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Developing a threat-specific contingency plan for the exotic disease angular leaf scorch

FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **SAR1201**

Project Supervisor: **Mark Sosnowski**
Principal Investigator: **Matthew Ayres**
Project Collaborator: **Wayne Wilcox**

Research Organisations:
South Australian Research & Development Institute
Cornell University, USA

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All enquiries

Dr Mark Sosnowski
South Australian Research and Development Institute
GPO Box 397, Adelaide, South Australia 5001
Phone: (08) 8303 9489; Mob: 0412148418
Email: mark.sosnowski@sa.gov.au

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1 Abstract

Angular leaf scorch (ALS), caused by the fungus *Pseudopezicula tetraspora*, is a high-priority disease threat to the $40 billion Australian wine industry. Research undertaken at the Cornell University New York State Agricultural Experiment Station in Geneva, NY, USA, has provided contingency plans for the Australian wine industry in the event of an ALS incursion. A diagnostic protocol has been developed to enable a rapid response. Cultivars widely grown in Australia are susceptible to *P. tetraspora*, highlighting the threat that an incursion of ALS presents to Australia, and provides information to underpin effective monitoring and surveillance. The fungicides; trifloxystrobin (Flint), pyraclostrobin (Cabrio) and tebuconazole (Folicur) were effective at controlling ALS and could be used for containment or eradication. A drastic pruning eradication strategy has been validated for ALS and, along with the diagnostic protocol, will be included in the Viticulture Industry Biosecurity Plan. This research has increased the biosecurity capability of the Australian viticulture industry and improved industry preparedness, with potential to save the wine industry many millions of dollars in lost production and vineyard re-establishment costs, whilst maintaining the competitive advantage conferred by freedom from exotic diseases.

2 Executive Summary

Angular leaf scorch (ALS), listed as a high-priority threat to the Australian wine industry, is caused by the fungus *Pseudopezicula tetraspora* and can cause yield losses of up to 90%. Developing contingency plans for ALS will contribute to maintaining freedom from exotic pests and diseases in Australia, providing financial benefit to the $40 billion per annum industry, and a distinct advantage in a very competitive world market.

Research was undertaken at the Cornell University New York State Agricultural Experiment Station in Geneva, NY, USA, to develop a diagnostic protocol for ALS and methodologies for undertaking research. Attempts to isolate *P. tetraspora* and induce development of fruiting bodies and sporulation on symptomatic leaves were largely unsuccessful, most likely due to the intensive fungicide spray program applied in commercial vineyards of NY, which may have reduced the viability of the fungus in infected leaves. Due to the inability to produce spores as an inoculum source, a method was developed for inoculation of leaves with mycelium of *P. tetraspora* grown in culture. This proved to be very effective, and provided a reliable inoculum source for subsequent greenhouse experiments and field trials.

Identification of *P. tetraspora* was achieved by microscopic analysis of symptomatic leaves, and through culture morphology and DNA sequencing of *P. tetraspora* (ATCC 62299). Submission of the DNA sequence data to GenBank will provide a reference for future diagnosis. A diagnostic protocol for ALS has been drafted and submitted to the Subcommittee on Plant Health Diagnostics from the Commonwealth Department of Agriculture and Water Resources, and once endorsed will be included in the Viticulture Industry Biosecurity Plan.

Grapevine cultivars vary greatly in their susceptibility to ALS but only a few *V. vinifera* cultivars have previously been evaluated. Greenhouse evaluations revealed that the seven cultivars most widely planted in Australia can be infected by *P. tetraspora.* Riesling was extremely susceptible, Sauvignon Blanc, Cabernet Sauvignon and Pinot Noir, moderately susceptible, and Chardonnay, Shiraz and Merlot, slightly susceptible. This highlights the threat that an incursion of ALS poses to Australia, and provides valuable information for effective monitoring and surveillance should an incursion occur.

Fungicides currently registered for use on grapes in Australia were evaluated in the laboratory and greenhouse for efficacy against *P. tetraspora*. Three fungicides; trifloxystrobin (Flint), pyraclostrobin (Cabrio) and tebuconazole (Folicur), belonging to three distinctive chemical groups that represent two different modes of action, were effective at controlling *P. tetraspora* and so can be used for control, containment or eradication of ALS in Australian vineyards, in the case of an incursion.

A drastic pruning strategy, previously validated for black rot disease, was evaluated in a simulated incursion of *P. tetraspora* in New York State. Over the three years following implementation, symptoms were recorded only on control vines, and not on treated vines, confirming the success of the eradication strategy for ALS. In the event of an incursion of *P. tetraspora* in Australia, the drastic pruning protocol has potential to save the wine industry many millions of dollars in lost production and vineyard re-establishment costs. The eradication protocol will be published and included in the Viticulture Industry Biosecurity Plan.

Australian researchers have gained expertise in the diagnosis, eradication and management of ALS and strengthened the existing collaborative partnership with Cornell University.

3 Background

Freedom from exotic pests and diseases provides the Australian wine industry with a distinct advantage in a very competitive world market. The Viticultural Industry Biosecurity Plan (VIBP) categorises the risk of exotic pests and diseases to the Australian industry and provides generic contingency plans through PLANTPLAN, which is a national set of incursion response guidelines for the plant sector. The VIBP proposes to complete threat-specific contingency plans for all high-priority grapevine pests and diseases as the information is generated through scientific research.

National diagnostic protocols are being undertaken for 13 high-priority viticultural pests and diseases. To ensure a rapid response in the case of incursion, it is crucial that the Australian wine industry has diagnostic capability for all high-priority pests and diseases. The diagnostic protocol for angular leaf scorch (ALS) was developed in this project.

The standard strategy for eradication of an exotic plant pathogen is based on the removal of whole affected plants, followed by burning and/or burial. However, this strategy may have significant economic and social impacts on the industry and communities, particularly with respect to older, high-value premium vineyards. Research led by SARDI and in collaboration with Cornell University, USA and DPI Victoria through the CRC for National Plant Biosecurity has developed and validated a drastic pruning strategy to eradicate the high-priority disease black rot, caused by the fungus *Guignardia bidwellii*, from grapevines, without the need to completely remove vines (Sosnowski *et al*. 2012). It is possible that a similar eradication strategy could be effective for other high-priority exotic diseases, such as ALS and Rotbrenner, based on similarities in biology of the causal fungi.

Angular leaf scorch is caused by the fungus *Pseudopezicula tetraspora* and was first described in the USA in 1986 (Korf *et al.* 1986). It is closely related to Rotbrenner disease, which is caused by the fungus *Pseudopezicula tracheiphila* and was first described in Europe in 1903 (Korf *et al.* 1986, Pearson *et al.* 1991). Both diseases cause leaf scorching (Figure 3.1), defoliation and pedicel infection which can lead to yield losses of up to 90% (Kassemeyer and Wilcox 2015). These fungi survive over winter in fruiting bodies called apothecia on dead leaves on the vineyard floor, from which ascospore inoculum is released in the following season to infect new growth (Figure 3.2).

Figure 3.1. Characteristic leaf symptoms of angular leaf scorch on cvs Chardonnay (a) and Concord (b).

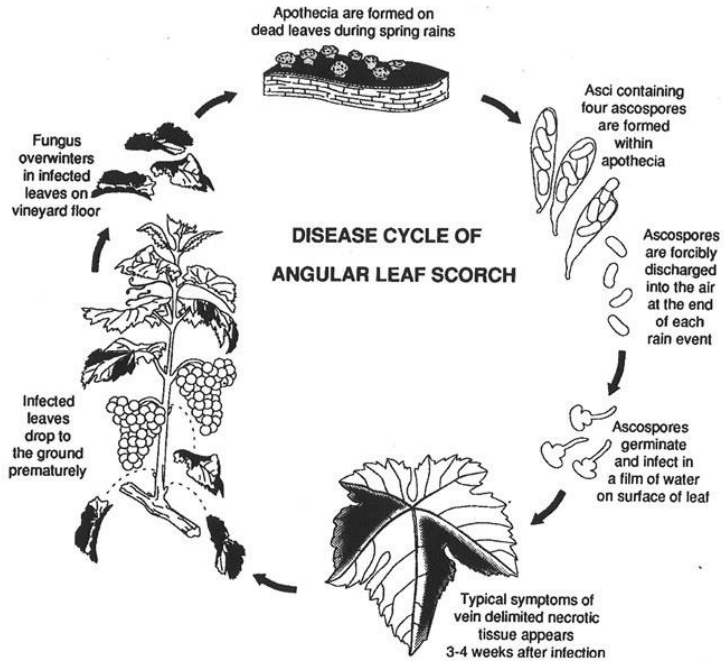

Figure 3.2. Disease cycle of angular leaf scorch, caused by *Psudopezicula tetraspora* (Pearson 1992)

Grape cultivars vary greatly in their susceptibility to ALS (Pearson *et al.* 1988, Pearson 1992). Those classified as highly susceptible are the interspecific hybrids Aurore, Chancellor, Chelois, DeChaunac, Rougeon and Ventura. Cultivars classified as moderately susceptible are Baco Noir, Canadice, Cayuga White, Elvira, Missouri, Rosette, Seyval, Steuben, Vignoles and *V. vinifera* cvs Chardonnay and Riesling. Those classified as slightly susceptible are Catawba, Concord, Delaware, Dutchess, Foch, Fredonia, Himrod, Ives, Niagara, Remaily Seedless, Vidal Blanc, and *V. vinifera* cvs Gewurztraminer and Pinot Noir. Virginia creeper (*Parthenocissus quinquefolia*) and the wild grape, *V. riparia*, are also susceptible to the disease and can serve as reservoirs of *P. tetraspora*. Extensive screening of *V. vinifera* cultivars predominantly grown in Australia had not been conducted prior to the initiation of this project.

Benomyl was shown to be the most effective fungicide for controlling ALS (Pearson *et al.* 1988), but is no longer available for use on grapevines in Australia. Mancozeb was also reported to have moderate efficacy and would require regular applications from early shoot growth through to flowering for effective control. Captan was shown to be ineffective. This research is 30 years old and the efficacy of more recent fungicides is unknown.

Managing the endemic diseases powdery mildew, downy mildew and botrytis and other bunch rots is estimated to cost the Australian wine industry $191 million per annum (Scholefield and Morison 2010). Australia's isolation and strict quarantine processes have kept us free from many of the other diseases which cause major economic impact in wine producing countries around the world. Increasing globalisation is elevating the risk of incursions. Ensuring Australia is prepared to respond effectively to incursions of exotic diseases, such as ALS, will avoid increased costs for disease management and maintain our competitive advantage in the ever growing global wine market.

4 Project Aims and Performance targets

Develop diagnostic protocol for angular leaf scorch
A diagnostic protocol for angular leaf scorch will be developed and submitted to Subcommittee on Plant Health Diagnostics (SPHD) for national endorsement.

Determine susceptibility of *Vitis vinifera* cultivars to angular leaf scorch
The main *V. vinifera* cultivars grown in Australia will be screened for susceptibility to angular leaf scorch in a greenhouse to establish the likely impact on Australian vineyards in the case of an incursion.

Evaluate fungicides for control of angular leaf scorch
The efficacy of fungicides on angular leaf scorch will be evaluated to provide alternative strategies to respond to an incursion.

