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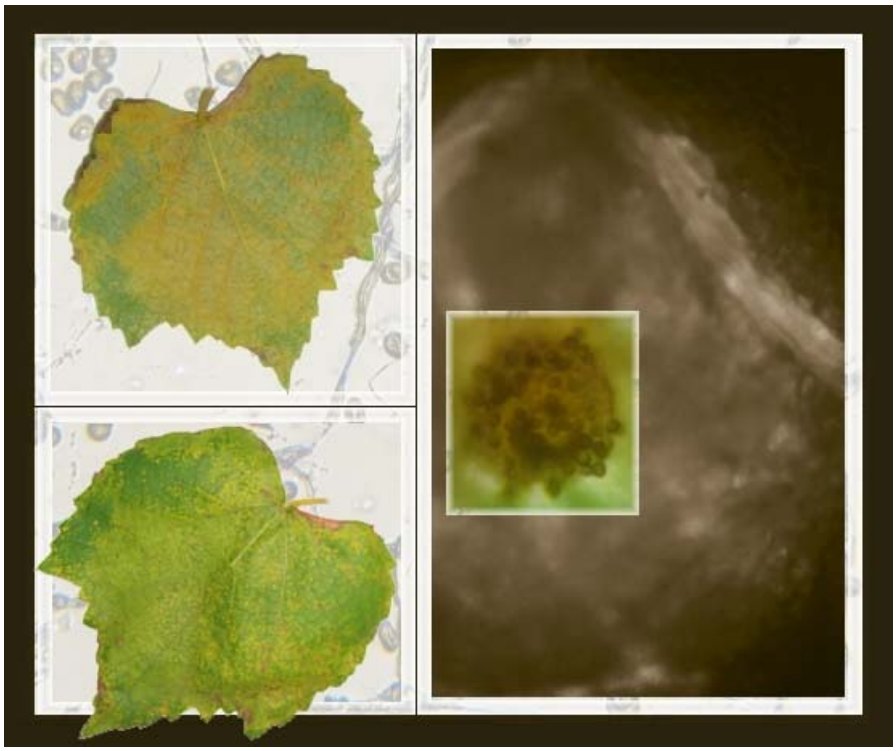
Grapevine Leaf Rust Project



FINAL REPORT

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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Research Organisation: *Northern Territory Department of Primary Industry, Fisheries and Mines*

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Grapevine Leaf Rust - Assessment of Cultivars for Resistance or Immunity and Fungicides Useful for control (Project1B) and Molecular Characterisation, Host Range and Biological Studies (Project 1C)

Andrew Daly & Chelsea Hennessy

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Abstract

411 genotypes of *Vitis* spp. were exposed to *P. euvitis* in the laboratory with four being moderately resistant or better. 91 unidentified *Vitis* spp. from suburban backyards and native Vitaceae plants (*Ampelocissus acetosa* and *A. frutescens*) all tested susceptible or highly susceptible.

Fungicidal control was assessed using 21 different products. 11 were effective in preventing infection and six reduced symptoms as post-infection treatments.

The optimal temperature for spore germination was 22°C. Latent period (of infection) in *Vitis* spp. leaves was shortest (six days) at 25°C. The optimal temperature for pustule production and sporulation was between 20°C and 21°C whilst six hours of leaf wetness significantly increased symptom development.

A protocol for identification of *P. euvitis* via PCR-RFLP analysis was successfully adapted and optimised for detection of the fungus at the Berrimah Farm Veterinary Laboratory.

There was no measurable difference between the two types of vine (*Vitis* spp. and native *Ampelocissus* spp.) in the microclimate of their canopies during a six-month period.

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Executive Summary

This research was conducted in response to an incursion of the Grapevine leaf rust pathogen, *Phakopsora euvitis,* in Darwin. It set out to enhance the prospects of eradication, protect against re-incursion and enable authorities to be more prepared for response to an incursion outside the Darwin quarantine zone (particularly in commercial grape growing areas).

Sources of resistance in cultivated and experimental selections of *Vitis* spp. were investigated for a potential suburban vine replacement program. This was viewed as an important quarantine measure because under the existing legislation, that covers exotic plant disease incursions (*Plant Diseases Control Act*, 1979), only diseased vines could be removed. In addition, many unidentified household vines and native plants within the grapevine family (Vitaceae) that hadn't succumbed to the disease were tested for their resistance. Chemical control was investigated to identify products that could be effectively used in the event that the disease was discovered outside the quarantine area. Also, a protocol for identification by DNA analysis was optimised and implemented and an effort to better understand disease behaviour was made by examining environmental influences on the pathogen and microclimate characteristics of cultivated vines (*Vitis* spp.) in backyards and bushland native vines.

Out of a total of 411 grapevine genotypes (principally *V. vinifera* obtained from CSIRO, Merbein and SARDI, Nuriootpa) four; namely '41 B', 'Aurora', 'Siebel 128' and '554-5 seedlings', showed moderate resistance or better. None was completely resistant or immune. 91 plants of local origin screened were found to be either susceptible or highly susceptible. Screening of native grapevines revealed that *Ampelocissus acetosa* and *A. frutescens* were as susceptible to the disease as the majority of the *Vitis* spp. However, *Cissus adnata* and *Cayratia maritima* were completely resistant.

Fungicide testing identified 11 products that could protect leaves from infection. Although none were able to completely prevent sporulation of the rust once infection had occurred, six products showed a high level of eradicative ability.

The DNA test to identify *P. euvitis* (published by Ono and Imazu 2001) was successfully adapted and optimised for use in Darwin to allow confirmation of the species in the event that a rust disease is found affecting grapevines in other locations.

Manipulation of temperature and leaf wetness in the laboratory was conducted to assess spore germination, infection, latent period and sporulation. It was found that the optimal temperature for spore germination was around 22°C. Symptoms of disease following infection (latent period) could appear as fast as 6 days at 25°C and six hours of leaf wetness significantly increased disease severity. Disease was most severe and spore production highest when infected leaves were exposed to temperatures between 20°C and 21°C.

In general, this work has generated a broad understanding of the susceptibility of cultivated *Vitis* species and other species of Vitaceae to Grapevine leaf rust and climatic conditions favourable for disease development. The discovery of a native alternative host influenced the sampling strategy and scope of the National Grapevine Leaf Rust Eradication Program (NGLREP), significantly enhancing its effectiveness. Numerous fungicides for effective disease control have been identified. In practical terms this information, together with a reliable DNA based test for identification, will enable a more efficient response to an incursion of the disease outside of the Darwin quarantine zone, increasing the chances of successful eradication or maintaining the disease free status of particular grape growing areas. An enhanced understanding of the pathogen and a more prepared position to deal with future incursions could be achieved by studies into the potential pathways of entry into the country as well as spread towards southern grape growing areas once established. Development of a test that can detect the presence of the pathogen in symptomless leaf tissue would also be very beneficial.

Background

Grapevine leaf rust (GLR) was discovered in Darwin in July 2001 (Weinert *et al.* 2003). The disease, caused by the fungus *Phakopsora euvitis*, had not been detected in Australia previously. As a result, the National Grapevine Leaf Rust Eradication Program (NGLREP) was initiated to eliminate the incursion. Delimiting surveys of areas in the NT between rural Darwin and Ti Tree (approximately 1100 km south of Darwin where table grapes are produced) and all other Australian states have determined that they are free of the disease. Grapevines are principally grown in Darwin within the Greek community for culinary purposes and there are no commercial vineyards in the region.

The disease is common in both Asia and Central America and can be very destructive if not controlled (Leu 1988). Spores of the pathogen are windborne and are presumed to have a high potential for spread by humans and machinery. They can cause disease over a wide range of temperatures and there is a risk that disease could establish in the majority of Australia's viticulture regions.

Previously all rust disease occurring on species of Vitaceae was attributed to one organism, *Physopella ampelopsidis*. However, recent studies by Ono (2000) determined that disease was a result of a complex of three distinct species, each limited to separate host genera. Within Vitaceae, *P. euvitis* was found to infect *Vitis* spp. only. Potentially, accurate studies into the diseases are lacking as a result of the previous taxonomic confusion.

This report presents the results of research into many aspects of *P. euvitis* including pathogen host range, identification, disease control and factors contributing to disease development. However the primary focus of the research was to screen germplasm of *Vitis* spp. as widely as possible to identify immunity to the pathogen.

