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Phylloxera infested grapevines have reduced chlorophyll and increased photoprotective pigment content – can leaf pigment composition aid pest detection?

A. L. Blanchfield

Primary Industries Research Victoria

Sharon A. Robinson

University of Wollongong, sharonr@uow.edu.au

L. J. Renzullo

CSIRO Land and Water

K. S. Powell

Primary Industries Research Victoria

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Abstract

Grape phylloxera is a root-feeding pest of grapevines. In Australia, phylloxera infested vineyards are subjected to quarantine restrictions and early detection remains vital for the timely implementation of post-outbreak quarantine protocols. Current detection methods rely on time-consuming ground surveying which involves detailed examination of grapevine (*Vitis vinifera* L.) root systems. Leaf pigment composition is often a sensitive indicator of plant stress. The increasing popularity of remote sensing systems, which exploit those changes in pigments observed with plant stress, offers a real possibility for the development of a phylloxera specific remote detection system. Our objective was to investigate changes in grapevine leaf pigments associated with phylloxera infestation and relate any changes to appropriate reflectance indices. This was achieved through a glasshouse experiment where the response of two vine cultivars (Cabernet Sauvignon and Shiraz) to phylloxera infestation was compared to their response to water- and nitrogen-deficiency. The response of leaf pigments to phylloxera infestation was also investigated in Pinot Noir and Cabernet Sauvignon grapevines grown under field conditions. A reduction in the leaf chlorophyll content and an increase in photoprotective pigment concentrations were observed in leaves of phylloxera infested grapevines compared to uninfested vines. The photochemical reflectance index (PRI) was found to be most closely associated with the ratio of total carotenoid to chlorophyll in these vines

Keywords

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Disciplines

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Phylloxera infested grapevines have reduced chlorophyll and increased photoprotective pigment content – can leaf pigment composition aid pest detection?

RUNNING TITLE: Phylloxera infestation changes leaf pigment composition in grapevines

Annette L. Blanchfield^{1,2} Sharon A. Robinson³ Luigi J. Renzullo^{2,4} and Kevin S. Powell^{1,2}

¹ Primary Industries Research Victoria (PIR Vic), Department of Primary Industries, Rutherglen Centre, RMB 1145, Chiltern Valley Road, Rutherglen, VIC 3685, Australia.

² Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, SA 5064, Australia.

³Institute for Conservation Biology, Department of Biological Sciences, University of Wollongong, Wollongong, NSW, 2522, Australia.

⁴CSIRO Land and Water, GPO Box, 1666, Canberra, ACT 2601, Australia

Corresponding author email: sharonr@uow.edu.au

Tel: +61(2)42215753

Fax: +61(2)42214135

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Abbreviations: HPLC, high-performance liquid chromatography; N, neoxanthin; Chl, chlorophyll_{a+b}; L, lutein; β -car, β -carotene, Tcar, total carotenoid, V, violaxanthin, A, antheraxanthin, Z, zeaxanthin, PRI, photochemical reflectance index, PIZ, Phylloxera Infested Zone, VAZ, xanthophyll cycle pigments.

Abstract

Grape phylloxera is a root-feeding pest of grapevines. In Australia, phylloxera infested vineyards are subjected to quarantine restrictions and early detection remains vital for the timely implementation of post-outbreak quarantine protocols. Current detection methods rely on time-consuming ground surveying which involves detailed examination of grapevine (*Vitis vinifera* L.) root systems. Leaf pigment composition is often a sensitive indicator of plant stress. The increasing popularity of remote sensing systems, which exploit those changes in pigments observed with plant stress, offers a real possibility for the development of a phylloxera specific remote detection system. Our objective was to investigate changes in grapevine leaf pigments associated with phylloxera infestation and relate any changes to appropriate reflectance indices. This was achieved through a glasshouse experiment where the response of two vine cultivars (Cabernet Sauvignon and Shiraz) to phylloxera infestation was compared to their response to water- and nitrogen-deficiency. The response of leaf pigments to phylloxera infestation was also investigated in Pinot Noir and Cabernet Sauvignon grapevines grown under field conditions. A reduction in the leaf chlorophyll content and an increase in photoprotective pigment concentrations were observed in leaves of phylloxera infested grapevines compared to uninfested vines. The photochemical reflectance index (PRI) was found to be most closely associated with the ratio of total carotenoid to chlorophyll in these vines.