Validate efficacy of drastic pruning for eradicating angular leaf scorch
The drastic pruning protocol developed for eradicating black rot will be evaluated as a means of eradicating angular leaf scorch, and if successful included in the Viticulture Industry Biosecurity Plan.

Increase the biosecurity capability of the Australian viticulture industry
Australian researchers will gain expertise in the diagnosis, eradication and management of angular leaf scorch and build on the collaborative partnership with Cornell University.

5 Preliminary experiments and diagnostic protocol

5.1 Introduction

Preliminary experiments were undertaken at the Cornell University New York State Agricultural Experiment Station in Geneva, NY. Beginning in the first year of the project, methodologies were developed for subsequent laboratory, greenhouse and field experiments on angular leaf scorch (ALS), along with a diagnostic protocol for *Pseudopezicula tetraspora*, the causal agent of ALS.

5.2 Isolation and sporulation from infected leaves

A vineyard in the Finger Lakes wine region of New York, known to have had ALS in previous years, was monitored for symptoms throughout the 2014 spring/summer season. Symptomatic leaves were observed on cvs Riesling and Cabernet Franc. Disease expression varied between the cultivars, with symptomatic leaves on the white cultivar displaying a yellow margin between the necrotic and healthy leaf tissue, and on the red cultivar, a red margin between the necrotic and healthy leaf tissue (Figure 5.1a).

Based on previous literature (Pearson *et al.* 1988, Pearson, 1992), methods producing fruiting bodies and ascospores were attempted. Symptomatic leaves on field vines were sprayed with sterile water and placed in closed plastic bags for 72 hours (Figure 5.1b). This attempt to induce sporulation was unsuccessful. Symptomatic leaves were also taken to the laboratory and incubated in humid conditions on wet filter paper enclosed in a Petri dish to encourage development of apothecia. This process was repeated several times but no fruiting bodies were produced. Symptomatic leaves were also collected and stored in paper bags for drying. The dried leaves were stored in a cold room (4°C) for future use in field trials.

Several attempts were made to isolate *P. tetraspora* using methods described by Pearson et al. (1988). Leaf sections were placed in a 1% sodium hypochlorite (bleach) solution for 1 minute prior to rinsing with sterile distilled water. In a laminar-flow cabinet, leaves were then dissected using sterile scalpels and small sections, taken from the margin between necrotic and healthy tissue, were plated onto malt extract agar (MEA) with streptomycin sulphate (100 mg/L) added. This was initially unsuccessful, usually due to fast-growing contaminants crowding the plates. In 2015, symptomatic leaves were collected from cv. Concord vines at the field site and returned to the laboratory and another attempt was made to isolate *P. tetraspora* using the methods described above. After 4 days, subcultures were taken from some of the organisms that had grown from the leaf sections and placed on agar plates. A colony morphologically resembling *P. tetraspora* was further subcultured onto ¼ strength PDA (¼ PDA) and MEA agar plates (Figure 5.1c-d). The culture was submitted to the Cornell University Institute of Technology, Biotechnology Resource Center for DNA sequencing, but was confirmed as the common yeast species *Aureobasidium pullulans*.

Some of the symptomatic leaves, and leaf material collected from the ground beneath vines from several vineyards (Figure 5.1e-g) that were observed to have symptoms of ALS in the previous season, were incubated in humid conditions on wet filter paper enclosed in a Petri dish to encourage development of fruiting bodies (Figure 5.1h). This process was repeated several times but no fruiting bodies developed.

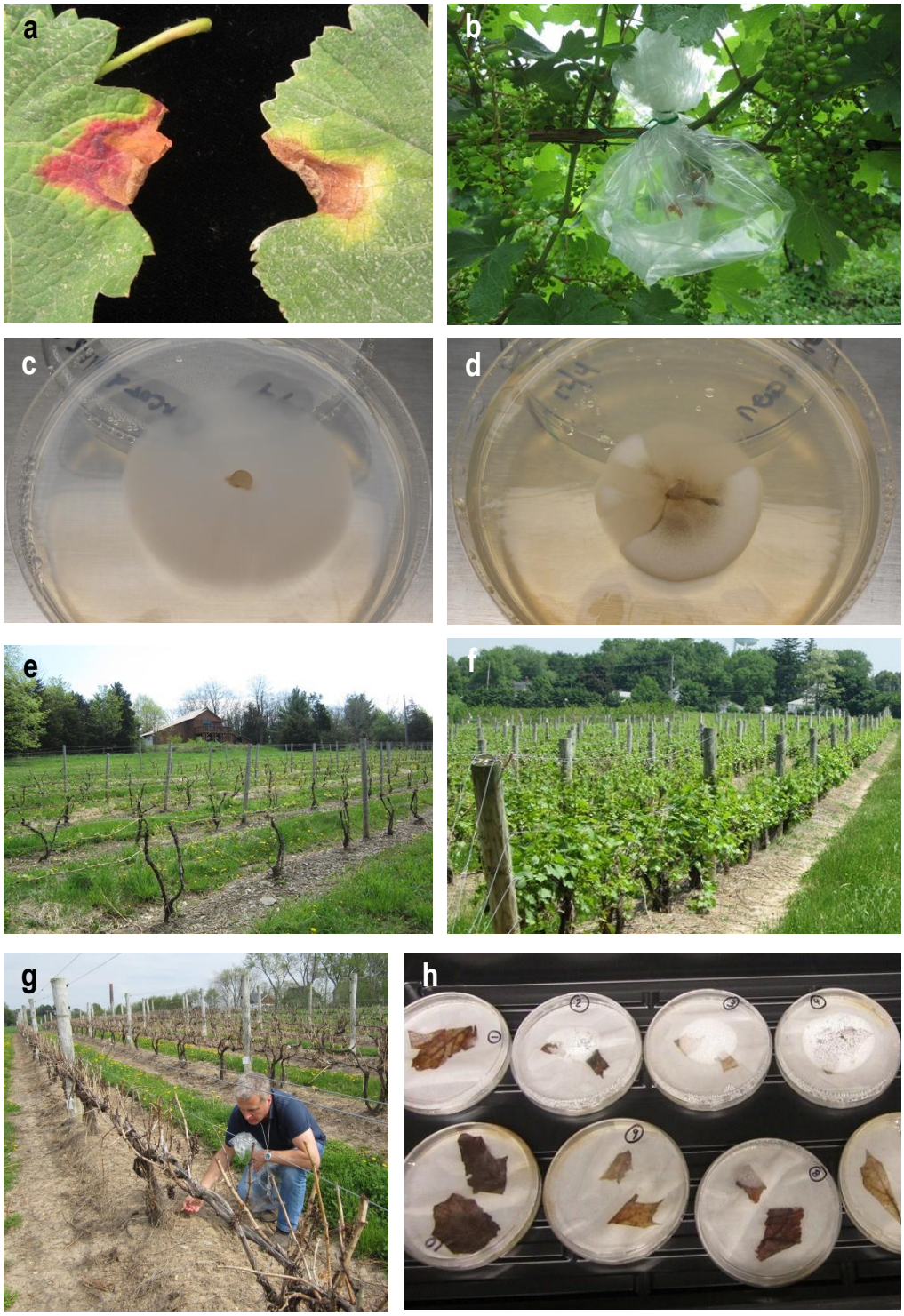

Figure 5.1a) Angular leaf scorch (ALS) symptoms on (left) Cabernet Franc and (right) Riesling grapevine leaves, b) symptomatic leaves placed in a moistened plastic bag for 72 hours to stimulate sporulation in the vineyard, culture isolated from symptomatic cv. Concord leaves in field trial on c) ¼ PDA and d) MEA, e-f) vineyards in the Finger Lakes wine region of NY, where ALS symptoms had been observed in the previous year, g) collecting leaf material from beneath vines and h) leaf material placed on moistened filter paper in Petri dishes in the laboratory (to induce sporulation).

5.3 Inoculation

In the absence of fungal spores for use as an inoculum source for greenhouse and field trials (Section 5.2), another method of inoculating plants was evaluated. An isolate of *P. tetraspora*, stored in the American Type Culture Collection (ATCC 62299; www.atcc.org) since 1986, was accessed and grown in the laboratory at approximately 22°C on a range of media including malt extract agar (MEA), potato dextrose agar (PDA) and ¼ strength PDA. This allowed the observation of culture characteristics for diagnostic purposes, and also provided mycelium which was used as an inoculum source for planned field, greenhouse and laboratory experiments. Photographs of the culture growing on the different media over several weeks were taken (Figure 5.2).

Based on previous literature (Pearson *et al.* 1988), another method of obtaining spores, which involved overlaying sterile grapevine leaf segments on the culture growing on MEA, in an attempt to colonise the leaf segments with mycelium, in the hope of developing apothecia. No apothecia were produced using this method.

A mycelial "slurry" (Figure 5.3a) was prepared by macerating *P. tetraspora* cultures on agar, with sterile distilled water (SDW) added, in a blender. Leaves on potted vines (cv. Riesling) were injured by scratching with forceps (Figure 5.3b), or left un-injured, then the mycelial slurry was spread on the leaves using a spatula (Figure 5.3c). In addition, small sections of agar cultures were placed on the leaves, mycelium side down, and secured with adhesive tape (Figure 5.3d). Bags were placed over all plants for 24 or 48 h to maintain high humidity, and placed in a controlled environment chamber (Biotron) to continue incubation at 20ºC, 14/10 h light cycle (Figure 5.3e). Within 7 days, leaves were observed to have necrosis around injury sites and under the tape, all assumed to be due to physical reactions, and very small lesions appearing on the non-injured leaves. Plants were monitored for a further 3 weeks for the development of characteristic ALS symptoms but none were observed.

The experiment was repeated on potted vines (cv. Riesling) with droplets of a mycelial slurry placed on injured and non-injured leaves as described above. Vines were then placed in a mist chamber (20°C, 12 h light cycle, constant mist (Figure 5.3f), for 72 h or 1 week. Control vines (injured or non-injured, non-inoculated) were included. After 72 h, some plants were removed from the mist chamber and placed in the Biotron, and after 1 week, mist was turned off and all plants were returned to the chamber and grown at 20°C, 12 h light cycle. After 20 days, characteristic ALS symptoms became apparent on all injured leaves misted for 72 hs and 1 week (Figure 5.3g). The symptoms continued to develop over the next few weeks. No symptoms developed on the non-injured leaves or on the control vines (Figure 5.3h).