Project Aims and Performance Targets

1. Conduct a mass screening of cultivated (including rootstock) and native grapevines to determine their reaction to *P. euvitis*	12-2003
2. Assess apparently non-susceptible grapevine material for resistance or immunity to the disease	03-2004
3. Identify grapevine material of unknown parentage shown to be resistant or immune to *P. euvitis*	06-2004
4. Assess fungicides for protective or eradicative action against *P. euvitis*	09-2003
5. Assess the effects of temperature and leaf wetness duration on disease development.	12-2003
6. Investigate the potential to differentiate strain(s) of *P. euvitis* present in Darwin from each other and from strain(s) in other countries by DNA analysis	09-2003
7. Develop the capacity to identify GLR by DNA analysis	08-2005
8. Determine the host range of GLR in Australian native *Vitaceae* and *Leeaceae*	08-2005
9. Investigate the biology of GLR and grapevines in the field	08-2005
10. Conduct a field assessment of fungicides in East Timor	07-2006

Methods

Laboratory Experiments

These experiments were conducted to assess the influence of disease resistance, temperature and leaf wetness on the pathogen, fungicidal efficacy and DNA identification. The trials were conducted in a secure laboratory environment to minimise the risk of compromising the eradication program. Leaves for the experiments were produced by disease free cuttings of numerous genotypes of *Vitis* spp. supplied by SARDI (Nuriootpa) and CSIRO (Merbein) grown in containers of potting mix in a shade-house at Berrimah Farm (Darwin).

- *In vitro* Culture:

Laboratory experiments which involved infecting grapevines were based on detached leaf culture. The method used was similar to that of Washington (1987). Leaves were cut into discs, placed on moist cotton wool pads with the under (abaxial) surface facing upwards and sealed individually in Petri dishes following treatment.

- Inoculation of Leaf Discs:

Leaf discs were inoculated on the under surface with an atomised spore suspension containing 30-40,000 spores/mL and Tween 80® (surfactant) to disperse the spores in suspension.

- Visual Ratings:

Where rating of infection was warranted, leaf discs were examined microscopically and given a visual rating based on a scale from 0-5 where:

0 = highly resistant, asymptomatic;

1 = resistant, <10 pustules;

2 = moderately resistant, 10-39 pustules;

3 = moderately susceptible, 40-69 pustules;

4 = susceptible, 70-100 pustules;

5 = highly susceptible, >100 pustules

1. Resistance Assessment

411 genotypes of *Vitis* spp. and four native Vitaceae species were tested for resistance. Four fully expanded leaves of any age were harvested from each genotype for the testing. Inoculated discs were kept at room temperature (25ºC) for seven days prior to being rated for disease symptoms. The process was repeated with another four leaves of each genotype

2. Assessment of Non-susceptible Material

This was not conducted as none of the genotypes tested were symptomless.

3. Assessment of Unidentified Vines

100 household vines of unknown parentage were also tested for resistance. Three leaves from each vine were used in the testing. Inoculated discs were kept at room temperature for seven days prior to being rated for disease symptoms.

4. Assessment of Fungicides

Leaf discs cut from fully expanded, mature leaves of susceptible genotypes were also used for assessment of fungicides. For the assessments of protective ability, leaf discs were immersed for 2 minutes in individual fungicides and air dried prior to inoculation. For assessments of eradicative ability, fungicide application (using the same procedure) was made 24 hours post-inoculation to allow infection to take place. Inoculated discs were kept at room temperature for seven days prior to being rated for disease symptoms. The fungicides used were: cyproconazole, azoxystrobin, triadimefon, difenconazole, chlorothalonil, pyraclostrobin, copper hydroxide, mancozeb, tetraconazole, trifloxystrobin, tebuconazole, flutriafol, benomyl, myclobutanil, oxycarboxin, pyrimethanil, kresomix-methyl, propiconizole, sulfur and pyrimethanil/fluquinconazole. All fungicides, including eradicatives, were tested in

the protective trials. Those products cited as having eradicative or curative abilities were used again in eradicative assessments. All were tested at their recommended rates indicated by their labels

5. Assessment of Temperature and Leaf Wetness

The effect of temperature on the total and rate of germination of urediniospores was assessed using water agar (Edwards *et al.*). Plates were placed in incubators and exposed to temperatures at 5°C intervals ranging from 5 to 35°C. Three replicates of 100 spores per temperature were counted on each plate every hour for the first six hours and a final count made after 24 hours. Germination was considered to have occurred when the germ tube was at least half the diameter of the spore in length (Edwards *et al*. 1998; de Vallavieille-Pope *et al.* 1995). The experiment was repeated twice.

To study the effect of leaf wetness duration, leaf discs were inoculated and kept wet for different periods of time including 0, 3, 6, 9, 12 and 24 hours. After each allocated time the discs were air dried and re-sealed. Inoculated discs were kept at room temperature for seven days prior to being rated for disease symptoms. The experiment was repeated.

The effect of temperature on the latent period (time between infection and symptom development) was assessed by using inoculated leaf discs kept at room temperature for 24 hours to allow infection. Sets of discs were subjected to temperatures at 5°C intervals ranging from 5 to 35°C and monitored to determine the number of days until symptom development.

The effect of temperature on *P. euvitis* sporulation (spore production) was assessed by inoculating leaf discs and incubating them at room temperature until pustule (symptom) development. Sets of discs were then subjected to different temperatures between 5 and 35°C, at 5°C intervals. Upon sporulation, the number of pustules on each disc was recorded. The spores were then washed from the discs and the total

number estimated (using a haemocytometer) to derive the spore production per pustule.

6 - 7. DNA Analysis

The methodology was based on that used during the research of Ono and Imazu (2001).

8. Host Range of GLR in Vitaceae and Leeaceae

This study was not conducted due to the requirement to adhere to quarantine restrictions during the eradication program. All known disease had been removed from within the Darwin quarantine zone at the time.

Field Assessments

9. Grapevine Biology

Aspects of the micro-climate of household (*Vitis* spp.) and native (*Ampelocissus* spp.) grapevines including temperature, humidity and leaf wetness were recorded for comparison. Instruments to measure these parameters were placed in the canopies of four grapevines in suburban yards and six native grapevines growing in remnant vegetation at CSIRO, Darwin. The instruments were set to record at 15 minute intervals.

Leaf retention and shoot growth rates of household *Vitis* spp. were also recorded. Six shoots each from three vines were tagged and recordings conducted on a monthly basis.

10. Assessment of Fungicides in East Timor

The efficacy of fungicides which provided good control of *P. euvitis* in the laboratory was to be validated in field trials in East Timor. A shade-house was constructed at the East Timor Ministry of Agriculture, Forestry and Fisheries (MAFF) facility in Comoro, Dili. Cuttings from locally grown grapevines were potted and hand watered by MAFF staff. However, in January 2006 mass civil unrest occurred in Dili and associated long lasting violence ensued. This forced the abandonment of the assessments before any fungicides had been applied.

11. Native Vine Field Trial

In response to the discovery that two native grape species were susceptible to *P. euvitis* (*in vitro*) a small field trial was conducted in 2004 to validate the results. Two containers of infected cuttings of *Vitis* spp. were placed within a strip of native vegetation that included around 40 plants of *A. frutescens* at a secure site on Berrimah Research Farm. Each plant was labelled, routinely sampled and examined microscopically for disease. At the completion of the trial in May (when the plants were in dormancy due to the “dry season”) the site was burned as part of the general fire management of the area. The labelled plants were sampled again following re-growth later the same year (September) to ensure that no disease from surviving residual spores had occurred.

Results & Discussion

1. Resistance Assessment

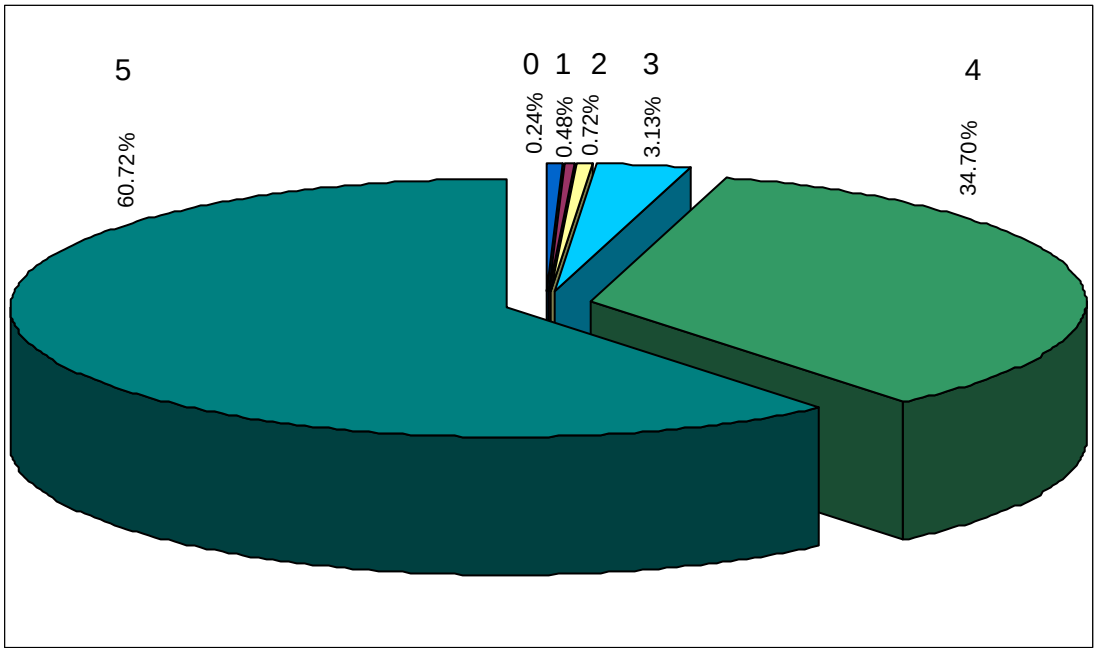

Figure 1. Median ratings of grapevines for resistance to *P. euvitis* (including natives). 0 = highly resistant; 1 = resistant; 2 = moderately resistant; 3 = moderately susceptible; 4 = susceptible; 5 = highly susceptible.