Introduction

Grape phylloxera (*Daktulosphaira vitifoliae*) is a root-feeding member of the Aphididae family. Feeding on the roots of grapevines it causes the formation of galls and feeding sites can also act as access points for secondary fungal infections (Omer *et al.* 1995). The resulting damage to and decay of the root system leads to a reduction in yield and quality of grapes, making phylloxera an insect pest of economic significance to viticultural industries worldwide. In Australia, phylloxera infested vineyards are subjected to quarantine regulations.

Phylloxera outbreaks and infestations in the states of Victoria and New South Wales (NSW), both historically (late 19th century) and more recently (December 2003), have highlighted the Australian viticulture industry's vulnerability and the need for vigilance, awareness and on-going research into early detection. Due to the cost of phylloxera-resistant rootstock material, the shortage of supply and the extra cultural management required for grafted vines, the proportion of total vineyard area in Australia planted as own-rooted vines is unlikely to decrease appreciably over the next 20 years.

To date, detection methods have relied predominantly on ground surveying techniques which involve detailed examination of the grapevine root system for the presence of insects and galls. However, ground surveying alone, which is still used in NSW, is by its very nature costly, time consuming and not very effective (Herbert *et al.* 2003). Above-ground, symptoms of phylloxera-infested vines are expressed as premature yellowing of the leaves or canopy reduction. However, this may go unnoticed for two-three years by which time there is irreversible and economically significant damage to the vines and a high risk of a breakdown in quarantine. In cases where a low virulence phylloxera genotype is present it could take much longer than three years before visible symptoms are expressed (Powell, *pers. comm.*).

In Australia, remote sensing techniques were first applied to phylloxera detection in Victoria in the mid 1990s using near-infra red aerial imagery (Frazier *et al.* 2004). Further improvements in remote sensing have led to the use of high resolution multispectral imaging sensors for surveying large areas of South Australia (Edwards *et al.* 2004), allowing reduction in costs associated with ground truthing by targeting 'weak spots' for ground inspection. According to the National Phylloxera Management Protocols (NVHSC 2003), viticulture regions must undertake expensive labour intensive ground surveys over three consecutive years in order to be eligible for 'phylloxera-free' status.

Current remote sensing phylloxera detection methods use multispectral airborne sensors to detect visual changes in the grapevine canopy induced by stresses imposed on the root system by phylloxera feeding. While these systems do highlight weak spots in vineyard blocks (Hall *et al.* 2002), such canopy variability is also caused by other abiotic or biotic variables such as water stress, nutrient deficiencies or drainage issues. Therefore, any positive identification of phylloxera infestation still requires follow-up ground surveying; the same protocol employed in the absence of a phylloxera-specific remote sensing system.

Leaf pigment composition is sensitive to plant stress, with a range of abiotic and biotic factors responsible for either loss of photosynthetic pigments (e.g. chlorophylls) or the production of photoprotective pigments (such as zeaxanthin or β -carotene, Demmig-Adams and Adams 1992). Changes in leaf pigment content of grapevines has previously been examined in response to growth light, water stress, cold-temperature stress and virus infection (Bertamini *et al.* 2004; Bertamini *et al.* 2005; Bertamini and Nedunchezian 2004; Chaumont *et al.* 1997; Flexas *et al.* 2000; 2001; Hendrickson *et al.* 2004; Maroco *et al.* 2002; Medrano *et al.* 2002; Sampol *et al.* 2003). Chlorophyll and carotenoid content clearly acclimate to changes in growth light (Bertamini and Nedunchezian 2004; Chaumont *et al.* 1995; Chaumont *et al.*

1997; Flexas *et al.* 2001; Medrano *et al.* 2002). However whilst significant reduction of chlorophyll content in response to drought has been shown in some studies (Flexas *et al.* 2000; Maroco *et al.* 2002) this is not a consistent response (Chaumont *et al.* 1997; Medrano *et al.* 2002). In addition, drought stress did not affect carotenoid composition in field grown vines in contrast to other Mediterranean species (Demmig *et al.* 1988; Medrano *et al.* 2002). Virus infected vines showed decreased chlorophyll, increased carotenoid/chlorophyll content and increased nonphotochemical quenching (Sampol *et al.* 2003), which was attributed to xanthophyll mediated heat dissipation (Balachandran *et al.* 1997). In general, grapevines maintain large pools of the photoprotective xanthophyll pigments and have been shown to utilise photoprotective heat dissipation via xanthophyll pigments to avoid photoinhibition (Chaumont *et al.* 1997; Hendrickson *et al.* 2004; Medrano *et al.* 2002).