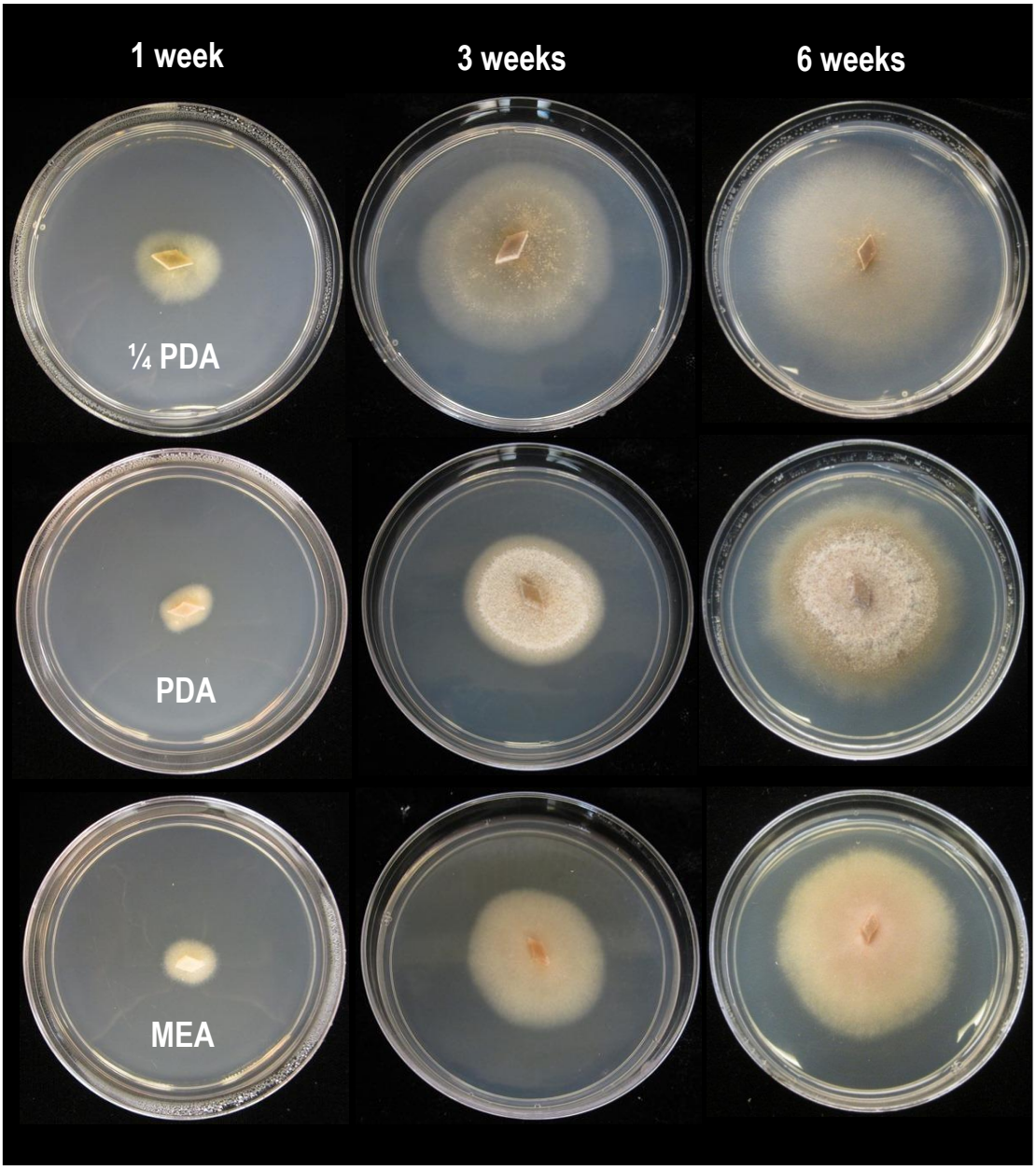

Figure 5.2 *Pseudopezicula tetraspora* cultures grown in the laboratory at approximately 22°C under fluorescent lights (16 h light cycle) on quarter strength potato dextrose agar (1/4 PDA), full strength potato dextrose agar (PDA) and malt extract agar (MEA) at 1, 3 and 5 weeks.

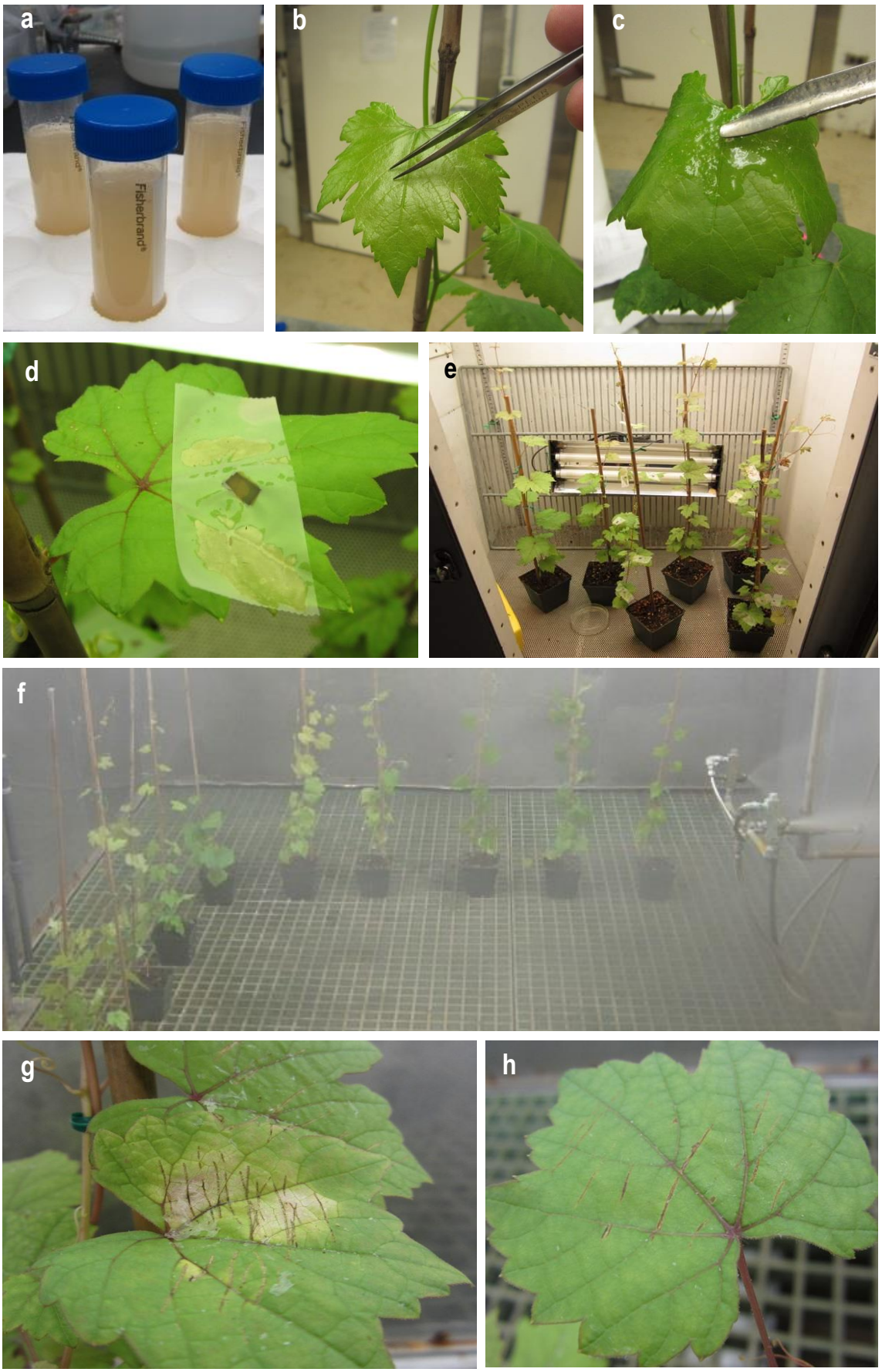

Figure 5.3 a) Mycelial slurry of *Pseudopezicula tetraspora*, b) injuring leaves on potted vines (cv. Riesling) by scratching with sharp forceps, c) inoculating vine leaves with mycelium slurry, d) mycelium plug placed on vine leaf and secured with adhesive tape, e) potted vines (cv. Riesling) in a controlled environment chamber (Biotron), f) potted vines in a mist chamber. g) Early symptom development of ALS on injured, inoculated vine leaves, h) injured, non-inoculated vine leaves without symptoms.

5.4 Identification

Microscopic identification of *P. tetraspora* hyphae growing within vascular tissue of affected leaves was undertaken using methods described by Pearson *et al.* (1988). Symptomatic field and mist-chamber leaf segments were boiled in 2% potassium hydroxide solution for 5 to 7 mins, then placed on a microscope slide and squashed under a coverslip. Hyphae, growing in a sine-wave pattern through the leaf structure, were observed when viewed under a light microscope (Figure 5.4). This confirmed the presence of *P. tetraspora* in the symptomatic leaves from the field and the mist chamber experiment.

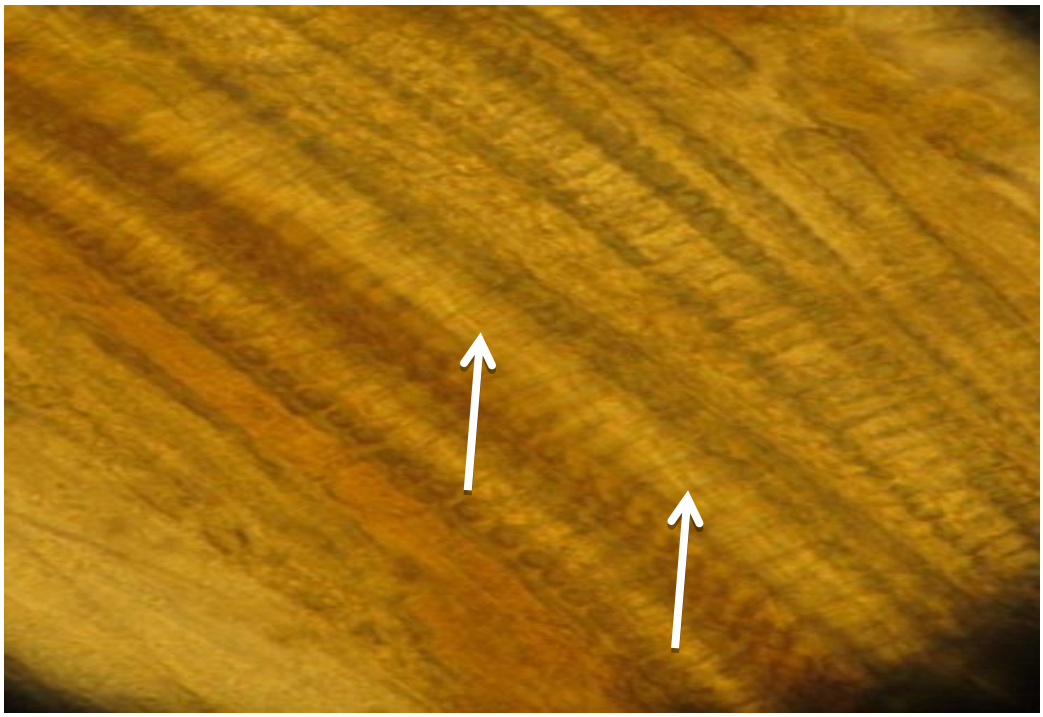

Figure 5.4 Sine-wave growth pattern of *Pseudopezicula tetraspora* hyphae (indicated by arrows) growing in a vessel within leaf tissue, viewed under a light microscope at 400x magnification.

A culture of *P. tetraspora* (ATCC 62299) growing on MEA was supplied to Cornell University Institute of Technology, Biotechnology Resource Center, NY, USA, for DNA sequencing using the Sanger sequencing method. The resulting final sequence of 539 bp was submitted to the National Center for Biotechnology Information (NCBI) GenBank (www.ncbi.nlm.nih.gov/genbank/) on 1 November 2017.