None of the grapevines inoculated were symptomless following the incubation period. The vast majority were either susceptible or highly susceptible.

Four genotypes showed resistance to the pathogen (Hennessy *et al* 2007). '41B', a hybrid rootstock, was classed as resistant whilst two other rootstocks ('128 Seibel' and '554-5 seedlings') and the hybrid cultivar, 'Aurore', were moderately resistant. Not one is a major wine or table grape variety.

Following inoculation of the native grapevines, neither *Cissus adnata* nor *Cayratia maritima* developed symptoms of disease. However, both *Ampelocissus acetosa* and *A. frutescens* were found to be highly susceptible. The infection levels of these species were similar to the majority of *the* Vitis spp. that were susceptible (Daly *et al* 2006).

3. Assessment of Unidentified Vines

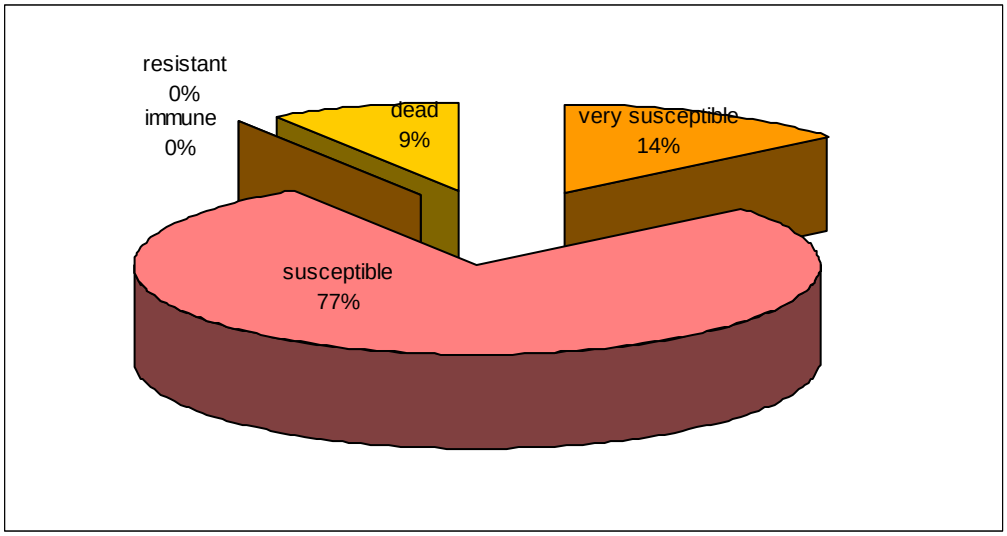

Figure 2. Susceptibility of household vines to *P. euvitis*.

During the eradication program many vines (up to 150) remained GLR disease free and it was decided to conduct some *in vitro* testing to determine if any were resistant. Six vines on different properties were originally targeted due to their healthy status, despite there having been other infected vines on those properties. Unfortunately these vines were found to be susceptible. Disease was subsequently detected in two of these vines during NGLREP surveys. Another 85 vines from other properties were also shown to be susceptible or highly susceptible. A small percentage of samples which were dehydrated, senescing due to age or due to infection with common diseases such as powdery and downy mildew decayed before they could be rated.

Although many vines being grown in home gardens were proven susceptible, under existing Northern territory legislation removal of these vines could not be undertaken without consent from the owner. Plants must be diseased *in situ* before forcible removal can legally take place. However many of the vine owners have volunteered their plants for removal during the program.

4. Assessment of Fungicides

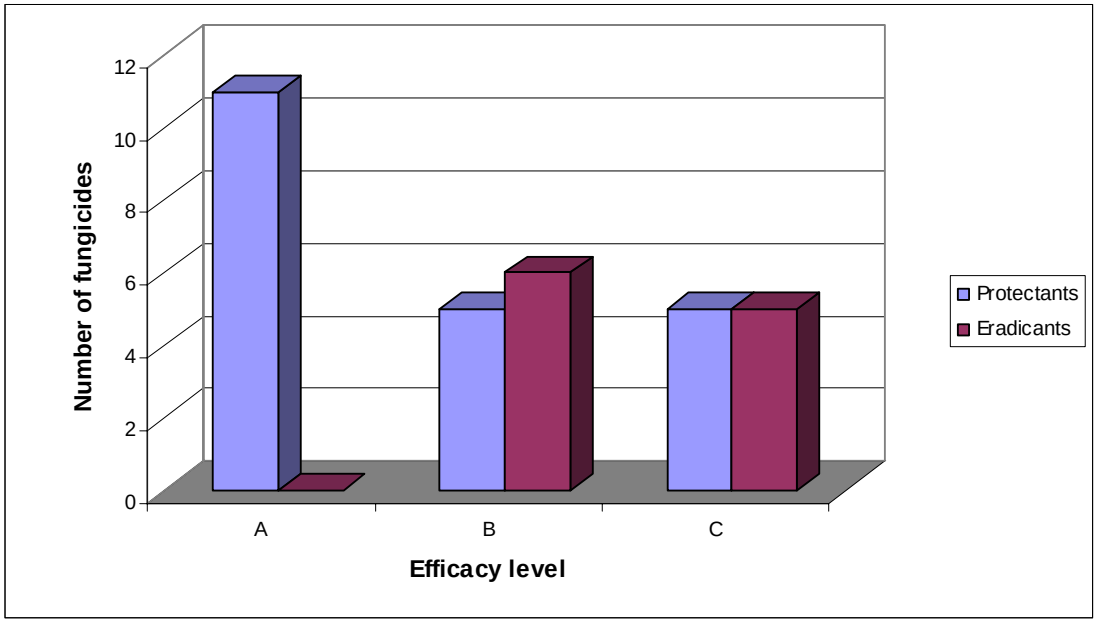

Figure 3. Groupings of protective and eradicative fungicides based on their efficacy against *P. euvitis*. A = completely effective, B = reduced infection and C = ineffective

In comparisons made with infected leaves not treated with a fungicide (positive controls), the protective assessments revealed three different groups of efficacy in the fungicides tested; those that were completely effective, those that were partially effective (reduced symptom development but infection and disease still occurred) and those that were ineffective. Some of the effective fungicides (such as mancozeb) are already routinely used in vineyards for other disease control.

None of the eradicative fungicides, applied after the leaves were inoculated and infected, were completely effective at preventing disease. However, some were substantially better than the control, limiting the infection to significantly lower levels. The most effective was oxycarboxin (Plantvax®)

5. Assessment of Temperature and Leaf Wetness

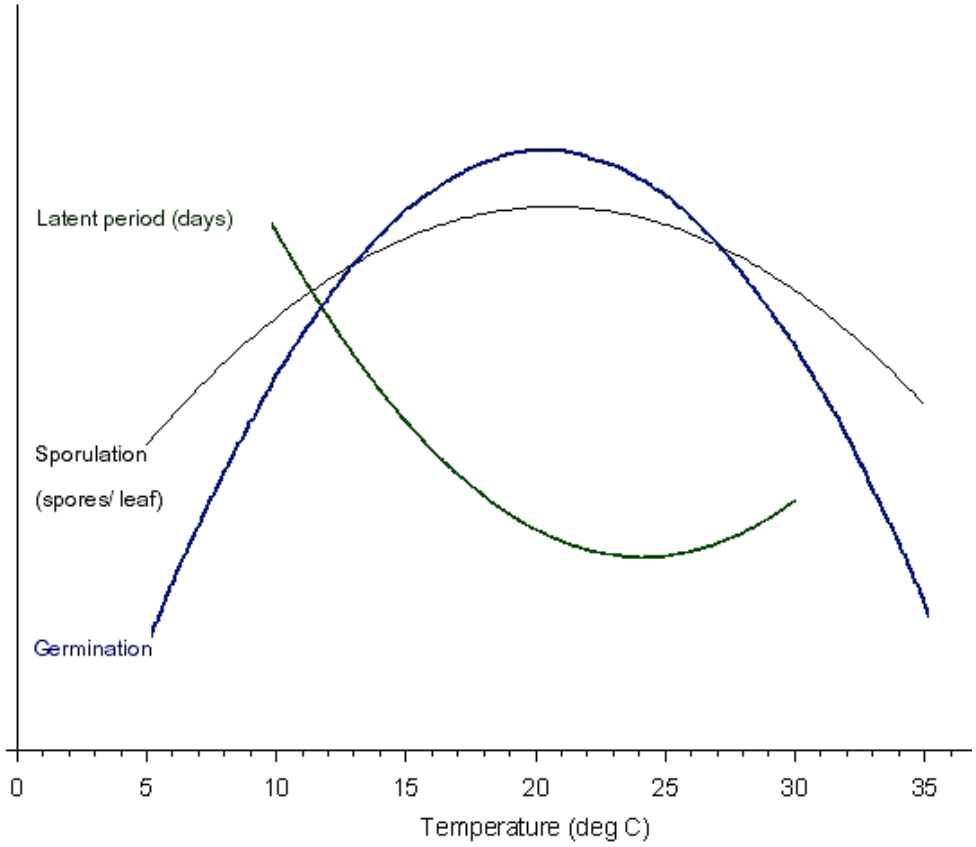

Figure 4. Effect of temperature on germination, length of latency and sporulation of *P. euvitis* urediniospores.

