring is carried out by detection or quantification of characterised resistance genotypes, commonly genetic mutations. The use of smart trap technologies, integrated with portable real-time sequencing tools, shows potential for use in resistance detection and monitoring. Worldwide incidence of resistance for many fungicide groups including the widely used Demethylation Inhibitors (DMIs) is increasing and spreading, as is the scientific understanding of the complexity of associated resistance mechanisms. Research aiming to determine the molecular mechanisms for the Succinate Dehydrogenase Inhibitors (FRAC group 7), Quinone inside Inhibitors (QiI, FRAC group 21) and QoSI (Quinone inside Inhibitors, FRAC group 45) were presented. Resistance management remains challenging, however, collaboration among chemical companies, research institutions and agricultural enterprises is driving research to improve understanding of resistance mechanism and improve management strategies. Data modelling to predict resistance risk is also contributing to the improvement of resistance management. Novel technologies to improve the effective delivery of fungicides, understand fitness costs in resistant cells and determine resistance mechanisms were also presented. A new DMI fungicide Revysol® from BASF, used for grape powdery mildew, is targeted for release in 2019/20 into Europe and has recently undergone APVMA assessment for approval for release in Australia.

Prior to the symposium, Dr McKay visited the field laboratories of BASF at Limburgerhof, Germany. Her visit was hosted by Dr Gerd Stammler, Principal Scientist of the Fungicide Resistance Research area. Dr Stammler and his team have been working on fungicide resistance of powdery mildew for many years and are very collaborative and readily share knowledge and techniques.

It is essential that Australian researchers working in disease management using fungicides and related chemicals attend this symposium. It is the main forum worldwide for academic and applied researchers as well as representatives from chemical companies to share new knowledge about fungicides. Attendees readily share knowledge and expertise, and present and discuss new developments in a collegiate and collaborative manner. Contacts with leading researchers in this area were maintained and developed, providing additional benefit for future collaboration. The exposure to all aspects of research relating to fungicides, particularly resistance, will continue to benefit a wide range of Australian agricultural industries, in this case the Wine Industry.

19TH REINHARDSBRUNN SYMPOSIUM

Held in Friedrichroda, Germany, for the previous 50 years, the 19th Reinhardsbrunn symposium took place between 7th and 11th of April, 2019. This symposium is held every three years and brings together scientists from academia, research institutes and industry all working in the area of fungicides. It is the main forum for scientists to present, listen to and discuss the latest research and information on all aspects of fungicide use, including development, efficacy, resistance monitoring and resistance management. The 19th symposium consisted of 5 keynote lectures, 63 oral presentations and 39 posters. Of the 11 sessions, eight focussed on aspects of fungicide resistance, including monitoring, risk assessment, molecular mechanisms, management and regulation, whereas the other three sessions focussed on biorational fungicides/biocontrol, new technologies and applications, and new fungicides.

The symposium attracted over 160 delegates from 23 countries (Cover picture and Fig 1). The four delegates from Australia presented research findings on fungicide resistance on pathogens of grapevines (Dr McKay) and cereals. The emphasis on resistance research continues to increase worldwide, involving the large agro-chemical companies, smaller niche-market companies and world-leading research institutes and universities. The number of submissions relating to resistance in this symposium has increased from 50% in 2013, 60% in 2016 to 65% in 2019. Only 10% of submissions related to new compounds, which highlights the increased the importance of early resistance detection, monitoring and management to protect the existing chemistries for as long as possible.

Contributions to the symposium will be published as Proceedings of the Reinhardsbrunn-Series "Modern Fungicides and Antifungal Compounds", printed by the German Phytomedical Society/Deutsche Phytomedizinische Gesellschaft.



Fig 1. Delegates at the 19th Reinhardsbrunn symposium, Germany, April, 2019

Fungicide resistance monitoring

Two sessions were held on this topic, comprising 11 oral presentations by researchers from universities and agrochemical companies. While research findings presented were on specific pathogen/ crop combinations, in particular Asian soybean rust and Septoria blotch of wheat, the techniques used and the outcomes of research were valuable and adaptable to resistance monitoring of different pathogens in other crops.