Several studies have shown that changes in pigment composition are quantifiable as subtle changes in crop-level reflectance (Carter and Knapp 2001; Held and Jupp 1994; Nichol *et al.* 2000). In addition, specific spectral indices have been developed that correlate with plant stress indicators such as a decline in photosynthetic efficiency (Gamon *et al.* 1997; Lovelock and Robinson 2002). In grapevines attempts have been made to establish remote methodologies for determining water stress (Evain *et al.* 2004; Flexas *et al.* 2000).

The aim of this study was to investigate whether changes in grapevine leaf pigments occur prior to visible symptoms of phylloxera infestation. High-performance liquid chromatography (HPLC) was used to analyse the pigment content of field grown, ungrafted, *Vitis vinifera* leaf samples collected from commercial vineyards in Phylloxera Infested Zones (PIZ) in north eastern Victoria, Australia. In a separate glasshouse experiment the response of grapevine pigments to infestation with phylloxera was compared to that produced by other stressors such as nitrogen or water deficiency. Pigment concentration and pigment ratios were

compared for phylloxera infested and uninfested grapevines with the aim of determining grapevine response to phylloxera infestation and to identify pigment changes that might provide additional tools for early detection of grapevine infestation, either as stand-alone markers or preferably to enhance the capability of remote sensing methodologies.

Materials and Methods

Glasshouse experiment

Plant Material

Three-year old rootlings of ungrafted *Vitis vinifera* L. cv Shiraz, clone PT23 and ungrafted *Vitis vinifera* L. cv Cabernet Sauvignon, clone LC10 were sourced from Boulevard Nurseries, Mildura, Victoria, Australia. The Shiraz vines were sourced one season prior to the Cabernet Sauvignon, hence were four and a half years old at the commencement of the study, whereas the Cabernet Sauvignon were three and a half years old. All plants were potted into 20 cm pots in a 3:1 ratio mix of potting mix and perlite and maintained in a shadehouse on the premises of DPI Victoria-Rutherglen. “Yates” Thrive - all purpose soluble fertiliser (Arthur Yates and Co. Limited, Homebush, NSW, Australia) was applied monthly, 8 g was dissolved in 4.5 L water, as per manufacturer’s recommendations. The final application being one month before the commencement of the trial. All plants were moved to a glasshouse one week before the commencement of the trial.

Trial Design and Infestation Method

Eight vines of each variety were used for each treatment group, total 96 vines. The six treatments included – unstressed control vines; nitrogen deficient; water deficient; phylloxera infested; phylloxera infested and nitrogen deficient; phylloxera infested and water deficient. The control vines and vines without phylloxera treatment were replanted with fresh potting mix and placed back into their original pots. Plants were then supplemented with either 2 g

ammonium nitrate or deprived of this fertiliser (termed “nitrogen deficient”). All treatments received 2.5 g “Yates” Trace Elements Essential Nutrients (Arthur Yates and Co. Limited, Homebush, NSW, Australia). All pots were sealed in a 53 μm mesh bag, secured at the base of the vine trunk and Tanglefoot™ was applied around the neck of the bag where it contacted with the vine trunk, to prevent phylloxera cross-contamination.

Vines to be infested were removed from their pots and one medium sized lignified root was teased out. Twenty phylloxera eggs of G4 genotype (Corrie *et al.* 1998) were sourced from excised root piece bioassays (de Benedictis and Granett 1993) maintained at DPI Victoria-Rutherglen laboratory and were placed on dampened filter paper strips approximately 40 mm x 15 mm. The filter paper was placed on polyamide gauze fabric of 53 μm mesh. The mesh was then rolled around the root so the eggs were in contact with the root piece, and secured with cable ties and Tanglefoot™ was applied (Burns and Powell submitted). Plants were repotted and received trace elements and nitrogen fertiliser, except for the nitrogen deficient treatment group, before being bagged, as before.

The plants were placed in a single glasshouse room and arranged in eight randomised blocks according to a 2x3x2 random factorial design created using GenStat. All plants except those of the water deficient treatment groups were watered for 2 mins daily via a drip irrigation system. Plants subjected to the water deficiency treatment were originally given half the water of the controls, however after the first week of the trial the watering regime for the water deficiency treatments was reduced to approximately a third of that supplied to the control group. Plants were visually assessed for symptoms of severe water stress and were given additional water when required. The glasshouse was maintained at approximately 24 °C throughout the day and 17 °C overnight and was illuminated using Son-T Agro 400 globes (Philips) for 12 hours daily, with an additional hour boost at 2:00 am to maintain appropriate

photoperiod. Leaf samples were collected nine weeks after initial infestation. Leaf disks were collected using a cork borer, 1.4 cm diameter, and immediately frozen in liquid nitrogen for later HPLC analysis. Whole leaf samples were taken for nitrogen content analysis. Leaves were oven-dried at 45°C for 48 hours. Nitrogen content analysis was completed by CSIRO commercial testing laboratories, Canberra, Australia.