The main infective units (spores) of *P. euvitis* are known as urediniospores. These are responsible for continual infections and rapid build-up of disease within grapevines. In tropical climates, the fungus survives throughout the year via successive generations of these urediniospores. The results of assessment into the effect of temperature on the lifecycle of these spores (from germination and infection of a spore through to production of new spores) are illustrated by Figure 4. The optimal temperature was found to be consistently between 20ºC and 25ºC for the various stages of the urediniospore lifecycle (germination, latent period and sporulation).

a) Temperature and Germination

Based on the assessment, the urediniospores can germinate at any temperature between 10ºC and 30ºC, but not at 5ºC or 35ºC. The optimum temperature was found to be between 21ºC and 22ºC. Although no germination occurred at the temperature extremes of the assessment, the spores survived and could germinate after being kept at room temperature following treatment.

b) Temperature and Latent Period

The result of the latent period assessment was similar to that for germination in that 10ºC and 30ºC were the upper and lower limits. No disease developed following incubation of infected leaves at 5ºC or 35ºC. There was a marked difference in latent period length as the temperature was decreased. At 25ºC it was an optimal 6 days between infection and symptom development, whereas at 10ºC it was 20 days. In an infected vine this would significantly affect the infection levels over time. For instance, based on the experimental results, urediniospores at a constant 25ºC could produce three generations of new spores in the time an infection by one urediniospore at a constant 10ºC would take to result in one generation of new spores.

c) Temperature and Sporulation

Once pustules are formed, they will sporulate at any temperature between 5º and 35ºC inclusive. However below the optimum 20-25ºC the numbers of spores produced declines significantly. Based on experimental data from this research, a pustule (resulting from infection by one spore) can produce approximately150 spores at 10˚C compared to 300 spores at 20-25˚C, all potentially infective. This would significantly increase the wind-borne spore load from an infected vine. If we fully extrapolate using this and previous information (whereby a temperatures of 25˚C can result in disease three-fold that of 10˚C), at 20-25˚C, anything up to 27 million spores could be produced in three generations following infection by just one spore.. However, this assumes that there is 100% infection rate for the spores, which is highly unrealistic. Nonetheless it highlights the effect of optimum temperatures on disease potential.

d) Leaf Wetness

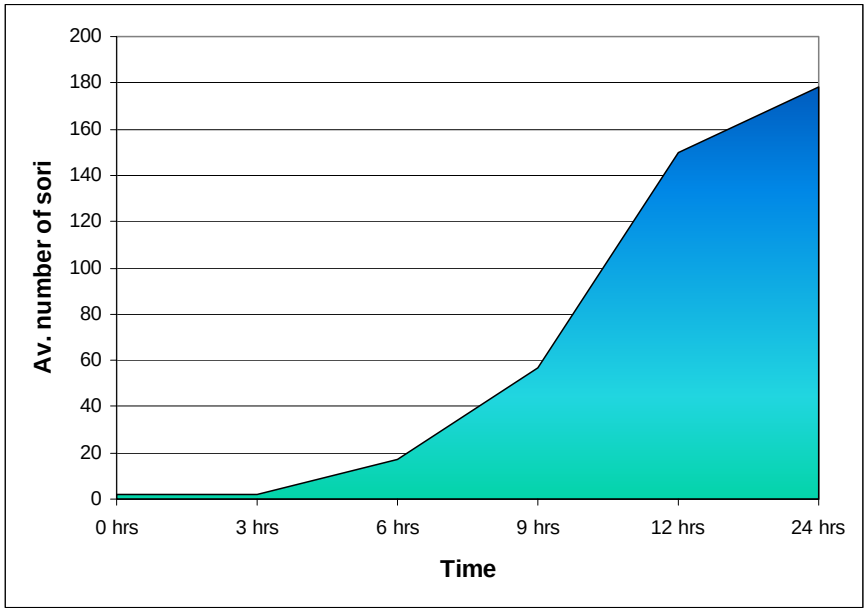

Figure 5. Effect of leaf wetness duration on infection.

Leaf wetness duration also had a significant effect on the lifecycle of *P. euvitis* and subsequent disease development. Whilst wetness for 0 or 3 hours resulted in one or two pustules, the number was increased by at least 90 times following 24 hours of wetness.

Given these results it is not surprising that *P. euvitis* flourishes in Darwin. However, it is interesting that spore germination and pustule sporulation slow down significantly at temperatures over 30ºC, since Darwin consistently has day time maximums of 33ºC or higher throughout the year. Temperatures most suitable for these functions would occur predominantly over-night, probably more-so in the "dry season" when the average minimum is 21˚C (the average minimum in the "wet season" being 25˚C). Also, darkness is known to favour urediniospore germination (Leu and Wu 1983). As such, the data is a useful indicator of "peak infection" periods in terms of eradication efforts, allowing the strategy for the NGLREP to be adjusted accordingly. It also gives an insight into the disease potential under the conditions which prevail in southern regions, should spread of the pathogen occur.

In cold climates when *Vitis* spp. become dormant another spore type, the teliospore is produced, capable of surviving lengthy periods over winter. Urediniospores are not designed for survival for long periods in adverse conditions. Once teliospores develop, they cannot re-infect grapevines the following growing season. They can only result in infection of the alternate host *Meliosma myriantha*, native to Japan (refer to figure 6). The alternate host is not found in the Northern Territory, as such the life-cycle is restricted to the urediniospore stage.

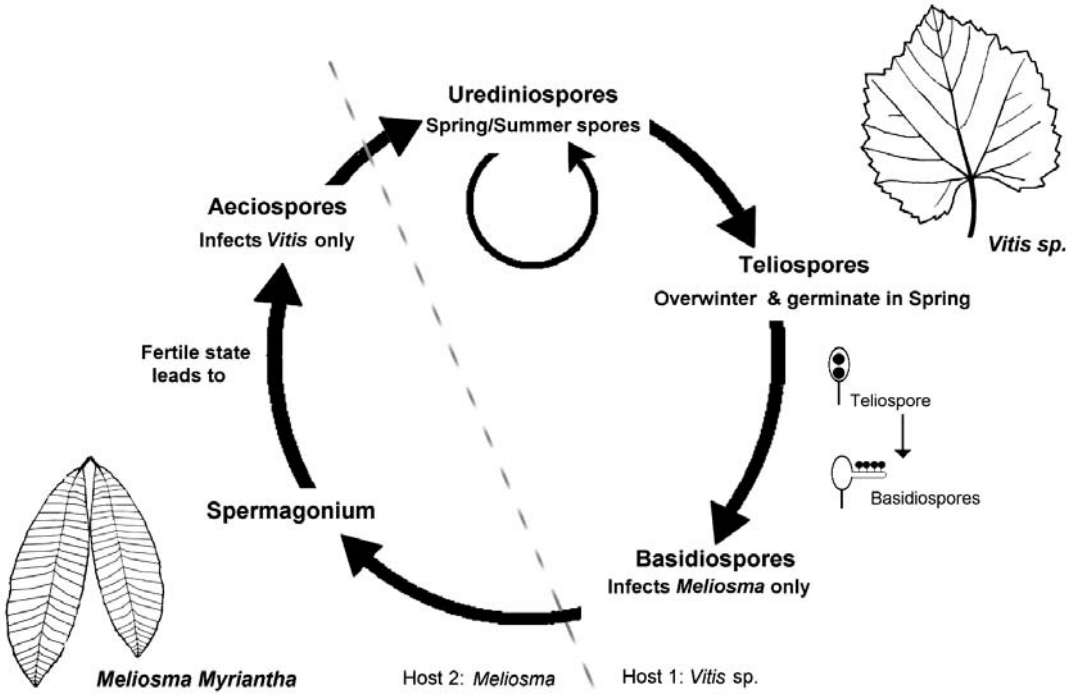

Figure 6. P. euvitis lifecycle. (C. Hennessy)

Hence, disease is not likely to persist in climates where *Vitis* spp. under-go a prolonged dormant period and the alternate host not present. However, despite urediniospores not being designed for survival in adverse conditions, experimentally they have been shown to remain viable for 180 days at 5°C and 30% relative humidity (Ozoe and Kadowaki 1971). Although very unlikely, this suggests that survival of some urediniospores could span the dormancy period in some milder grape growing areas of Australia if the disease was to establish during the growing season. A risk, albeit extremely small, of these spores re-infecting vines the following spring may therefore exist. Furthermore, the existence of localised areas where some vines might maintain a small percentage of their foliage for most or all of the year may present an opportunity for the disease to persist once established.