The accession reference is: MG385670, version: MG385670.1

5.5 Discussion

In order to produce spores for use as inoculum for greenhouse experiments and field trials, several attempts were made to induce sporulation on symptomatic leaves in the laboratory and field, based on previously described methods (Pearson *et al.* 1988). All these attempts were unsuccessful. Several attempts were also made to isolate *P. tetraspora* from ALS symptomatic leaves but these were also unsuccessful.

A possible explanation for the lack of success in obtaining fruiting bodies, spores or isolating the pathogen may be the fungicide spray program currently applied in commercial vineyards, which involves different active ingredients from those applied in 1988, when the previous research resulting in *P. tetraspora* sporulation was conducted. The Finger Lakes region has a humid climate with high summer rainfall, which is conducive to foliar diseases, necessitating frequent fungicide applications throughout the growing season. Following infection by *P. tetraspora*, it takes three weeks of incubation before ALS leaf symptoms become apparent, so the vines may have been sprayed since infection, killing the pathogen. This could also have affected the production of fruiting bodies and spores on fallen leaves collected from under the vines.

In the absence of spores to use as an inoculum source, the development of a mycelial slurry inoculum in this project allowed for the completion of all greenhouse experiments and field trials.

A technique described by Pearson *et al.* (1988) provided microscopic observation of *P. tetraspora* hyphae growing in a characteristic sine-wave pattern in segments of symptomatic leaves, confirmed the presence of the pathogen in inoculated leaves from field trials in this project.

Differences in morphology of the known reference *P. tetraspora* isolate (ATCC 62299) growing on different media were observed and provided a means with which to compare the various organisms that were isolated from symptomatic leaves. The ATCC 62299 cultures also provided material for DNA sequence analysis, and submission to GenBank provides a reference for diagnosis.

Based on these preliminary experiments, a diagnostic protocol for *P. tetraspora* has been developed and submitted to the Subcommittee on Plant Health Diagnostics from the Commonwealth Department of Agriculture and Water Resources, and will be included in the Viticulture Industry Biosecurity Plan.

6 Cultivar susceptibility

6.1 Introduction

Grapevine cultivars vary greatly in their susceptibility to ALS (Pearson *et al.* 1988, Pearson 1992). However, these reports were based on anecdotal observations in the field, involving mainly interspecific hybrids that are predominantly grown in north-eastern USA. Only a few *V. vinifera* cultivars were included; Chardonnay, Riesling, Gewurztraminer and Pinot Noir, which were classified as slightly to moderately susceptible. In Australia, *V. vinifera* cultivars are grown, either on own roots or on hybrid rootstocks, the most common being Shiraz, Cabernet Sauvignon, Chardonnay, Merlot, Pinot Noir, Sauvignon Blanc and Riesling. In order to understand the threat of an ALS incursion to the Australian industry, evaluation of these cultivars was undertaken at the Cornell University New York State Agricultural Experiment Station in Geneva, NY.

6.2 Methods

Grapevine canes were collected from vineyards in the Finger Lakes wine region of NY during the 2013/14 pruning season and stored in a cool room at 3-4°C. In May 2014, canes were prepared for propagation by trimming and placing in crates lined with cheesecloth and filled with perlite (Figure 6.1a). The canes included *V. vinifera* cultivars commonly grown in Australia: Shiraz (Syrah), Cabernet Sauvignon, Chardonnay, Merlot, Pinot Noir, Sauvignon Blanc and Riesling, and the local interspecific hybrid cultivar Chancellor was also included as a control, due to its high susceptibility to ALS. The canes were placed in a greenhouse at 22°C where they were callused and produced shoots and roots. After 8 weeks, rootlings were removed from the crates and placed individually into 20 cm pots filled with potting soil (Figure 6.1b). At the end of the season, vines were pruned and pots, with vines in a dormant state, were stored over winter in a barn.

In April 2015, potted vines were removed from storage and placed in the greenhouse at 22°C. Once shoots had at least three fully opened leaves, vines were inoculated with *P. tetraspora* (ATCC 62299) as described in Section 5.3. On 3 June 2015, three leaves each on two shoots per plant were scratched with sharp forceps and inoculated with a mycelium slurry, and the plants were then placed in a humidity chamber (20°C, 14/10 h light cycle, constant mist) for 72 hs (Figure 6.1c), prior to being returned to a greenhouse maintained at approximately 23°C (Figure 6.1d). The experiment was established as a randomised block design, with 10 replicates of each cultivar, and an additional three replicates of non-inoculated control plants. On 2 August 2015, vines were assessed for incidence and severity of ALS symptoms by measuring and recording the area of necrotic lesion on leaves. The experiment was repeated on 8 June 2015, with vines assessed for foliar symptoms on 3 August 2015. Data were subjected to analysis of variance and least significant difference (LSD) at the 5% level was used for all pairwise comparisons.

6.3 Results

Symptoms of ALS started to develop within 20 days of inoculation and over the following 5 weeks lesions expanded to different extents on leaves of different cultivars (Figure 6.1e-h). Statistical analysis revealed that there was no difference between experiments, so data was combined and is presented in Figure 6.2. Results showed that all cultivars could be infected by *P. tetraspora*, but there were differences in severity of symptoms between cultivars. Riesling was the most susceptible cultivar of those evaluated, significantly more so than the susceptible control cv. Chancellor. The least susceptible cvs were Merlot, Shiraz and Chardonnay, being significantly less susceptible than cvs Sauvignon Blanc, Chancellor and Riesling.

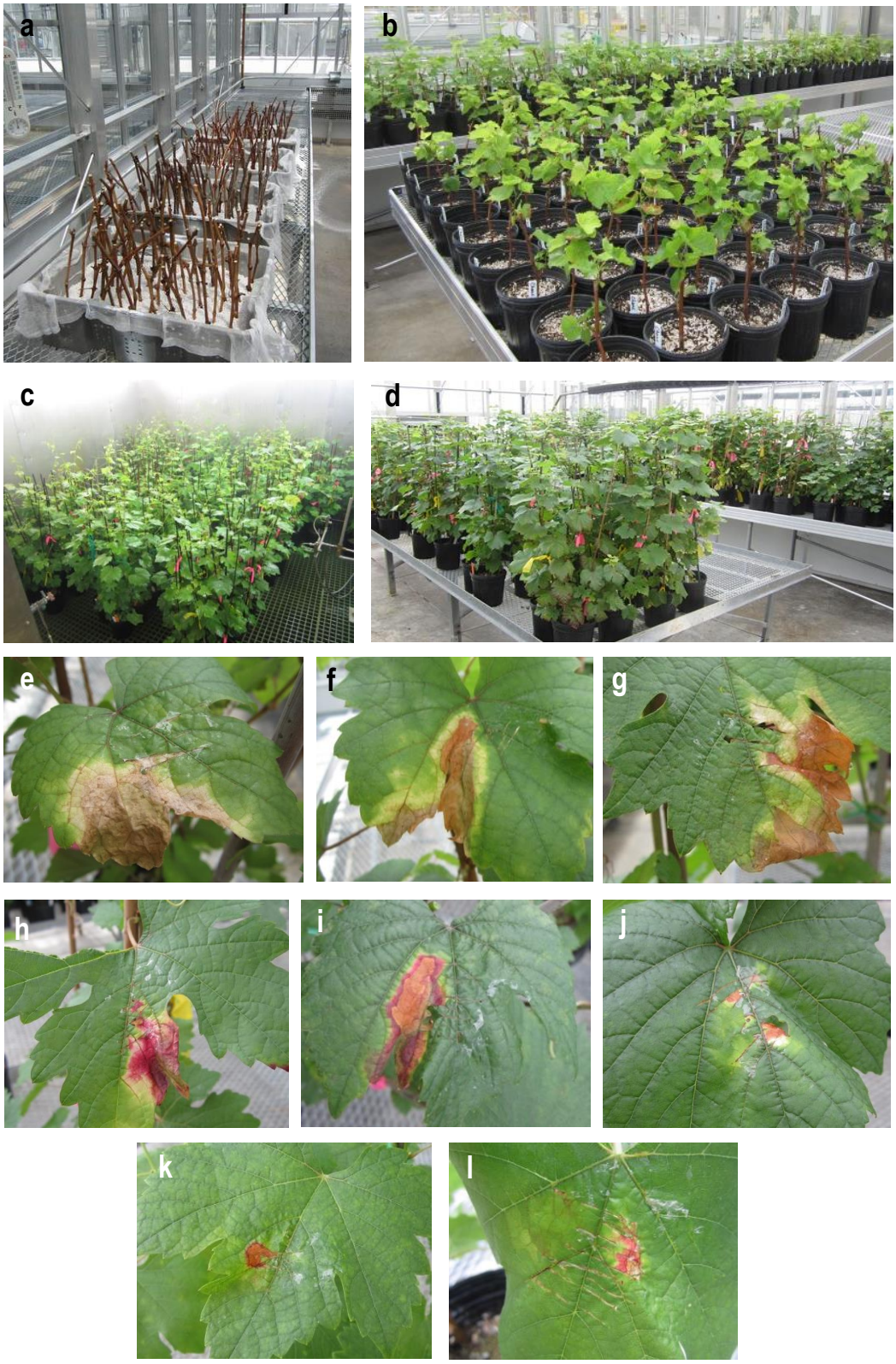

Figure 6.1. Vine propagation in greenhouse at Cornell University New York State Agricultural Experiment Station in Geneva, NY for cultivar susceptibility trial. Canes were callused and rooted in perlite (a) and then transplanted into pots of soil (b). Potted vines were placed in a humidity chamber (c) following inoculation with *Pseudopezicula tetraspora*, and then transferred to a greenhouse (d). Angular leaf scorch symptoms 6 weeks after inoculation on leaves of cultivars; Riesling (e), Chancellor (f), Sauvignon Blanc (g), Cabernet Sauvignon (h), Pinot Noir (i), Chardonnay (j), Shiraz (k) and Merlot (l).

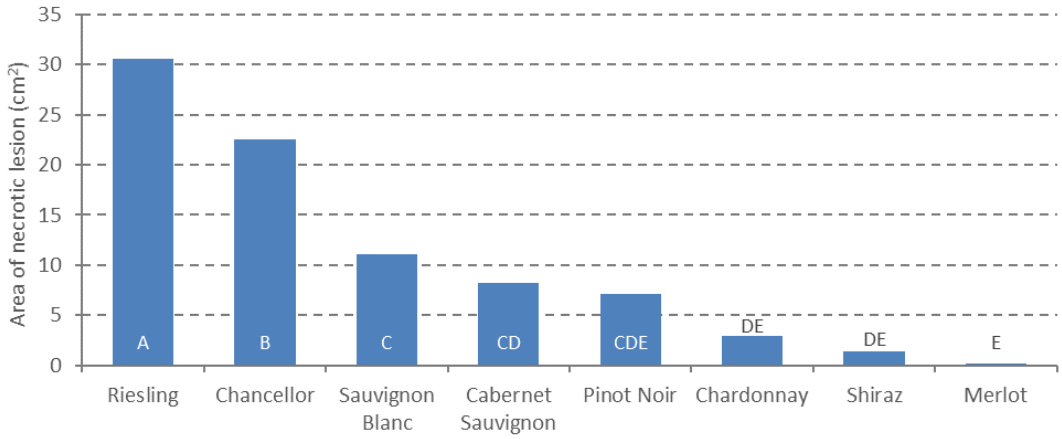

Figure 6.2. Differences in susceptibility to infection by *Pseudopezicula tetraspora* between cultivars of *Vitis vinifera* and the susceptible interspecific hybrid cv. Chancellor as determined by angular leaf scorch foliar symptom lesion area. Values with the same letter are not significantly different from one another (*P* = 0.05).