Demethylation Inhibitor (DMI) fungicides (FRAC group 3)

Overall, resistance to DMIs is increasing and spreading worldwide. Resistance is associated with various molecular mechanisms, most commonly multiple mutations in the CYP51 gene, however, many detectable mutations are not necessarily correlated with sensitivity or resistance to DMIs. Summary of key points:

- Reduction in DMI efficacy to control *Phakopsora pachyrhizi* (Asian soybean rust), is associated with at least six CYP51 mutations and overexpression (Brazil).
- Reduced DMI sensitivity in cereal pathogens, *Zymoseptoria tritici* and *Pyrenophora teres*, developed over 30-40 years and is associated with 10-11 mutations. The effect of each mutation varies between each pathogen and with different fungicides. The mutation dynamics show temporal variation as mutations peak and disappear over time, however, in general with time and repeated fungicide application, the number of mutations accumulate for each fungicide/pathogen combination (UK).
- Resistance of *Z. tritici* to both DMI and Succinate Dehydrogenase Inhibitors (SHDIs) fungicides appears to be increasing and spreading eastwards through Europe. Multiplex qPCR (using double-dye probes) has been developed to track the spread of genotypes and estimate allele proportions for pooled field samples. This technology is being used from airborne spores collected from Burkhard spore traps (Belgium, Switzerland and Germany).
- UK research has found over 50 variants of the CYP51 gene have been associated with decreased sensitivity of DMIs in *Z. tritici*. North and South American isolates are generally less sensitive than European isolates due to the presence of less complex CYP51 variants (UK).
- Multi-resistant populations of *Cercospora beticola* (cercospora leaf spot in sugar beet) to DMIs, Methyl benzimidazole carbamates (MBCs, FRAC group 1) and Quinone outside Inhibitors (QoIs, FRAC group 11) have been detected. Nine haplotypes of CYP51 were identified but no correlations with sensitivity or resistance to DMIs was found (Serbia).
- Mutations in the CYP51 and/or promotor inserts (leading to overexpression) have accumulated in the UK populations of *Pyrenopeziza brassicae* (leaf spot of canola) resulting in continued decline of azole sensitivity (UK).

SDHI fungicides (group 7)

- SDHIs were used for the first time in Brazil for control of *P. pachyrhizi* in 2013/14 and reduced sensitivity first detected in 2015/16. Decreased sensitivity is associated with C-186F gene mutation(s) (Brazil).
- Mutations in the sdh subunits B, C and D are associated with reduced sensitivity of SDHIs in *Z. tritici*, *P. teres* and *Ramularia coll-cygni* with a diverse pattern of mutations for each pathogen. The mutation CH15R is associated with a high resistance factor, however this mutation results in a fitness penalty so the mutant cells do not survive from year to year. Pyrosequencing is used to monitor mutations and EC_{50}

values of >0.3 µg/mL and 1.0 µg/mL are the threshold values for phenotypic resistance for *Z. tritici* and *P.teres*, and *R. coll-cyngi* respectively (Germany).

Group 29 (uncouplers of oxidative phosphorylation)

- After 6 years of commercial introduction of Fluazinam® in to the United States for control of dollar spot disease of turf, populations remain stable in terms of sensitivity (USA).

MBC fungicides (group 1)

- Resistance of cucurbit powdery mildew (*Podosphaera xanthii*) and *C. beticola* to MBC fungicides is associated with E198A genotype (Spain and Serbia). Although not quantitative, Loop mediated isothermal amplification (LAMP) has the potential to be a specific and reproducible tool for detection and monitoring of MBC resistance in the field (Spain).

Group 22 (thiazole carboxamides)

- Resistance mechanisms, comprising point mutations in the β-tubulin gene, have been first described for ethaboxam, used to control *Phytophthora sojae*. Methodology involved induction of laboratory mutants on amended media as well as using the CRISPR/Cas9 mediated transformation system (China).