6 - 7. DNA Analysis

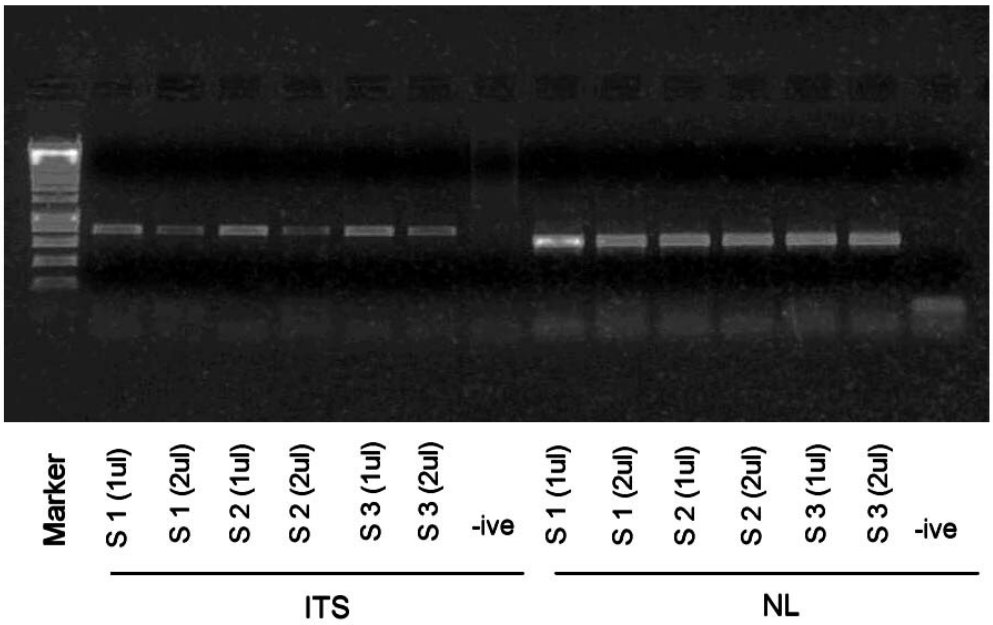

Figure 7. P. euvitis DNA fingerprint. Three different GLR samples (S1, S2 and S3), using the ITS and NL primers each with a negative control showing no DNA.

The diagnostic DNA test for *P. euvitis* was altered slightly from the method employed by Ono and Imazu (2001). During amplification, HotStar Taq™ enzyme (and associated chemicals such as dNTP's and Magnesium) was used instead of AmpliTaq Gold™ enzyme. The temperatures used during the PCR cycles were consequently adjusted to compensate for the use of the alternative enzyme (refer to the attached PCR protocol).

9. Grapevine Biology

	Sample 1	Sample 2	Sample 3	**AV.**
Growth/day (mm.)	31.06	32.52	20.17	**27.92**

Table 1. Average daily growth rates for each sample.

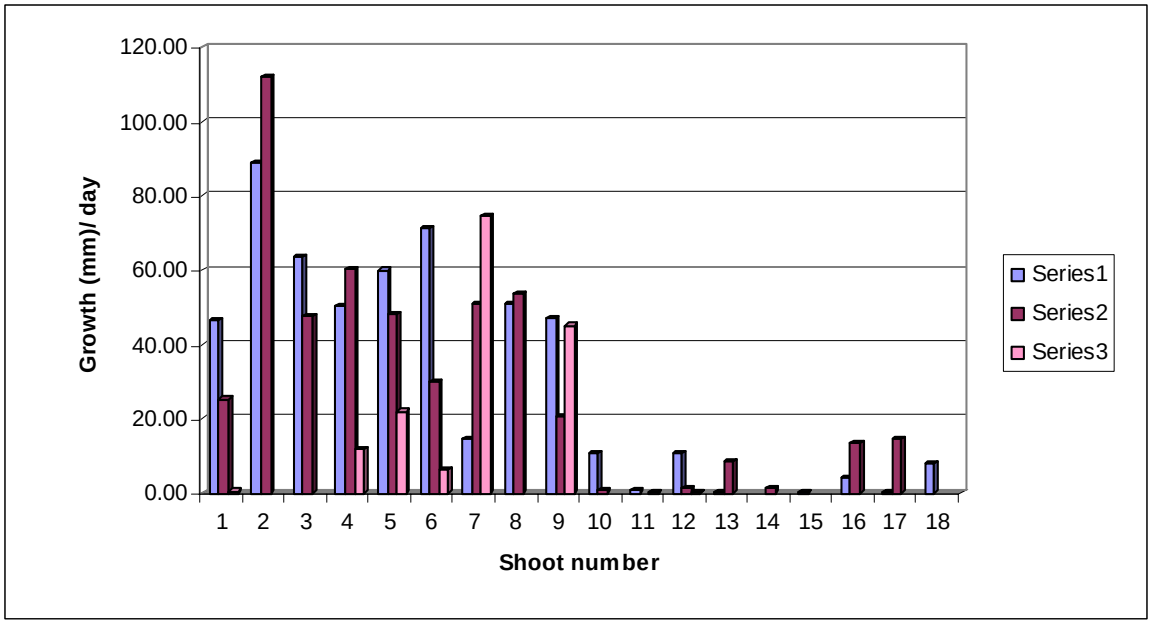

Figure 8. Individual shoot growth rates.

As discussed previously, the prevailing environmental conditions in the Darwin region means that grapevines are evergreen.

The average shoot growth for the 3 vines was almost 28mm per day for the first three months of the wet season (October to December inclusive). However, as shown by the graph individual growth could be more than 100 mm per day and on occasions no growth at all was recorded. Perhaps significantly, lower growth was recorded in the hottest month of the year (November). During these vine recordings it was apparent that there were essentially two types of shoots, those which grew rapidly and those which grew extremely slowly. This was reflected in the appearance of the shoot tips and the canes. The fast growing shoots consisted of healthy looking soft, green material with large, light green leaves and the slow growing tips consisted of weak looking hardened material and small, dark green leaves.

During the course of the experiment all three vines were trimmed at the owners discretion and leaves picked for consumption. Up to 20 leaves per shoot were picked for this purpose on a regular basis, significantly affecting the leaf retention of the vine. The leaves that weren't harvested were found to be capable of living for longer than 3 months. In the majority of cases the defining factor in leaf retention was a characteristic of the lifestyle of the vine owners rather than the vines themselves. As most vine owners trim their vines back at least twice a year (before and after the wet season to encourage new growth) leaf retention and continuous shoot growth would not usually exceed 6 months.

Grapevine canopy micro-climate data was collected to allow a comparison between *Vitis* and *Ampelocissus* spp. and to illustrate which period(s) of the year are likely to be the most conductive to disease based on the results of laboratory studies of the effects of temperature and leaf wetness on *P. euvitis'* lifecycle. In addition, if infection occurred in one of the vines being monitored, it would useful to know the conditions existing within the canopy at the time that might have contributed to disease development.

Throughout the experiment there were significant problems experienced with the data-logging instruments. The manufacturing of the temperature and humidity devices was sub-standard and they were not watertight. The wet season rains penetrated and destroyed most of them. Replacements were provided half way into the trial. In addition to this, the instruments used to record leaf wetness data included a cable in their design. Many of the ones placed in the native grapevines had their cables chewed through by rats or possums.

Unfortunately due to the instrument failure detailed above, not enough data was generated from this experiment to provide a rigorous scientific study. However analysis of the surviving canopy data showed during January to April ("wet season") temperatures were generally between 25 and 35ºC with occasional maximums up to 40ºC. The average minimum temperature was 27˚C and the overnight humidity was 86- 90%. Similar data was obtained from the native plants. However there were problems with extremely high temperature spikes when exposed to full sun which spoiled the maximum temperature values.

By May the native plants had died back to a tuber, leaving only the household vines to be monitored. From May to June ("dry season") the overnight temperature was maintained between 20 and 22˚C in the canopies. During July the minimum fell to an average of around 18˚C. The overnight humidity was comparable to that recorded during the "wet season" (from January to April), possibly as a result of dew, leaf transpiration and/or watering by the owners. However, during the day the humidity fell significantly.

Similarly, leaf wetness was a nocturnal event during the "dry season", with an average of 6.5%, but with periods of up to 30- 50 %. In comparison the "wet season" average was much higher and up to 100% overnight.