6.4 Discussion

These greenhouse experiments have revealed that the *V. vinifera* cultivars most commonly grown in Australia can be infected by *P. tetraspora*. Furthermore, cv. Riesling developed more severe symptoms than cv. Chancellor, which is known to be highly susceptible to ALS (Pearson *et al.* 1988). In the same study, Pearson *et al*. (1988), categorised Riesling as only moderately susceptible, which could perhaps due to the use of mycelium for inoculum in this study rather than spores, as occurs naturally. This research highlights the risk that an incursion of ALS might have in Australia, given the high to moderate susceptibility cultivars that make up a large proportion of the total vineyard planting.

Observation of ALS symptoms in a commercial vineyard in New York State in 2015 also revealed that cv. Riesling was more susceptible than cv. Cabernet Franc. Of 100 vines counted in two rows, 30 Riesling vines had ALS symptoms, while, in two adjacent rows of Cabernet Franc, under the same management regime, and similar age, only two vines had symptoms. This further confirms the high susceptibility of Riesling.

7 Fungicide evaluation

7.1 Introduction

Fungicides have been evaluated for control of angular leaf scorch (ALS) and benomyl was shown to be the most effective (Pearson *et al.* 1988). However, benomyl is no longer available for use on grapevines in Australia. Mancozeb was also reported to have moderate efficacy and would require regular applications from early shoot growth through to flowering for effective control. Captan was shown to be ineffective. As this research is 30 years old, it is important to evaluate the efficacy of fungicides currently used in Australian viticulture to prepare the Australian wine industry for containment and eradication of the disease in the event of an incursion. A series of laboratory and greenhouse experiments were conducted at the Cornell University New York State Agricultural Experiment Station in Geneva, NY, to evaluate fungicides for control of ALS.

7.2 *In vitro* experiment

7.2.1 Methods

An *in-vitro* experiment was established in 2014 using *P. tetraspora* (ATCC 62299) grown on ¼ strength PDA amended with six fungicides representing three different chemical activity groups (Table 7.1) at a range of concentrations; 10, 1, 0.5, 0.25, 0.125, and 0.0625 ppm a.i. salicylhydroxamic acid (SHAM) was added to the agar amended with trifloxystrobin and pyraclostrobin at 1 ml/L agar, in order to inhibit bacterial growth. The experiment included non-amended control plates and was set up as a randomized block design with 4 replicates. On 3 July 2014, a 4-mm plug of actively growing mycelium of *P. tetraspora* was placed on the agar in each plate and incubated under fluorescent lights (16/8 h light cycle) in the laboratory at approximately 22°C. Six weeks later, the diameter of cultures was measured, twice perpendicularly, and the mean diameter recorded for each plate. The experiment was repeated at the same time with cultures incubated under a separate bank of fluorescent lights. Data were subjected to analysis of variance and least significant difference (LSD) at the 5% level was used for all pairwise comparisons; standard error of the means was calculated.

Table 7.1. Fungicides evaluated for efficacy against *Pseudopezicula tetraspora* in *in-vitro* trials.

Fungicide group	Chemical family	Active ingredient
Demethylation inhibitors (DMI)	triazole	tebuconazole
		difenoconazole
		myclobutanil
Strobilurins (QoI)	oximino acetate	trifloxystrobin
	methoxy carbamate	pyraclostrobin
Succinate dehydrogenase inhibitor (SDHI)	pyridine carboxamide	boscalid

7.2.2 Results

Plates were inspected and photographed 3 weeks after commencement of the experiments (Figure 7.1). At this stage, trifloxystrobin and pyraclostrobin completely prevented growth of *P. tetraspora* at all concentrations, and all other fungicides less effective, preventing growth only at higher concentrations. Figure 7.2 shows the mean diameter of cultures 6 weeks after commencement of the experiments. Again, trifloxystrobin and pyraclostrobin completely prevented growth at all of the concentrations tested. Difenoconazole completely prevented growth at the two highest concentrations and tebuconazole only at the highest concentration, with all other concentrations inhibiting growth up to 77% relative to the control. Myclobutanil prevented growth at the highest concentration, but efficacy decreased exponentially as concentration decreased, inhibiting growth by between 90 and 3%. Boscalid had no significant effect except at the highest concentration (10 ppm) where it inhibited growth by 86%.

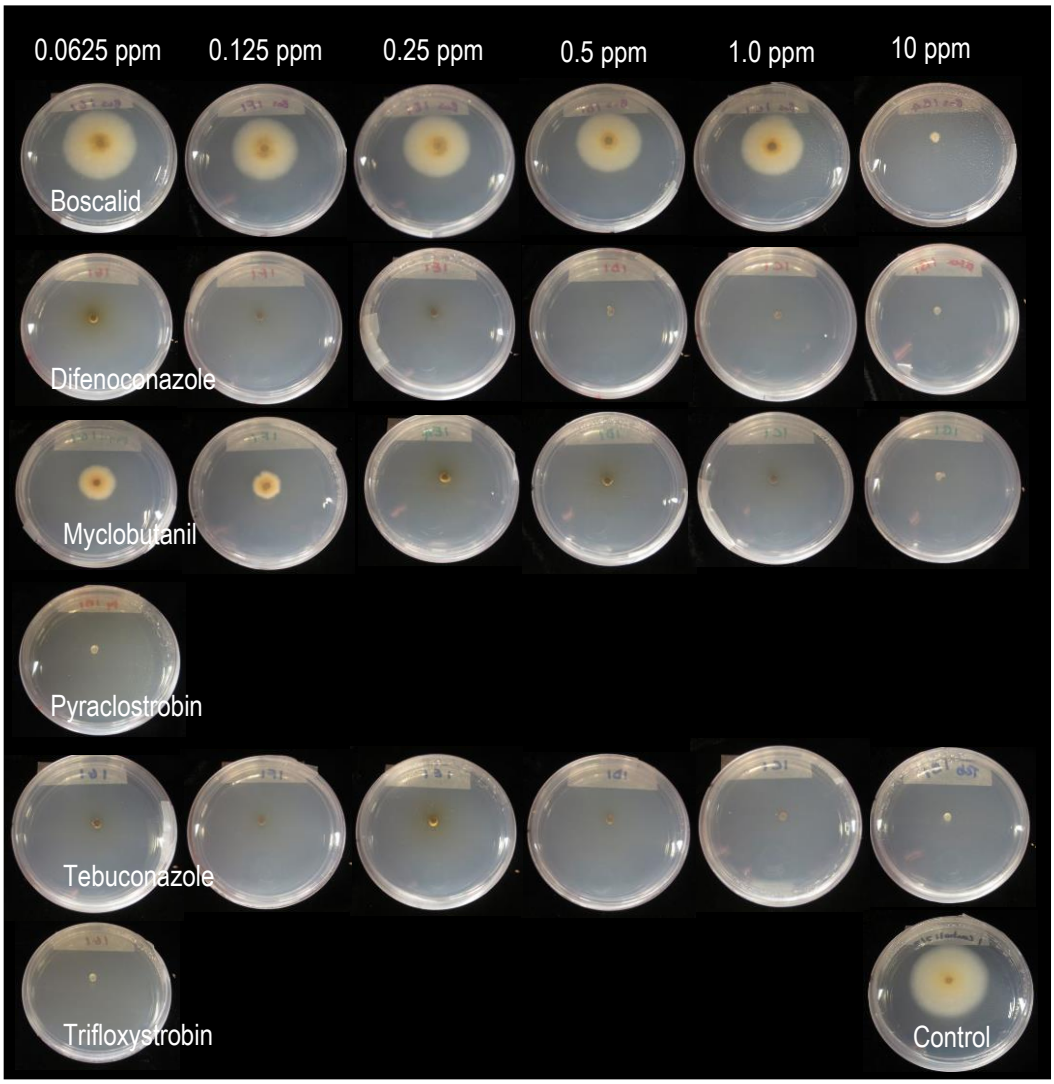

Figure 7.1. *In vitro* fungicide Experiment 1, colonies 3 weeks after plugs with mycelium were placed on ¼ strength PDA amended with various concentrations of fungicides. The unamended control is shown in the bottom right-hand corner.

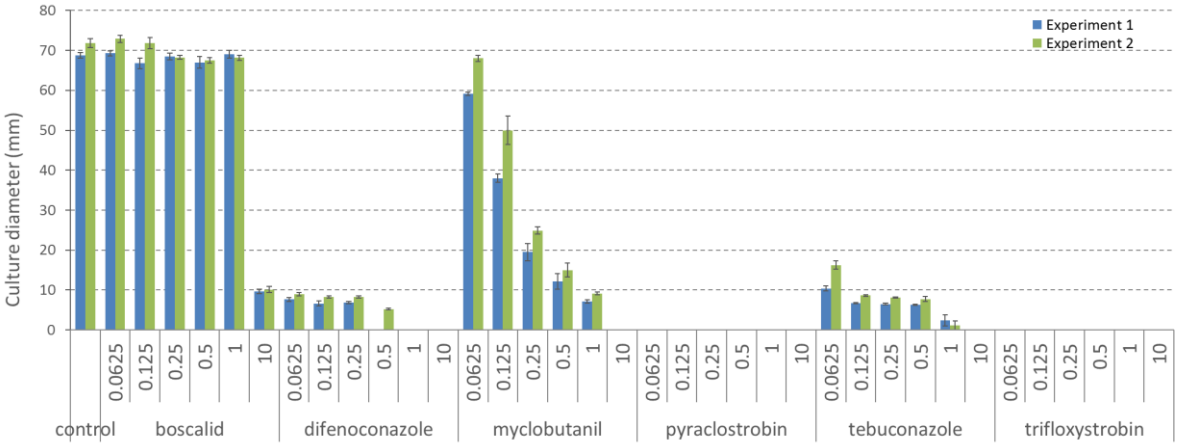

Figure 7.2. Diameter of *Pseudopezicula tetraspora* cultures grown for 6 weeks on ¼ strength PDA plates amended with fungicides at a range of concentrations (ppm a.i.). Bars represent standard error of the mean.

7.3 Potted vine experiments

7.3.1 Methods

Based on *in-vitro* experiments conducted in 2014 (Section 7.2) and current registration for grapes in Australia, the fungicides; trifloxystrobin, pyraclostrobin and tebuconazole were chosen for further evaluation in July 2015. Cv. Chancellor vines were propagated as described in Section 6.2. Three fungicide treatments (Table 7.2) were applied to potted vines with a hand sprayer (pressurised), in a fume extraction hood, separately, to prevent any cross-contamination. On the next day, two to four shoots on each vine were tagged and all leaves scratched and inoculated with *P. tetraspora* (ATCC 62299) as described in Section 5.3. Inoculated vines without fungicides applied were used as controls. Potted vines were placed into a mist chamber for 72 h (20°C, 14/10 h light cycle, constant mist), then returned to the greenhouse and monitored for disease symptoms. The experiment was arranged as a randomised block design with seven replications. The experiment was repeated on the same day, using a different mist chamber, and vines were placed in a different section of the greenhouse.