M5 (multi-site, chloronitriles)

- Chlorothalonil remains central to the control of *R. coll-cygni* (RLS) in barley. The potential withdrawal of chlorothalonil from use within Europe will likely have adverse effects on control of RLS (Ireland).

QoI (group 11)

- Reduced sensitivity detected in *P. pachyrhizi* is associated with the F129L mutation and not G143A, which has never been detected (Brazil).
- Resistance in *C. beticola* is associated with G143A (Serbia).

Other

- Due to increased resistance to single-site actives, the use of multi-site fungicides e.g. mancozeb, chlorothalonil and copper, has increased to control *P. pachyrhizi* (Brazil).

Resistance management

Management of resistance remains a challenge that varies widely depending on the regulations of each country, the size and nature of the farming enterprise, crop type and the fungicides used. It is clear that the research community and chemical companies are increasingly working together in a collaborative and cooperative manner, as it is widely recognised that resistance management does not rest with one organisation or company.

- Regulatory measures were introduced in Brazil to manage resistance in soybean rust. These measures aim to reduce inoculum between crop seasons by having a soybean free period, avoiding late-season sowing and the incorporation of resistant soybean lines (Brazil). Complicating the management of resistance is that cross-resistance among the compounds in the DMI and SDHI groups is often incomplete, which

increases the complexity of advice to growers. In addition, over-expression of CYP51 is implicated in resistance but the in-field impact of this mechanism is not fully understood (Germany).

- Asia-FRAC was formed in 2017, supported by CropLife Asia, to improve resistance management throughout Asia. Support for the establishment of national FRACs aim to drive educational and communication activities (Asia).
- Europe wide management of resistance of *Z. tritici* is difficult due to the following factors:
    - Efficacy of various azoles varies among countries
    - Multiple CYP51 mutations
    - Old azoles still provide useful levels of control
    - Population structures of CYP51 mutants is very dynamic leading to larger variations among regions
    - Links between specific mutations and EC_{50} values is not always clear (Denmark).
- A monitoring resistance program established for *Botrytis cinerea* in strawberry in USA, found:
    - three new species of *Botrytis*
    - no change in resistance to boscalid, cyprodonil and iprodione over the recent years
    - despite an increase in fludioxinil use, there hasn't been a corresponding increase in resistance
    - an increase in multi-drug resistance over the recent years with some isolates showing resistance to 6 or 7 different mode of action groups (USA).
- The mutation G1105S/V is associated with resistance in *Plasmopara viticola* to mandipropamid from the Carboxylic Acid Amides (CAAs FRAC group 40). A spray program with and without mandipropamid was used on field populations containing either sensitive or resistant strains. Both programs gave equivalent control and unexpectedly the program containing mandipropamid did not result in a large increase in the frequency of insensitive strains. However, due to sexual recombination in this pathogen, the resistant strain frequency varies considerably irrespective of fungicide exposure (Italy).
- Fitness of *Alternaria alternata* from pistachio containing SdhB and -C mutations was stable in laboratory assays. The findings were confirmed in field experiments when even after 3 successive years of discontinuing SDHI applications, no reduction in sensitivities or mutant frequencies was observed (USA).

Molecular mechanisms of fungicide resistance

Considerable research effort is going in to understanding molecular mechanisms~~,~~ of resistance. In most cases, ongoing research reveals an increasing complexity of the mechanisms, including multiple mutations, contributing to phenotypic resistance. However, determining and understanding the molecular mechanisms underpinning resistance means that molecular markers can be found enabling early detection and ongoing monitoring of resistance.

<u>DMI fungicides (group 3)</u>

- DMI resistance in *Z. tritici* has been evolving over the past 30+ years resulting in an extremely complex web of resistance mechanisms including multiple CYP51 variants.