With high humidity and significant leaf wetness being recorded overnight during the "dry season", this is likely to provide ideal conditions for germination and infection of spores and thus for disease to prevail, particularly with the lower temperatures and the fact that darkness favours germination (Leu and Wu 1983). In contrast, the extended periods of 100% leaf wetness during the "wet season" might inhibit disease to a large extent since spores don't germinate in free water (Leu and Wu 1983). Also, heavy rain may frequently lead to spores being washed from the leaves and onto the ground.

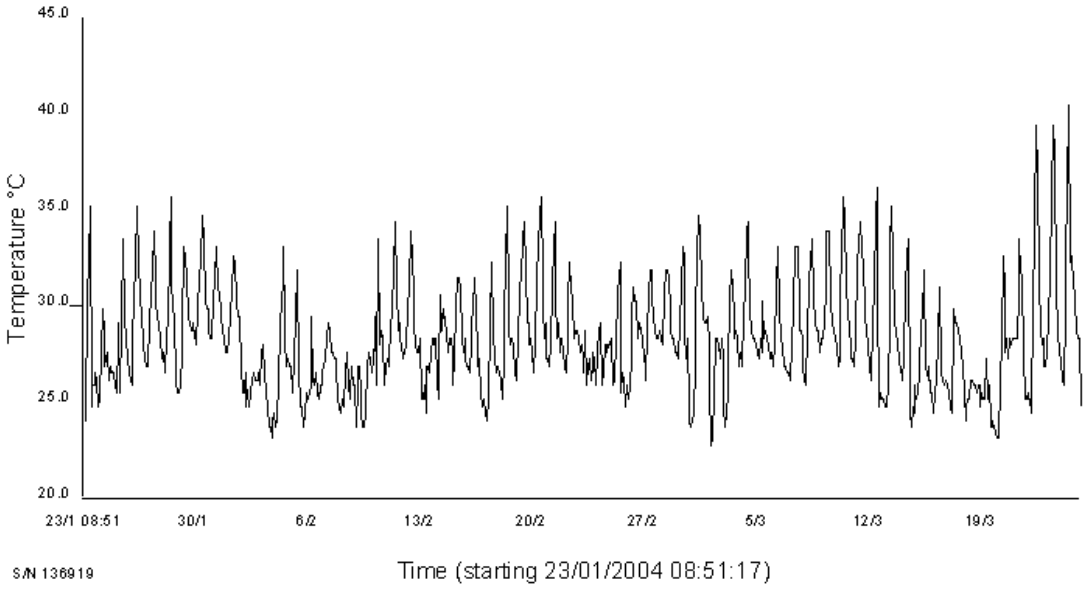

Figure 9. Temperature in a grapevine canopy

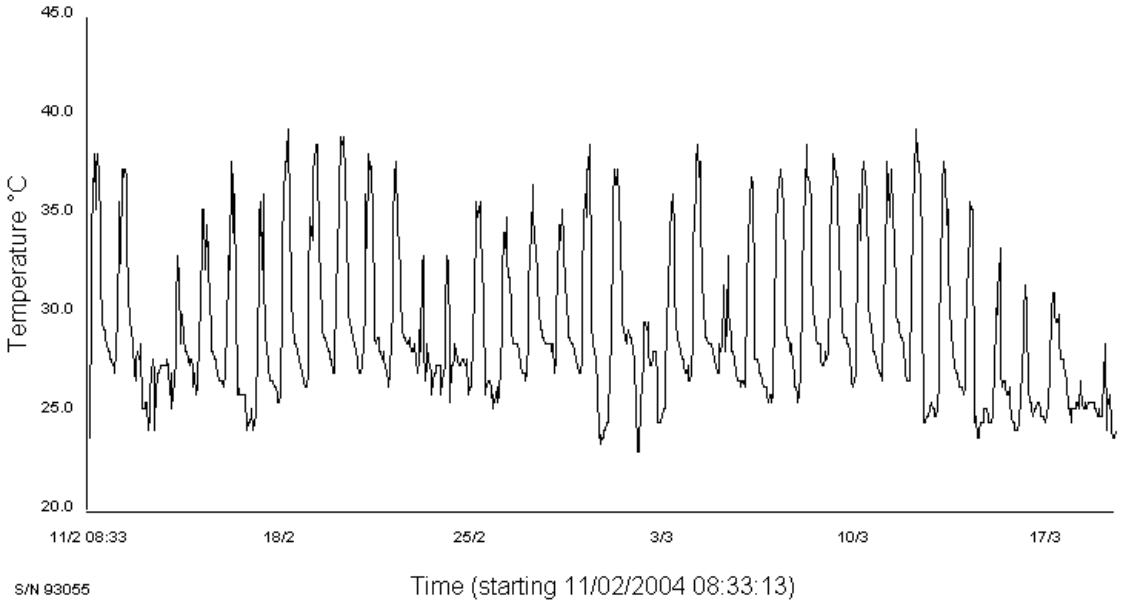

Figure 10. Temperature in a native (*Ampelocissus sp.*) canopy

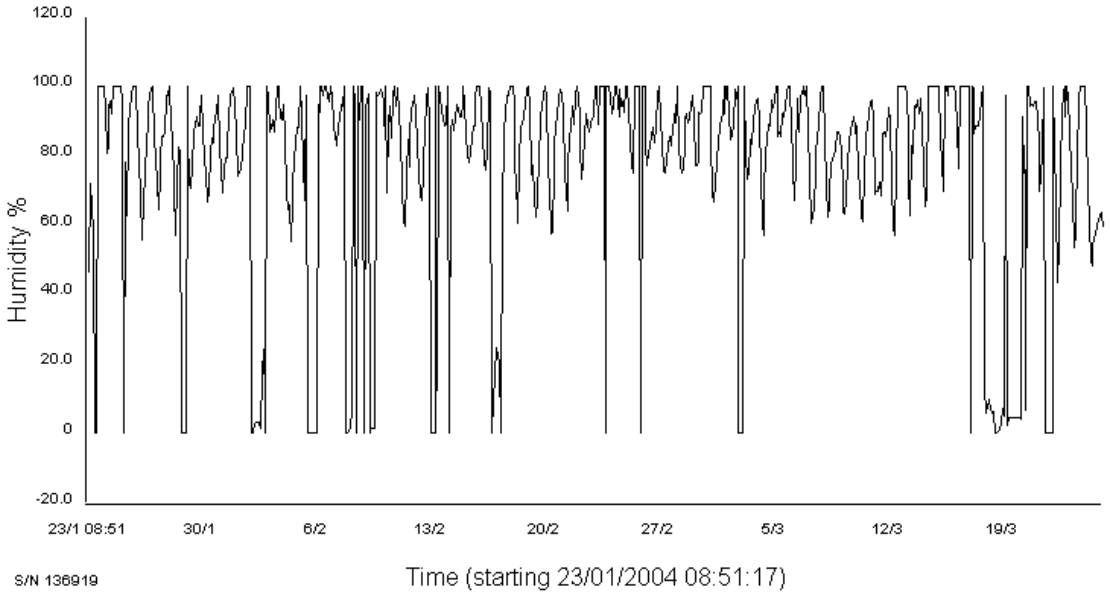

Figure 11. Humidity in a grapevine canopy

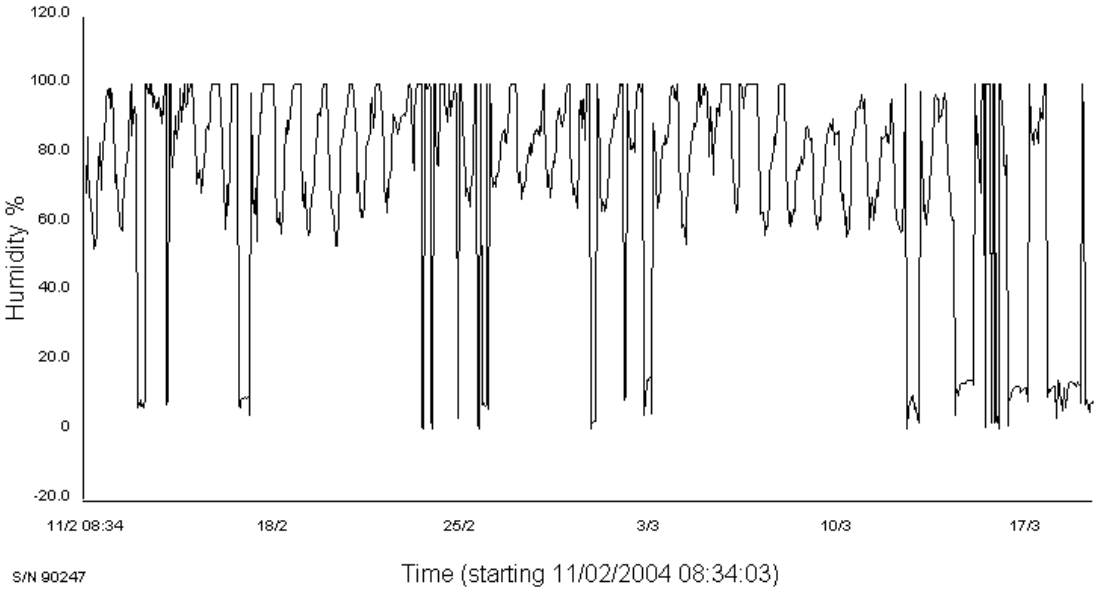

Figure 12. Humidity in a native (*Ampelocissus sp*.) canopy

11. Native Vine Field Trial

	% Positive		
	Sample 1	Sample 2	Sample 3
Natives	38	40	50
Grapevines	41	41	75

Table 2. Incidence of *P. euvitis* infection in grapevines and native *Ampelocissus* spp.