Table 7.2. Fungicides used in potted vine experiment.

Fungicide (USA)	Active ingredient	Product rate	Fungicide (Australia)
Elite 45 DF	450 g/L tebuconazole	28.66 g/100 L	Folicur
Flint 50 WG	500 g/L trifloxystrobin	15 g/100 L	Flint
Cabrio EG	200 g/kg pyraclostrobin	50 g/100 L	Cabrio

The experiments were conducted again in May 2016, using the highly susceptible cv. Riesling and with a revised experimental protocol in which plastic bags were used to cover each potted vine to prevent fungicide vapour activity interfering with adjacent treatments (Figure 7.3a).

The fungicides; trifloxystrobin, pyraclostrobin and tebuconazole, were applied with a hand sprayer (pressurised) on 10th May 2016 to potted vines (cv. Riesling). Leaves were inoculated with *P. tetraspora* (ATCC 62299) as described in Section 5.3, and untreated vines were included as controls. Each vine was then covered with a plastic bag secured around the base of the pot and placed in growth room at 22°C, 14 h light cycle, for 72 h prior to being returned to the greenhouse maintained at approx. 22°C, and bags removed. The experiment was arranged as a widely-spaced (approx. 1 m, Figure 7.3b) randomised block design with seven replications. The experiment was repeated on the same day, with the treated vines placed in a separate growth room, and then relocated to a different section of the greenhouse. The plants were continually monitored for ALS disease symptoms, with a final assessment eight weeks after establishment.

7.3.2 Results

In the first set of experiments that were conducted in 2015, no ALS symptoms developed on any of the control or treated plants.

In the second set of experiments, conducted in 2016, ALS symptoms had appeared on untreated control plants after 4 weeks (Figure 7.3c). After 8 weeks, ALS symptoms had developed on 94% and 100% of inoculated shoots on the untreated control plants in the first and second experiments, respectively. No symptoms were observed on any of the fungicide-treated vines (Figure 7.3d).

Figure 7.3. a) Potted vines in plastic bags in a growth room; b) potted vines positioned with wide spacing in a greenhouse; c) control (untreated) vine showing ALS foliar symptoms 4 weeks after inoculation and d) fungicide-treated vine with no symptoms 4 weeks after inoculation.

7.4 Discussion

In the initial potted vine experiments, conducted in 2015, symptoms were not observed on any of the control vines. It is likely that vapour activity of the fungicides used against *P. tetraspora*, inhibited development of ALS symptoms on the control vines, which had been placed in close proximity to the fungicide-treated vines in the misting chamber and greenhouse. Similarly, Kennelly *et al.* (2007) reported that grapes inoculated with the pathogen *Plasmopara viticola* (downy mildew) were controlled solely by mefenoxam fungicide vapour activity. In the 2016 experiments, to overcome the vapour effect, plants were individually bagged for 72 h prior to returning to the greenhouse, and then placed on benches with at least 1 m separation between vines. This resulted in symptoms developing on the untreated control vines, confirming the efficacy of fungicides on the symptomless, treated vines.

In addition, the interspecific hybrid cv. Chancellor, known to be highly susceptible to ALS (Pearson *et al*. (1988), was used in the 2015 experiments, whereas in the following year, cv. Riesling, shown to be even more susceptible than cv. Chancellor (Section 6), were used. This may have also contributed to the symptom development on controls in 2016.

Three fungicides; trifloxystrobin (Flint), pyraclostrobin (Cabrio) and tebuconazole (Folicur), each belonging to distinctive chemical groups that represent two different modes of action, and already used in Australia on grapevines, were shown to be effective in controlling ALS. This information will be valuable in assisting containment or eradication of ALS in Australian vineyards, should an incursion occur.

8 Drastic pruning for eradication

8.1 Introduction

A drastic pruning strategy, based on cutting trunks and removing all upper parts of the vine, was developed to eradicate 'surface' diseases, which affect only green tissue and/or fruit on grapevine. This was done using the model endemic black spot disease, caused by *Elsinoë ampelina*, in research undertaken in Australia (Sosnowski *et al*. 2012). The strategy was subsequently validated for the high-priority grapevine disease black rot, caused by the fungus *Guignardia bidwellii*, in research conducted in New York, USA, in collaboration with Cornell University. The standard strategy for eradication of an exotic plant pathogen is based on the removal of whole affected plants, followed by burning and/or burial. However, the drastic pruning strategy eliminates the need to completely remove vines, which can have significant economic and social impacts on the industry and communities, particularly with respect to older, high-value premium vineyards.

It is likely that a similar eradication strategy could be effective for other high-priority exotic diseases, such as angular leaf scorch (ALS) and Rotbrenner, based on similarities in biology of the causal fungi and their disease cycles. This research aims to validate the efficacy of drastic pruning for eradicating ALS.

8.2 Methods

A block of vines (approximately 40 years old) at the Robbins Research Farm, New York State Agricultural Experiment Station, Cornell University, Geneva, New York, USA (42°52'10.84"N, 77°02'46.57"W) was utilised for this trial (Figure 8.1). The same block was used for the previous project to validate the drastic pruning eradication strategy for black rot disease (Sosnowski *et al*. 2012). The block comprised vines of interspecific hybrid cultivars with varying susceptibility to infection by *P. tetraspora* (Table 8.1). Vines between plots and rows had been removed in the previous trial to provide spatial separation (of at least 4 m) between control and treatment plots (Figure 8.2).

Table 8.1. Interspecific hybrid grapevine cultivars used in the eradication trial and their relative susceptibility to angular leaf scorch (Pearson et al. 1988)

	Cultivar	Susceptibility
1	Concord	slight
2	Catawba	slight
3	Niagara	slight
4	Ives	slight
5	Foch	slight
6	Delaware	slight
7	Cayuga White	moderate
8	De Chanauc	extreme
9	Chancellor	extreme

In May 2014, all vines were cane pruned and trained to an 'Umbrella Kniffen' style trellis (Figure 8.3a) leaving approximately 50 buds per vine. Vines were maintained throughout the season with minimal herbicide and pesticide applications where necessary, avoiding the use of fungicides that may inhibit ALS symptom development.

On 18 July 2014, to simulate an incursion, all vines were artificially inoculated as follows. Four to six leaves on four tagged shoots per vine were scored using sharp forceps (Figure 8.3b). A slurry, consisting of actively growing *P. tetraspora* mycelia in agar mixed with sterile distilled water (see Section 5.3), was placed onto the wounded leaves using a spatula (Figure 8.3c), the leaves were sprayed with distilled water and the shoots covered in plastic bags (Figure 8.3d). The treated vines were then covered with 2 layers of reflective shade-cloth (Figure 8.3e). After 72 hs, the shade-cloth and bags were removed from the vines.

Figure 8.1. Robbins Research Farm trial plot at the Cornell University New York State Agricultural Experiment Station

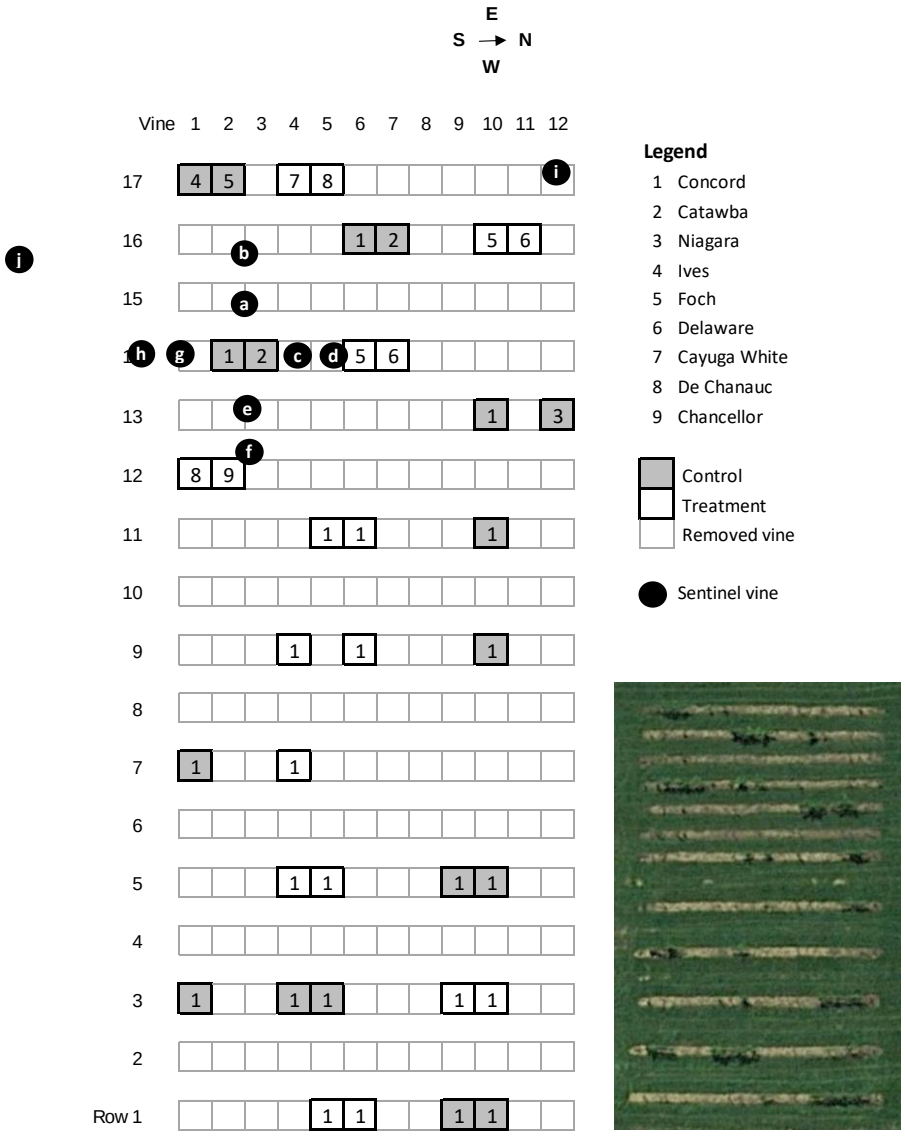

Figure 8.2. Eradication field trial plots located in a vineyard block with various interspecific hybrid cultivars at the Robbins Research Farm, Cornell University New York State Agricultural Experiment Station. Treatment vines were subjected to drastic pruning, control vines were pruned to 'Umbrella Kniffen' style trellis and sentinel vines comprised potted vines (cv. Chancellor in 2015 & 2016; cv. Riesling in 2017). Bottom right is a satellite image of the trial (Google Earth).

Figure 8.3. Angular leaf scorch trial eradication trial in 2014. a) Trial vines trained as 'Umbrella Kniffen' style trellis, b) scraping grapevine leaves with sharp forceps, c) applying mycelial inoculum to grapevine leaf with spatula, d) bags on inoculated shoots, e) inoculated vines under shade cloth.