Other resistance mechanisms include overexpression of the CYP51 enzyme and increased efflux activity in the cell. Although, our understanding that the resistance mechanisms are becoming more complex, an holistic approach still needs to be taken to further understand sensitivity changes (Germany).

- Parallel evolution of multiple CYP51 mutations has occurred in target and non-target fungal spp. The question was posed: If resistance in an off-target spp e.g. *Trichoderma* could be induced, could these resistant strains then be used in conjunction with fungicides to provide disease control (UK).
- Different mechanisms may govern resistance to the different DMI fungicides for *Venturia inequalis* (apple scab pathogen). Relative expression of CYP51A gene was greater for difenconazole resistant isolates than for isolates resistant to myclobutanil only (USA).
- There are no obvious fitness penalties for isolates with higher EC_{50} values of *Cercospora* on sugar beet, with similar amounts of disease expressing despite having different EC_{50} values. No mutations were correlated with EC_{50} values however, expression of CYP51 was related. Resistant isolates have high natural expression of CYP51 and have the ability to ramp up expression when exposed to fungicides. Isolates with EC_{50} values of > 1.0 µg/mL are classified as resistant (USA).
- To determine the efficacy of DMI fungicides when exposed to Y136F, results from detached leaf and potted plant assays were compared to data from a heterologous yeast expression system, where yeast was transformed with either wild type (Y136) or mutant (Y136F). Of the fungicides registered in Australia for control of *Erysiphe necator* (grapevine powdery mildew), difenconazole was the most effective with high levels of Y136F whereas triademenol was the least effective with Y136F however, all were similarly effective with Y136/wild type (Australia, Fig 2).



Fig 2. Dr McKay presenting research findings about DMI efficacy on grapevine powdery mildew at the 19th Reinhardsbrunn symposium

SDHI fungicides (group 7)

- Although cross-resistance is generally observed among the SDHIs, it is not always uniform for every plant pathogen system e.g. benzovindiflupyl was clearly suppressive to strains of *Corynespora cassiicola* (cucumber leaf spot pathogen) that were highly resistant to boscalid, demonstrating variation in cross-resistance among the SDHIs for this pathogen (Japan).
- In *Clarireedia* spp. (dollar-spot of turfgrass) multiple point mutations result in differential sensitivities to the SHDIs and each mutation is linked with different cross-resistance profiles (USA). Similarly, for *Alternaria* spp. in pear, variable cross-resistance is observed depending on the mutations. Sensitivity appears to be a continuum as it was difficult to classify strains in to resistant or sensitive groups. Testing revealed that decreased sensitivity to some newer SDHIs is being detected before their commercial introduction. This suggests that either less-sensitive variants already exist in the pathogen populations and/or resistance is building on the previous selected mutations already responsible for SDHI resistance (USA).Although, each mutation results in differing resistance to the various products, the resistance management advice for farmers is the same (Germany).

QoI, QiI and QoSi fungicides (groups 11, 21 and 45 respectively)

- Fenpicoxamid (Inatreq®, DowDuPont) is a new Quinone inside Inhibitor (QiI) soon to be introduced in to the EU for control of cereal pathogens. As fenpicoxamid binds to the 2nd ubiquinone site of cytb, no cross-resistance with QoIs is not anticipated. Laboratory mutagenesis assays found that the likely mechanisms of resistance are:
    - Target site modification, i.e. G37V mutation
    - Increase efflux
    - Alternative pathways
    - Default inactivation

  Determining the potential resistance mechanisms may help to optimise anti-resistance strategies when the fungicide is introduced to the market (France).

- *P. viticola* populations from France showed decreased sensitivity to ametoctradin (a QoSi fungicide) and cyazofamid (a QiI fungicide). The S34L mutation is associated with decreased sensitivity of ametoctradin and two mutations (V204? and G205?) were associated with cyazofamid resistant strains (France).
- Overexpression of the alternative oxidative pathway may contribute to QoI resistance in *Sclerotinia sclerotiorum* (Germany).