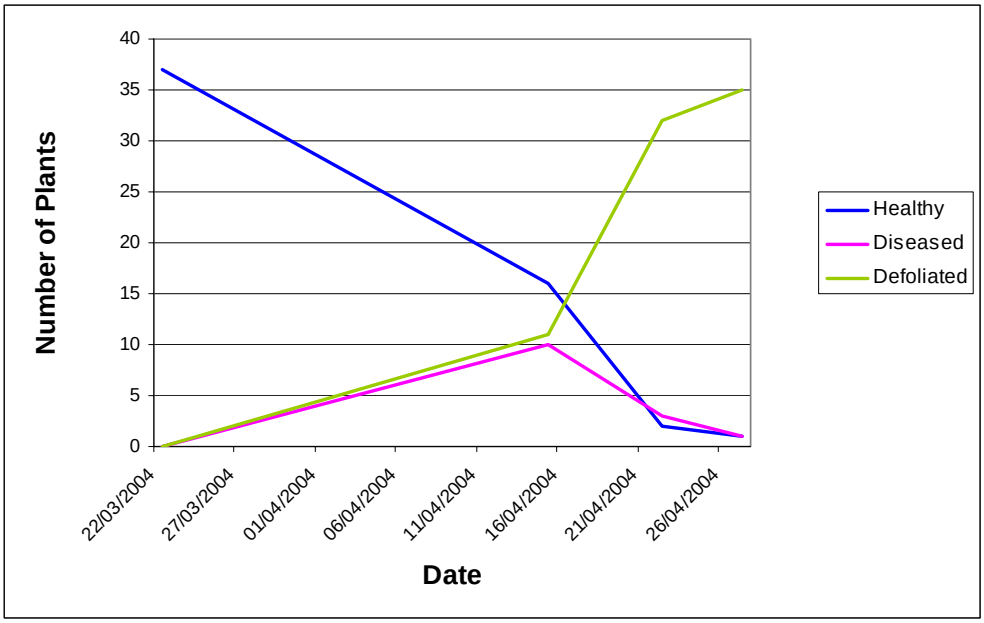

Figure 13. Infection status of native Ampelocissus spp.

The results of the field trial validated the laboratory results that *Ampelocissus* spp. are susceptible to *P. euvitis* (Daly *et. al.* 2005). The incidence of disease on *Ampelocissus frutescens* plants was initially similar to that on the sentinel *Vitis* spp. plants but by the third sampling, the number of infected *Vitis* spp. was much greater. However, this was due to the deciduous nature of the native species (as illustrated by Fig. 12) which die back to an underground tuber during the "dry season". The number of leaves of *Ampelocissus* spp. available for sampling was significantly reduced towards the end of the trial. The greatest distance of spread of disease was approximately 20m to the plant furthest away from the disease source.

Although the field trial confirmed that *Ampelocissus* spp. are susceptible when growing naturally in an outdoor environment, no plants of these species growing in other wild populations had ever been found to be infected during NGLREP surveys.

However, a natural infection was finally confirmed in April 2005. The native vine was located growing in a yard in close proximity to an infected *Vitis* spp. vine (Daly & Hennessy 2006).

Outcomes & Conclusions

The research has made a number of specific findings which are beneficial to industry in the event of future outbreaks. Significantly, these findings include the extent of susceptibility of *Vitis* spp., where screening included all of the commercially important wine and table-grape varieties. Outcomes during the course of the NGLREP, such as the discovery that the native *Ampelocissus* species were capable of infection, were important and of immediate use and significantly enhanced the prospects of eradication. This was highlighted by the discovery of an infected native vine on the same property as an infected *Vitis* sp. vine (Daly & Hennessy 2006).

The research has also uncovered a number of viable control options should the disease be discovered in southern viticulture regions. Supplementary to this has been to establish the influence of vital environmental conditions on the lifecycle of the rust. This information helps to identify conditions in other important grape producing regions which are conducive to disease and has been useful for the NGLREP in the development of monitoring and sampling strategies.

Another important result is the refinement of the PCR diagnostic test. This will allow confirmation of GLR disease within an 8 hour period following detection in new areas. Previously specimens had to be sent overseas. Furthermore, a protocol has been developed so that the test can be replicated in other laboratories for validation of results in future incursion situations.

Appendices

Appendix 1- Communications

Hennessy C., Daly A. and Hearnden M. (2007) Assessment of grapevine cultivars for resistance to *Phakopsora euvitis.* *Australasian Plant Pathology* (In Press).

Daly A., Hennessy C (2006) Agnote I65: Natural Infection of a Native Grape Species with Grapevine Leaf Rust, DPIFM

Daly A., Hennessy C., Schultz G., (2005) New host record for the grapevine leaf rust fungus, *Phakopsora euvitis*. *Australasian Plant Pathology* **34**; 415- 416.

Poster presented at the 12th Annual Australian Wine Industry Technical Conference, Melbourne (2004)

Appendix 2- References

Anonymous (2001) *Phakopsora euvitis*. In "CABI Crop protection compendium, global module (3rd edition)" CABI International, Wallingford, UK.

Leu, L.S. (1988). Rust. **In:** *Compendium of Grape Diseases.* Eds. Pearson, R.C. and Goheen, A.C., APS Press, St Paul, Minnesota. pp 28-30.

Leu LS and Wu HG (1983) Urediniospore germination, infection and colonisation of grape rust fungus, *Phakopsora ampelopsidis.* Plant Protection Bulletin, Taiwan, **25**(3); 167-175

Ono Y. & Imazu M., (2001) Variation in the D1/D2 region of nuclear large subunit ribosomal DNA in *Phakopsora ampelopsidis, P. euvitis* and *P. vitis* (Uredinales). *Bulletin of the Faculty of Education, Ibaraki University.*

Ono Y. (2000) Taxonomy of the *Phakopsora ampelopsidis* species complex on Vitaceous hosts in Asia including a new species, *P. euvitis. Mycologia*. **92 (1)**; 154-173.

Ozoe S and Kadowaski Y (1971) Ecology and control of grape rust disease. *Shokubutsuboueki* **25**: 401-40

Washington W (1987) Susceptibility of *Rubus* species and cultivars to blackberry leaf rust (*Phragmidium violaceum*) and its control by fungicides. *Journal of Phytopathology* 118, 265-275

Weinert M., Shivas R., Pitkethley R. & Daly A., (2003) First record of grapevine leaf rust in the Northern Territory, Australia. *Australasian Plant Pathology*. **32**; 117-118.

Appendix 3- Staff

Rex Pitkethley (Supervisor)
Andrew Daly (Chief Investigator)
Chelsea Hennessy (Research Officer)
Mark Hearnden (Biometrician)