Vines were assessed for symptoms on 19 August 2014 (Rows 1-11, cv. Concord) and 19 September 2014 (Rows 12-17, mixed cultivars). Each symptomatic leaf was rated for severity using a scale where 0 = no expanding necrosis beyond the inoculation injury sites; 1 = small expanding lesion or lesions, up to 1 cm beyond inoculation site; or 2 = lesion/s expanding > 1 cm beyond the inoculation site.

At the end of September 2014, symptomatic leaves were collected from the control vines and taken to the laboratory for storage. In December 2014, following leaf-fall, when the vines were dormant, the collected symptomatic leaves were secured in wire-mesh holders and placed under the control vines, to simulate infected leaves falling to the ground beneath the vines, ensuring an inoculum source for re-infection in 2015, mimicking the typical disease cycle (Figure 8.4a).

In May 2015, the eradication protocol was performed on the treatment vines by cutting each vine at the crown with loppers or a hand saw, leaving only an unbranched trunk approximately 1 m high (Figure 8.4b). Control vines were subjected to the standard pruning regime described above (Figure 8.4a). Plots were arranged in a randomized block design, with individual or pairs of vines allocated to either control or eradication treatment (Figure 8.2).

All vines were monitored for ALS symptoms throughout the season. Early leaf symptoms were observed in July and leaf samples were removed for re-isolation in the laboratory using methods described in Section 5.2.

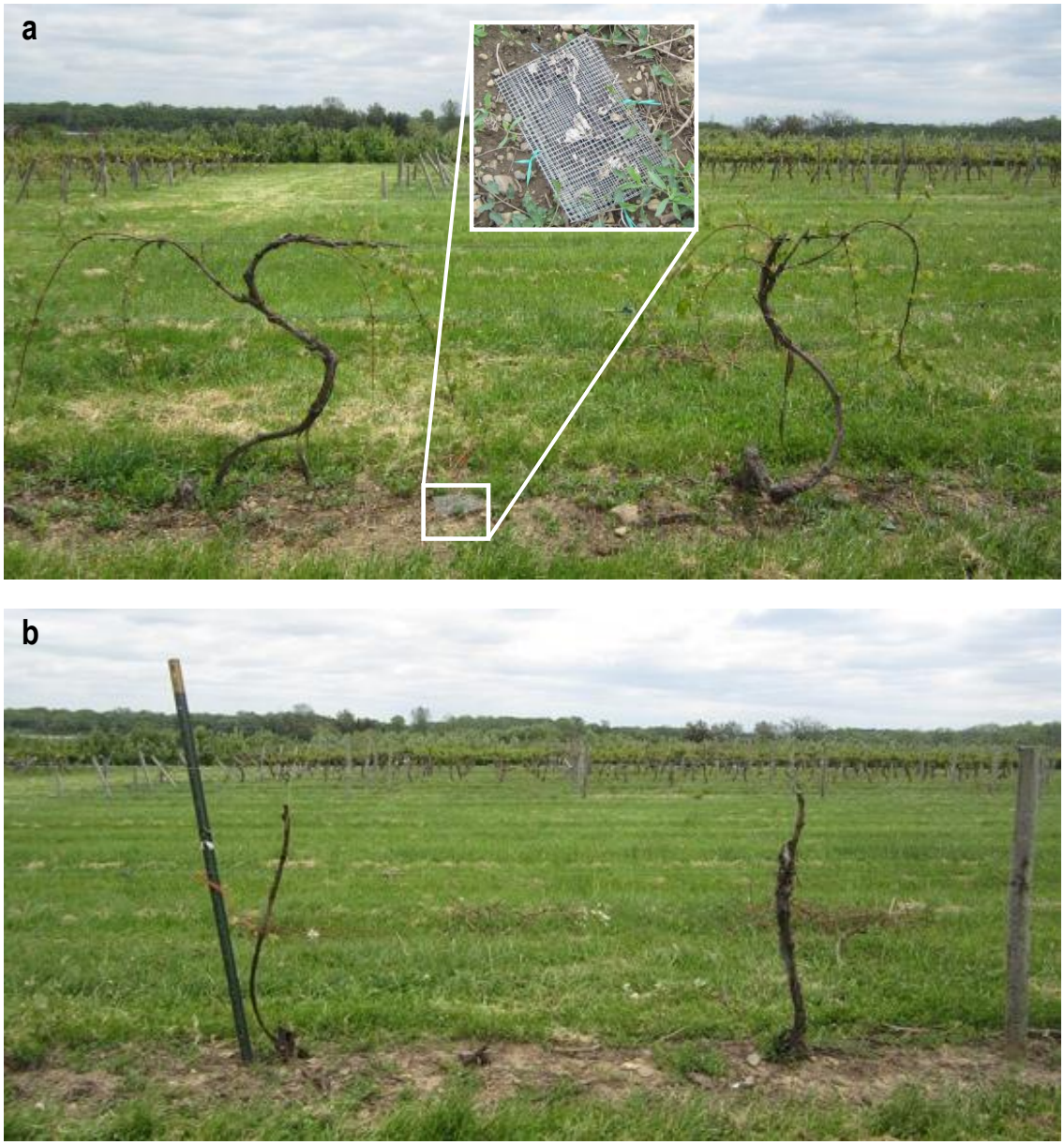

Figure 8.4. Angular leaf scorch trial eradication trial in 2015. (a) Wire cage containing dried, symptomatic leaves placed beneath control vines and (b) vines subjected to drastic pruning treatment.

A final assessment was made on 21 September 2015 by counting the number of symptomatic leaves per vine. At the end of September 2015, the symptomatic leaves were removed from the vines and taken to the laboratory for storage. In December 2015, symptomatic leaves were placed in wire holders underneath the vines to simulate the typical disease cycle, as described above.

In May 2016 and 2017, all vines in the field trial were subjected to the standard pruning regime and maintained throughout each season, as described above. In December 2016, symptomatic leaves collected from greenhouse trials were placed in wire holders underneath the control vines to simulate the typical disease cycle. All vines were monitored for ALS symptoms throughout each season and a final assessment was undertaken on 16 October 2017 by counting symptomatic leaves.

Sentinel vines

To monitor for spore movement between plots and from external sources, 10 potted sentinel vines (cv. Chancellor in 2015 & 2016; cv. Riesling in 2017, propagated as described in Section 6.2) were placed for three discrete 6-7-week periods per growing season at strategic positions within the experiment site, radiating at approximately 2-m intervals in different directions from a selected control plot (Figure 8.5, row 14, vines 2 and 3), with two additional pots placed peripherally, at least 3 m from any control vines in the

experiment (Figure 8.2). Before placement in the trial, potted vines were maintained in the greenhouse, isolated from any vineyards that might serve as a source of *P. tetraspora* inoculum, and new sets of 10 vines were placed at the experiment site for each monitoring period. At the end of each period, sentinel vines were returned to the outdoor nursery.

Figure 8.5. Sentinel vines (—) placed strategically around a control plot (X), radiating at approximately 2-m intervals in different directions, in the ALS eradication trial.

8.3 Results

In 2014, ALS symptoms became apparent on leaves within 3 weeks of inoculation, and continued to develop throughout the season (Figure 8.6a-b). Disease severity ratings conducted on 19 August (Rows 1-11, cv. Concord) and 19 September (Rows 12-17, mixed cultivars) showed that symptoms were evenly spread between control and treated plots, with similar percentages of inoculated leaves recorded for each category (Table 8.2).

Table 8.2. Disease assessment for plots designated as either control or eradication treatments following inoculation in 2014, showing mean percentage of inoculated leaves in each disease assessment category (0 = no expanding necrosis beyond inoculation site; 1 = small expanding lesion/s up to 1 cm beyond inoculation site; 2 = lesion/s expanding >1 cm beyond inoculation site.

		Assessment category		
Vine plot	Treatment	0	1	2
Concord	control	58	29	13
	treated	60	33	7
Mixed cultivar	control	52	6	42
	treated	43	11	46

In 2015, faint chlorotic patches began to appear by mid-June on two field trial vines indicating early ALS foliar symptoms. By early July 2015, necrotic lesions typical of ALS were observed on a total of 10 leaves, on seven different vines. Symptoms continued to develop and by the end of July, a total of 37 leaves on 10 of the 12 control plots were observed to have advanced ALS symptoms (Figure 8.6c). In the final assessment on 21 September 2015, 42 leaves were recorded with ALS symptoms on 11 of the 12 control plots in the trial. No symptoms were recorded on any of the treated or sentinel vines. The mean number of symptomatic leaves per vine for treated and control vines is shown in Figure 8.7.

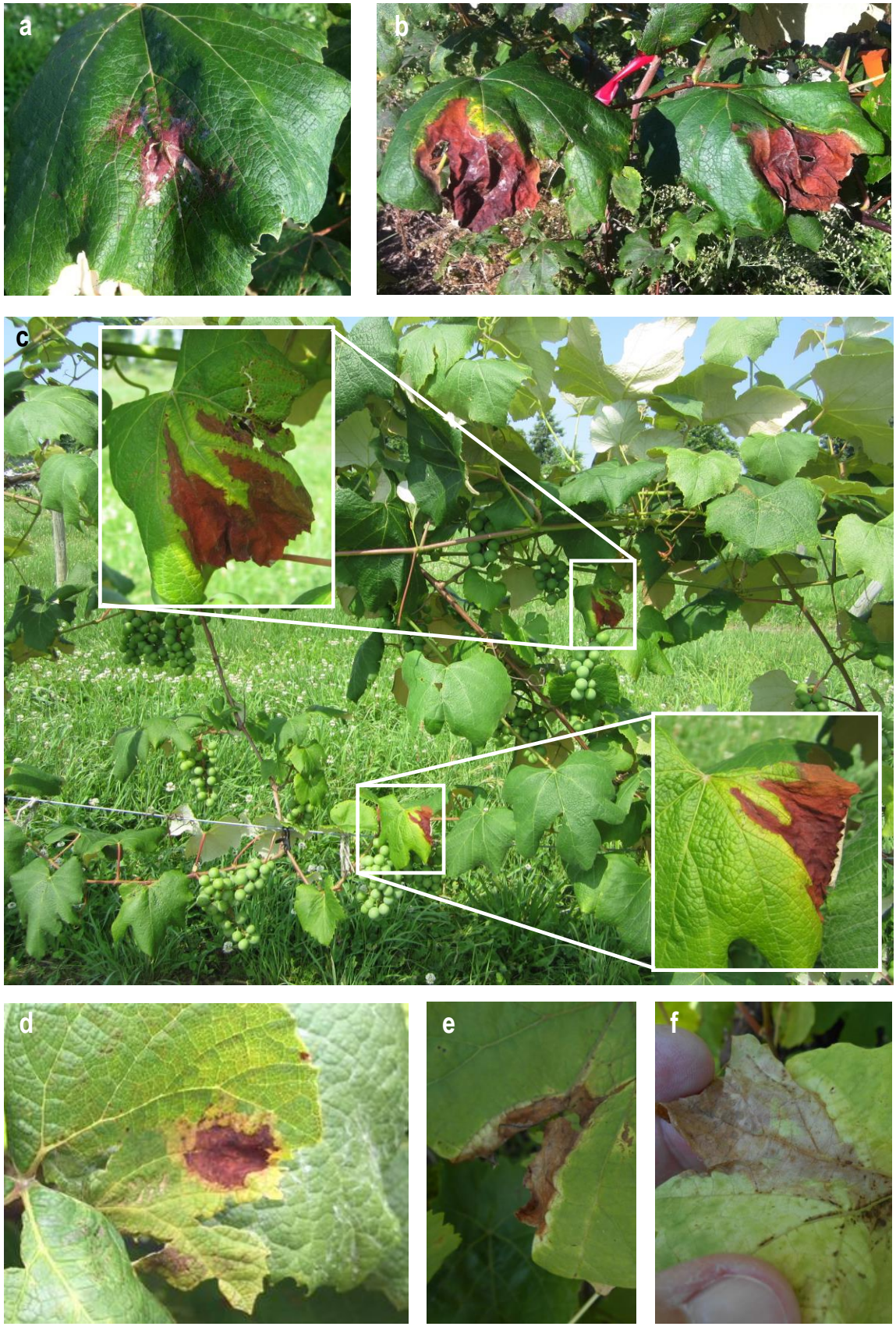

Figure 8.6 Angular leaf scorch foliar symptoms on cv. Concord in the field trial following inoculation with *Pseudopezicula tetraspora* in 2014 (a-b), in controls during 2015 (c) and 2017 (d). Symptoms observed on sentinel vines (cv. Riesling) in June 2017 (e-f).