Other

- Insertions in the promoter region of the membrane transporter gene, MFS1, are responsible for the multi-drug resistant phenotype of *Z. tritici*, with no apparent fitness penalty. This mechanism is independent of the frequent target site mutation mechanisms. Isolates with both mechanisms have a significant potential for increased resistance risk (France).

A new simple and unified pesticide classification system was proposed. The project is collaborative and freely available on Open Science Framework at https://osf.io/ubhr5/ (France).

Regulatory aspects

- Foliar application of fungicides results in a greater amount of total active ingredient (a.i.) of fungicide than does seed treatments with ratios of 1:1 e.g. fluxapyroxid for wheat pathogens to 1:34 e.g. tebuconazole for wheat *Fusarium* spp. or even greater for other fungicide/pathogen combinations. Although this might appear intuitive, it is not the case for insecticides where seed treatments deliver more a.i. than foliar applications. Where possible, seed treatments may provide a means to decrease overall fungicide use in line with obligatory IPM measures in the European Union (Poland).
- DMI resistant *Aspergillus fumigatus* isolates have been obtained from humans who were naïve to DMIs. DMI resistant isolates may originate from 'hot spots' in the agricultural sector e.g. compost, flower bulb farms, and transfer to humans as a 'collateral' effect. A risk assessment revealed a strong correlation among all the DMIs used in agriculture, medicine and material protection for the majority of *A. fumigatus* isolates studied. This finding as well as the fact that identical DMI compounds are used in both agriculture and medicine, highlights the need for collaboration amongst sectors in terms of resistance monitoring and management (Switzerland).

Resistance modelling

- A host- pathogen interaction database, PHI-base, www.phi-base.org, is publicly available. The database stores information about phenotypes on genes implicated in virulence for 263 pathogens on 194 hosts. However, it does not appear to contain information on powdery or downy mildew on *Vitis* spp. (UK).
  Reference: Urban et al. (2017). PHI-base: a new interface and further additions for the multi-species pathogen-host interactions database. Nucleic Acids Res. doi: 10.1093/nar/gkw1089
- Models using *Z. tritici* demonstrate trade-offs between the various resistance management strategies. Key points:
  - If the mixing partner is effective then using a mixing partner compared to alternation leads to decreased selection of resistant genotypes
  - If mixing partner is relatively ineffective, then alternation is better
  - Fitness phenotypes drive population growth rates: "evolution doesn't care about mutations, overexpression"
  - Concept of 'dose-splitting'. i.e. a grower has a maximum dose they can use in a season and they can decide how to use this dose
  - Increased rate of resistance development with sexual recombination than asexual reproduction (UK).

  References:

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  3. van den Bosch et al. (2014). Governing principles can guide fungicide resistance management tactics. Annual Review of Phytopathology, 52, 175-195.
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Digital pathogen sensing and resistance risk assessment

- There is considerable potential to use smart airborne spore traps for resistance detection and monitoring. Several projects worldwide are assessing the technology for use in resistance monitoring, including for grapevine pathogens in Australia (UK).
- Potential to integrate lateral flow assays e.g. MinION (portable real-time DNA and RNA sequencing devices) with airborne spore traps, then relay results in real-time using Wi-Fi (UK).

Biorational fungicides/Biocontrol

- Application of *Bacillus subtilis* QST713 (Serenade®, Bayer) in alternation with fluopyram (an SDHI) provided good control of *B. cinerea* on greenhouse tomatoes, as well as contributed to the elimination of strains resistant to SHDIs (in particular sdhB mutants (Greece).
- There is increasing pressure to use less fungicides whereas the use of biological and related controls is encouraged. However, the effects of this phenomenon are not well understood. Many biological control agents produce secondary metabolites which may be highly toxic and/or carcinogenic. Also the interaction between the biological control agent and the pathogen may produce unwanted effects e.g. toxin production, of which we are yet unaware (Germany).
- The essential oil of *Anethum graveolens* (dill) seed showed antifungal growth properties against *B. cinerea* in *in-vitro* assays (Lithuania).