Appendix 4- Raw Data

Rating	Cultivars
1	41B
2	128 Seibel, 554-5 seedlings, Aurore
3	1202, 188-04 Castel, Agadaj, Americano, Black Malaga, Black Sultana, Chambourcin, Colombard, Crimson Seedless, Hunisa, Lilierila INRA, Muscat Gigas, Tinta Ameralla
4	101-14 – HT 100-3, 107-11, 10868 Seibel, 3306, 34 EM, 62-66, 99 Richter, Abouriou, Agestsage blanc, Aleatico, A x R 1, Aualdena No. 1, Auldana No. 3, Baco noir, Bankside Acorn, Barlinka, Baufranc, Baxter's Sherry, Beauty Seedless, Biancone, Boal, Buckland's Sweetwater, Burgrave X, Canada Muscat, Canocazo, Cape Currant, Cardinal, Carina, Carmine, Cascade, Centennial Seedless, Centurion, Cesanese, Chardonnay, Christmas Rose C, Cinsaut, Constantia, Couderc noir, Crouchen, Danlas, Danugue, Diamond, Djandal Kara, Dolcetto, Early Muscat, Egiodola, FER, Fiesta, Flora, Foch, Freisa, Fuji Muscat, Furmint, Gamay – Beaujolais 200A, Ganson, Glenora, Gold, Golden Muscat, Gouais, Goyura, Gramon, Granache BVRC 5, Grenache, Gros Colman, Gros Meslier, Harmony, Harslevelu, Irsay Oliver, Italia, J S 23-416, Keknyelu, Kishmishi, Les de L'el, Limberger, LN 33, Madresfield Court, Malvasia Istria, Mammolo, Melvasia Rei, Mission Seedling, Molinara, Monerac, Morio Muscat, Moss Sultana, Mueller Thurgau, Muscat a petits grains, Muscat Gordo Blanco, Muscat Hamburg, Nebbiolo 111 CVT, Nebbiolo Bourgu, Nyora, O'Hanez, Ondenc, Opuzensia Rana, P 76 – 19, Paulsen 1103, Perle De Csaba, Pink Sultana, Pinot Blanc, Pinot noir, Procupak, R 99, Raboso Piave, Raffiet de Moncade, Red Globe, Red Lady's Finger, Reichensteiner, Ribier, Ribol, Riparia Gloire, Rkaziteli, Rosaki, Royal Ascot, Royalty, Ruggeri 140, Rupestris St. George, Sabalkenskoi, Saturn Ex Northfield, Schwarzmann, Semebat, Seyval, Shiraz, Sylvaner, Symphony, Tannat, Teleki 8B, Terret Noir, Thompson Seedless, Tinta Carvalha, Touriga, Traminer, Trieste 4X, Trollinger, Tulillah, Tunn Currant, Valdepenas Tempranillo, Varousset, Verdicchio, Villard blanc, *Vitis caribaea*, *Vitis cordifolia*, *Vitis longii*, Zante Currant – BC 0158.
5	1613, 1616, 21 B Trier, 333 EM Foex, Alden, Alicante Bouschet, Angostenga Blanc, Ansonica, Antigona, Aramon, Arinarnoa, Arneis 15 CVT, Arrilobe, Aurelia, Autumn Black, Auxerrois, Baco Blanc - C10V12, Baileys Aucerot, Balluti, Banatski Muskat, Barbera, Baresana, Baroque, Bastardo, Bedgradske Besemena, Bellino X, Bianca D'Allessane, Biancolella, Bicane, Black Alicante, Black Frontignac, Black Mammoth, Black Muscat, Blush Seedless, Bonvedro Cl., Brown Frontignac, C.G. 26-879, Cabernet Sauvignon, Calitor noir, Calmeria, Campbell's Early, Canadice, Canner, Canon Hall Muscat, Carignan, Carnelian, Carolina Blackrose, Catawba, Cayuga White, CG 1481, CG 1730, CG 4320, Chancellor, Chasan INRA, Chasselas Dore, Chenin Blanc, Clairette, Clairette Blanche, Clersole Logine, Concord, Corvina Veronese, Criolla negra, Crystal, Daira Seedling, Dawn Seedless, Delaware, Delight, Demir Kapija, Dizmar, Dog Ridge, Doradillo, Dourado, Durif, Dutchess, Elvira, Emerald Riesling, Emerald Seedless, Emperor, Enhresfelser, Exotic, Fantasy Seedless, Fercal, Fernao Pires, Fetyeska, Fiano, Flame Seedless, Flame Tokay, Folle Blanche, Freedom, Fresno, Garronet, Gascon, Graciano, Green Veltliner, Greg Rose, Grocanica, Gropello Gentile, Helena, Henab Turki, Heptakilo, Herbemont, Herbert, Himrod, Illinois, Iona (grafted on Dogridge), Isabella, J 17-48, J17-69, Jacquez, K51-32, Kadarka, Kavadarski Drenak, Kober 125 AA, Kober 5BB, Kyoho, Lady Downe's Seed, Lady Patricia, Lady's Finger, Lagrain, Lambrusco H9V12, Leon Millot, Lider, Lignan, Loose Perlette, Maccabeu, Malbec, Malta Seedless, Malvasia Bianca, Mantley, Marechal Foch, Marroo Seedless, Marsanne, Mataro, Melon, Menavacca, Merbein Seedless, Merlot, Meunier, Michurinets, Mondeuse, Montepulciano, Montils, Monukka, Mrs. Pince's Muscat, Mtsvase, Muscadelle, Muscadelle du Bordelaise, Muscat Blanc, Muscat Ottenel, Muscat Rouge, Nebbiolo Fino, New York Muscat, Odola, Olivette Noir, Orange Muscat, Ortruge, Palomino, Pannaonia Gold, Parellada, Parsley Leaf Chasselas, Pearl of Csaba, Pedro Ximenez, Perdea, Perlette, Petit Meslier, Petit Verdot, Picolit, Pinot gris, Piquepoul noir, Portan, Putzscheere, Queen, Quick's Seedling, Rabener, Radmilovaski Muscat, Ramsey, Red Emperor, Red Malaga, Red Palamino, Red Prince, Rhine Riesling, Richter 110, Riesling 237 Gm, Rolle, Rose Cross ex Drumborg, Rosulus, Rousanne, Rubired, Ruby Cabernet, Ruby Seedless, Russian Seedless, Sangiovese, Santa Paula, Saperavi, Sauvignon blanc, Sauvignonasse, Scarlet, Scheurebe, Schuyler, Semillon, Senecca, Shtur Angur, Siegarrebe, SO 4, SORI - 92-14, Souzao, St. Macaire, Suffolk Red, Sugraone, Sultana (H12), Sultana H 25, Sultana M12, Sultana Moschata, Sultanina Monococco, Sumoll, Taminga, Tandannya, Tarrango, Teleki 5A, Teleki 5C, Temprase, Teroldego, Thomuscat, Tinta Cao, Tinta Molle, Touriga ex Rutherglen, Trabbiano LRC 15, Trajadura, Traminer X Riesling, Trebbiano, Trentham Black, Ughetta, Urbana, Valdiguie, Valensi blanc, Venus, Verdelho, Verdelot, Villard Noir, Viognier, *Vitis labrusca*, Waltham Cross, White Muscat, Wood's Red Muscat, Xarelle, Zante Corinth, Zinfandel.

Table 3. Resistance rating of grapevine genotypes (*Hennessy et al 2007*).

Appendix 5- Budget Reconciliation

GRAPE & WINE RESEARCH & DEVELOPMENT CORPORATION
FORM B

Trust Fund :RESEARCH TRUST FUND	FUNDING	$
	0607	
Project No :NT02/01b	Salaries	
Grantee : NT Department of Primary Industry Fisheries and Mines	Travel	
Title of Project:Grapevine Leaf rust assessment of cultivars	Operating	
(project 1B)	Capital	
	Total Funding	0

EXPENDITURE

	Salaries $	Travel $	Operating $	Capital $	Total $	
A Uncommitted (c/f 1 July)	27179.54	-12442.49	-6675.36	0.00	8061.69	UNEARNED
B Outstanding Commitments (c/f 1 July)	0.00	0.00	0.00	0.00	0.00	
C Refunds of funding Deferral of final report	3476.70	0.00	0.00	0.00	3476.70	92cm000639
D(1)(2) Revenue Received From Trust Fund	0.00	0.00	0.00	0.00	0.00	
E Approved transfers (from Form C)	0.00	0.00	0.00	0.00	0.00	
F Funds available (A+B-C+D±E)	23702.84	-12442.49	-6675.36	0.00	4584.99	
G Expenditure	1336.67	0.00	1900.87	0.00	3237.54	EXPENSES
H Outstanding Commitments (30 June)	0.00	0.00	0.00	0.00	0.00	
I Total funds Committed (G-H)	1336.67	0.00	1900.87	0.00	3237.54	
J Uncommitted (30 June) (F-I)	22366.17	-12442.49	-8576.23	0.00	1347.45	BAL UNEARNED in GAS
K Other income (Paid to Trust Funds)	0.00	0.00	0.00	0.00	0.00	

NOTE(1) Please note the NT Government now operates under an accrual system of financial reporting

I hereby certify that this statement of expenditure is correct.		
……	ANN HEDGER……..……14/02/2007	
Signature	Printed Name AO6 Budgets Officer	Date

Table 4. Statement of receipts and expenditure for the period ending 31 December 2006.

Grape and Wine Research & Development Corporation

Revenue Budget	salaries	travel	operating	**total**	
1a	7620			**7620**	
0304 1b	71298	1500	8300	81098	
0405 1b	69533	3000	19000	91533	
0506 1b	11589			11589	
0607 1b				0	
	160040	4500	27300	**191840**	
Actual Expenses	salaries	travel	operating	**total**	
0304	53457		4423.83	57880.83	
0405	53053.87	1213.74	23358.28	77625.89	
0506	23230.59	15503.75	9537.25	48271.59	
0607	1336.67		2174.67	3511.34	
	131078.1	16717.49	39494.03	**187289.7**	
calculated balance	28961.87	-12217.49	-12194.03	**4550.35**	unearned
GAS balance	22366.17	-12442.49	-8850.03	**1073.65**	unearned
					credit note92cm000639
Difference	6595.7	225	-3344	**3476.7**	issued due to delayed final report

Table 5. DPIFM Reconciliation of funds (as at 31 January 2007).