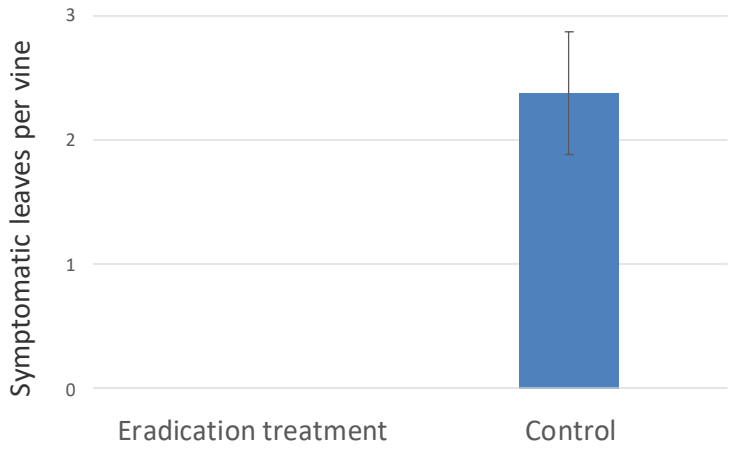

Figure 8.7. Mean number of leaves per vine with angular leaf scorch symptoms on 21 September 2015 in the eradication trial at the Robbins Research Farm.

In 2016, north-east USA experienced a severe drought which led to conditions not conducive for *P. tetraspora* to infect, compared with the two previous years (Table 8.3). Vines in the trial were monitored throughout the season but no symptoms developed. In 2017, precipitation was above average. Vines in the trial were monitored regularly, and very few symptoms were observed. On 16 October 2017, leaf symptoms (Figure 8.6d) were recorded on only two control vines: Row 3, Vine 5 (three leaves) and Row 5 Vine 10 (one leaf). No symptoms were recorded on any of the treated vines.

Symptoms resembling ALS were recorded on five sentinel vines (cv. Riesling), located in positions c, d, e, f and g (Figure 8.2), located in the trial from 23 May to 5 July 2017. Symptoms were first observed on 20 June, and photos were taken on 3 July (Figure 8.6e-f).

Table 8.3. Precipitation (mm) over the summer months for Geneva, NY USA (www.usclimatedata.com)

	2014	2015	2016	2017	Average
June	98	222	22	97	93
July	186	117	39	172	88
August	91	67	67	85	77
Summer total	**374**	**407**	**128**	**354**	**258**

8.4 Discussion

The validation trial undertaken in New York demonstrated the efficacy of the eradication protocol for ALS on grapevines. An inoculation technique that was developed at the beginning of this project using a mycelial slurry (Section 5.3) successfully simulated an incursion of ALS in the trial block. Subsequent assessment of the vines near the end of the season confirmed an even distribution of symptoms across control and treatment vine plots.

In the first season following the eradication, when environmental conditions were conducive for ALS according to precipitation records in the region, symptoms were recorded on most control vines, but not on vines that were subjected to the drastic pruning treatment. In the second season, no symptoms developed on any vines, control or treated, due to the drought conditions experienced in that summer. In the third summer season, precipitation was above average, but symptoms were recorded only on two control vines, and not on treated vines. The low levels may be attributed to the reduced inoculum load due to the lack of infection that occurred in 2016 and the prevalence of cv. Concord in the trial, which has previously been reported as only slightly susceptible. However, this result confirms the success of the eradication strategy, 3 years after it was implemented.

ALS symptoms were not observed on sentinel vines in the first two years of the experiment, suggesting no disease spread between plots or from vines beyond the trial in 2014, and the non-conducive weather conditions in 2015. In 2017, five sentinel plants were recorded with ALS symptoms, when no symptoms were recorded on the respective control vines that they surrounded. This may be due to the use of the highly susceptible cv. Riesling as sentinels in 2017 compared to the use of cv. Chancellor in the previous two years, which was shown in this study to e less susceptible to ALS (Section 6).

If an incursion of ALS occurred in Australia, the drastic pruning protocol has the potential to save the wine industry many millions of dollars in lost production and vineyard re-establishment costs. The eradication protocol will be included in the Viticulture Industry Biosecurity Plan, in addition to the protocol already published for black rot disease (Sosnowski *et al.* 2012). It is also highly likely that the drastic pruning strategy will be effective for Rotbrenner disease (*P. tracheiphila*), given the close relationship between the causal pathogens and their respective disease cycles.

9 Outcomes and recommendations

9.1 Develop diagnostic protocol for angular leaf scorch

A diagnostic protocol for angular leaf scorch will be developed and submitted to Subcommittee on Plant Health Diagnostics (SPHD) for national endorsement.

Preliminary experiments were undertaken at the beginning of the project in order to develop a diagnostic protocol for ALS, which provided an opportunity for development of methods for subsequent laboratory, greenhouse and field experiments.

Attempts to induce development of fruiting bodies and sporulation on ALS symptomatic leaves (Pearson *et al.* 1988) collected from vineyards were unsuccessful. Similarly, attempts to isolate *P. tetraspora* from leaves were also unsuccessful. It is likely that the intensive fungicide spray program applied in commercial vineyards may have reduced the viability of the fungus in infected leaves.

Due to the inability to produce spores as an inoculum source, methods were developed for making a mycelial slurry from *P. tetraspora* cultures grown *in vitro.* This proved to be effective for infection of vines, providing inoculum to conduct subsequent greenhouse experiments and field trials.

A technique described by Pearson *et al.* (1988) provided confirmation of *P. tetraspora* in inoculated leaves from field trials in this project. Documentation of the morphology of the reference isolate *P. tetraspora* (ATCC 62299) on several artificial culture media provides a means of identifying suspected cultures. Furthermore, DNA sequencing and subsequent submission to GenBank provides a reference for diagnosis. A diagnostic protocol for ALS has been drafted and submitted to the Subcommittee on Plant Health Diagnostics from the Commonwealth Department of Agriculture and Water Resources, and once fully endorsed will be included in the Viticulture Industry Biosecurity Plan.

9.2 Determine susceptibility of *Vitis vinifera* cultivars to angular leaf scorch

The susceptibility of the main *V. vinifera* cultivars grown in Australia to angular leaf scorch will be screened in a greenhouse to establish the likely impact on Australian vineyards in the case of an incursion.

Greenhouse experiments revealed that the seven cultivars most widely planted in Australia can be infected by *P. tetraspora.* Riesling was extremely susceptible, Sauvignon Blanc, Cabernet Sauvignon and Pinot Noir moderately susceptible, and Chardonnay, Shiraz and Merlot slightly susceptible. This highlights the threat that an incursion of ALS presents to Australia, and provides valuable information for effective monitoring and surveillance should an incursion occur.

9.3 Evaluate fungicides for control of angular leaf scorch

The efficacy of fungicides on angular leaf scorch will be evaluated to provide alternative strategies to respond to an incursion.

Three fungicides; trifloxystrobin (Flint), pyraclostrobin (Cabrio) and tebuconazole (Folicur), belonging to distinctive chemical groups that represent two different modes of action, were shown to be effective at controlling *P. tetraspora*. These fungicides, already registered for use on grapevines in Australia, can be used for control, containment or eradication of ALS in Australian vineyards, so will better prepare the wine industry in the case of an incursion.

9.4 Validate efficacy of drastic pruning for eradicating angular leaf scorch

The drastic pruning protocol developed for eradicating black rot will be evaluated as a means of eradicating angular leaf scorch, and if successful included in the Viticulture Industry Biosecurity Plan.

A drastic pruning strategy, previously validated for black rot disease, eliminates the need to completely remove vines, providing significant benefit to industry in the case of an incursion. In a simulated incursion of *P. tetraspora* in NY, a similar eradication strategy was evaluated for ALS. In the first season following the eradication, symptoms were recorded on control vines, but not treated vines. By the third season, again symptoms were only recorded on a few control vines, but no treated vines, confirming the success of the eradication strategy for ALS.

In the event of an incursion of *P. tetraspora* in Australia, the drastic pruning protocol has potential to save the wine industry many millions of dollars in lost production and vineyard re-establishment costs. The eradication protocol will be published and included in the Viticulture Industry Biosecurity Plan, along with the protocol already published for black rot disease (Sosnowski *et al.* 2012). It is also highly likely that the drastic pruning strategy will be effective for Rotbrenner disease (*P. tracheiphila*), given the close relationship between the causal pathogens.

9.5 Increase the biosecurity capability of the Australian viticulture industry

Australian researchers will gain expertise in the diagnosis, eradication and management of angular leaf scorch and build on the collaborative partnership with Cornell University.

This project has strengthened collaboration with researchers at Cornell University, who are world authorities on management of grapevine diseases. It has provided training for Australian researchers and greatly boosted the expertise of scientific personnel, who would be at the forefront of efforts to deal with an incursion of angular leaf scorch in Australia. The project has provided much new information on ALS regarding cultivar susceptibility and fungicide control, and also delivered both diagnostic and eradication protocols, which will be included in the Viticulture Industry Biosecurity Plan, to ensure industry preparedness.

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11 Communication

11.1 Conference papers

Ayres M, Wilcox W and Sosnowski M (2016) Angular leaf scorch, biosecurity implications for Australia. Poster 120 - The 16th Australian Wine Industry Technical Conference, Adelaide Convention Centre, SA, 24-28 July 2016. P 76.

11.2 Industry journals and articles

Ayres M, Sosnowski M and Wilcox W (2015) Investigating angular leaf scorch disease in the USA and implications for Australian biosecurity. *Wine and Viticulture Journal* 30(4), 49-50.

11.3 Presentations

Ayres M, Wilcox W and Sosnowski M (2017) Angular leaf scorch of grapevine: biosecurity implications. SARDI Seminar Series, Plant Research Centre, Urrbrae, 16 November 2017.

12 Staff

Project leader/supervisor: Mark Sosnowski

Principal investigator: Matthew Ayres

Collaborator and advisor: Wayne Wilcox

Cornell University technical assistance: Judy Burr

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