New technologies

- A novel technology has potential application for the safe and efficient delivery of low doses of pesticides for use in plant protection. BiFuPeps contain an anchor peptide that adheres an antimicrobial peptide to the plant surface. Also, anchor peptides can be 'decorated' with microgels that contain pesticides. These microgels then adhere to a plant surface and release the pesticide slowly and continuously over long periods of time (Germany).
- Live-cell imaging using lasers is being used as a quantitative method to investigate unknown modes of actions of fungicide; all in real time (UK).
- Small RNA (sRNA) molecules have been shown to be effective in disease control, by either use in transgenic plants (host-induced gene silencing, HIGS) and/or spray induced gene silencing (SIGS). Exploration of the mechanistic basis has found that exosome-like or extracellular vesicles are key transporters of the HIGS and SIGS-related sRNAs (Germany).
- Fitness costs linked with mutations in fungal genes are the most important factor in driving resistance evolution. A non-destructive, biochemical method measuring cell respiration was developed to assess the fitness of fungicide resistant mutants (Germany).
- A light-weight digital PCR was used in conjunction with a two-step field-based DNA extraction method to detect and quantify both resistant and wild-type alleles of *Blumeria* spp. of barley. Mutation levels as low as 0.2% in both genomic DNA and field samples were detected. Potential use of this technology is for early detection so growers can amend spray programs (Australia).

New fungicides

- A new triazole, Revysol®, from BASF, is scheduled for market introduction in Europe in 2019/20. Targets include grape powdery mildew. Revysol® has limited cross-resistance with other azoles, and even when target site mutations exist it shows activity with these resistant fungal strains. It has fast leaf uptake but slow translocation within the plant, resulting in a reservoir of the chemical in the inner leaf so it has long-lasting residual activity and some potential curative activity e.g. *Botrytis* latent infection (Germany).
- A new QoI, in the tetrazolinone chemical class, called Pavecto®, from Sumitomo Chemical Company, is available for *Z. tritici* on wheat and *P. teres* on barley. Pavecto® has a higher intrinsic activity than pyraclostrobin and is also active against both QoI resistant strains i.e. G143A and F129L due to the unique shape and size of the active ingredient (Japan and Germany).
- Isotianil, a plant defence inducer, has strong efficacy against *Magnaporthe oryzae Triticum* (wheat blast fungus). As isotianil has no molecular target within fungal cells, there is no risk of resistance development (Germany).
- Ametoctradin, used for grapevine downy mildew control, appears to bind to both Qo- and Qi- binding sites of the mitochondrial complex. However, ametoctradin differs from the other Qi- binding site fungicides of cyazofamid and amisulbrom (Germany and France).

VISIT TO BASF, LIMBURGERHOF, GERMANY.

The headquarters of BASF Crop Protection is located at Limburgerhof, Germany, west of Heidelberg (Fig 3). It is the hub for worldwide research and development operations, registrations as well as marketing. Here, new molecules are screened *in-planta*, then progress to product development, followed by in-field evaluation. If successful the product then goes through rigorous testing to obtain registration for the various markets. Resistance risk management strategies are an essential component of product registration. After products are launched in the market, resistance monitoring is also continuously carried out.



Fig 3. BASF Agricultural Field Center, Limburgerhof, Germany.

My visit was hosted by Dr Gerd Stammler, Principal Scientist of the Fungicide Resistance Research area. I toured the facilities focussing on new-product trials on a wide variety of crop types in the greenhouses. Robotics and closed mechanised systems are used extensively in the greenhouses to sort, move and spray plants with fungicide treatments, making high throughput screening for efficacy and resistance possible. I met and held discussions with various research and technical staff, with a particularly useful discussion with Gabi Berthold, who works on resistance testing of grape powdery mildew. We shared knowledge and information about subculturing, storage, sensitivity testing of *E. necator*, analyses and interpretation of results. BASF carry out some in-house phenotype resistance monitoring for various plant-pathosystems, including grape powdery mildew (Fig 4) but some is also outsourced to an external provider. Extensive resistance monitoring is also carried using DNA-based molecular techniques, in particular, pyrosequencing (Fig 5). Time in the laboratory was spent observing phenotype assays for sensitivity for the obligate biotrophic pathogen, *Phakopsora pachyrhizi* as well as DNA preparation and

pyrosequencing for detection of resistance alleles. It appears that little, if any, research is carried out to understand relationships between field efficacy and laboratory phenotype and genotype results. In discussions with researchers about this issue, it is considered unravelling the many relationships that contribute to field efficacy is extremely difficult, and direct relationships between laboratory results and field failure are, in most cases, difficult to determine. The vast majority of research is focussed on understanding molecular mechanisms of resistance, early detection and monitoring for incidence and severity of resistance, which is most commonly carried out by detection and/or quantification of mutations associated with resistance.



Fig 4. Phenotyping assay for grape powdery mildew resistance testing.



Fig 5. Pyrosequencing for the detection of resistance alleles.

BASF have approval for the release of Revysol®, a new DMI fungicide, for use in Europe on cereal pathogens. It is anticipated that further registrations will allow use in other pathosystems, including grape powdery mildew in Australia.

OUTCOMES

New knowledge and understanding about worldwide trends in fungicide resistance research and management have provided Dr McKay with a deeper understanding of the complexity of resistance and challenges that agricultural industries face in the future. This knowledge and understanding will benefit existing and future research projects on fungicide resistance, by adapting the focus and project aims to take in to account current research. New information is being communicated to the Australian grape and wine industry in conjunction with the current extension of the resistance project, through presentations, workshops, an article and personal communication.

Existing collaboration with international researchers, particular with Dr Gerd Stammler and team from BASF, Germany, Dr Jon West from Rothamsted Research, UK, and Dr Themis Michailides from the University of California, USA, was strengthened. New contacts were made with researchers working on resistance management in research organisations as well as with BASF, Bayer, Corteva and Syngenta.

COMMUNICATION

New information from the workshop was disseminated to the 'Fungicide Resistance, SAR 1701-1.2' project team and Industry Reference Group at the Fungicide Resistance project meeting in May 30 and 31st 2019.

Information gained from the symposium will be incorporated in presentations given to industry about fungicide resistance.

An article will be prepared for the industry journal Australian Viticulture.

A synopsis of key points will communicated to the Agrochemical working group, AWRI

The Contributions of the Symposium will be published as Proceedings of the Reinhardsbrunn-Series "Modern Fungicides and Antifungal Compounds", printed by the German Phytomedical Society/Deutsche Phytomedizinische Gesellschaft.

RECOMMENDATIONS

Relationships established during the workshop should be maintained and similar visits by scientists to international workshops need to be supported to:

- keep Australian scientists up to date with latest developments elsewhere
- allow professional development for scientists working in the Wine Industry
- promote collaborative work and exchange of ideas among researchers and foster the development of new ideas
- ensure that the research providers remain an active in the international research community, to provide the best outcomes for the Australian grape and wine industry.

I recommend that the current Fungicide Resistance project, SAR 1701-1.2, further pursue how best to use and build on the currently available technologies and methods, to work towards in-field detection tests and/or area wide resistance monitoring for those fungicides where genetic mechanisms are known. As new technologies are emerging around detection and quantification of resistance, using predominantly genotypic analysis, widespread resistance monitoring is being increasingly used in Europe, South America and North America for cereal, soybean and some horticultural crops with selected fungicide groups (where genetic mechanisms are known and can be used as markers for resistance detection). Although the marriage of sequencing technologies e.g. MinION, with spore traps and being able to send real time data back using Wi-Fi is considered the 'holy grail', there are useful technologies currently available and the Fungicide Resistance project team, following consultation with the Industry Reference Group of SAR 1707-1.2 and other scientists, should develop a proposal with recommendations of how best pursue work in this area to meet the needs of the Wine Industry.