Field experiment

Field sites and varieties

Samples were also collected for pigment analysis from grapevines growing in two commercial vineyards in PIZs in North Eastern Victoria, Australia. Ungrafted, *Vitis vinifera* L. cv Cabernet Sauvignon were situated at a site in the Upton PIZ where phylloxera was first detected in April 2000. Ungrafted, *Vitis vinifera* L. cv Pinot Noir were located at a second site in the Buckland Valley where phylloxera was first detected in December 2003. Above-ground symptoms of phylloxera infestation, including reduced vigour and yield, were first noticed by vineyard staff. Sampling was conducted over the season from bunch closure (January) to just prior to harvest (March) in 2004. Four vines were identified in each of three rows in phylloxera infested and uninfested areas at each field site. Phylloxera affected vines chosen for sampling showed reduced vigour but not leaf chlorosis. Leaves of uniform size and green colour were collected between 11:00 and 15:00 h on clear, sunny days, from the side of the grapevine receiving maximal exposure to the sun. Leaf disks were collected using a cork borer, 1.4 cm diameter, and immediately frozen in liquid nitrogen for later pigment analysis by HPLC. Root samples were collected from each of the grapevines once during the growing season and examined using a binocular microscope to verify the phylloxera infested or uninfested status of each vine included in the study.

Pigment extraction and quantification by HPLC

Pigments were extracted from vine leaves using three sequential acetone extractions (100%:80%:80%) following the methodology described in Dunn *et al.* (2004), and were quantified by HPLC using the method of Gilmore and Yamamoto (1991) as described in Dunn *et al.* (2004) using a SphereClone 5 μ m ODS1 column (Phenomenex, Sydney, Australia). Solvent A was adjusted to acetonitrile:methanol:Tris-HCL buffer 0.1M pH 8.0; 85:9:6, whilst Solvent B remained methanol:hexane 80:20.

Reflectance measurements

Individual leaf reflectance spectra of both glasshouse and field leaf samples were acquired using an ASD FieldSpec-Pro® spectroradiometer coupled with a LiCor Integrating Sphere as described in Renzullo *et al.* (2004). The photochemical reflectance index (PRI, $(R_{531}-570)/(531+570)$; Gamon *et al.* 1997) was calculated for each sample.

Statistical analysis

Differences in pigment content and pigment ratios as a function of variety, phylloxera infestation and treatment (glasshouse trial only) were analysed by Analysis of Variance (ANOVA) using Genstat. ANOVA (Genstat) was used to compare nitrogen content of leaves from each treatment group (glasshouse trial only). Linear regression was used to examine the association between the PRI and relative leaf carotenoid composition (including Tcar/Chl, β -car/Chl, VAZ/Chl, AZ/Chl, Z/Chl, AZ/VAZ and Z/VAZ).

Results

Glasshouse experiment

In the glasshouse experiment pot grown Cabernet Sauvignon and Shiraz varieties were subjected to water and nitrogen deficiency in the presence or absence of phylloxera infestation for nine weeks before sampling. Although some vines showed visible decline due

to treatment effect, such as reduced vigour and leaf chlorosis, leaves targeted for collection did not show any chlorosis. The treatment groups where nitrogen was withheld showed significantly less nitrogen content in their leaves than the other treatment groups ($p < 0.001$) - mean % nitrogen of total dry leaf weight: control 2.2, water deficiency 2.3, nitrogen deficiency 1.9, phylloxera infested control 2.3, water deficiency and phylloxera 2.2, nitrogen deficiency and phylloxera 1.9.

Cabernet Sauvignon leaves had higher chlorophyll_{a+b} and total carotenoid (Tcar) content and higher chlorophyll a/b ratios than Shiraz leaves (Fig 1; $P < 0.001$). However, the Tcar/Chl of Shiraz leaves was higher than that of Cabernet Sauvignon (1.39:1.48, $P = 0.0043$).

Both grapevine varieties responded similarly to reductions in water or nitrogen deficiency by reducing leaf chlorophyll and total carotenoids (Fig 1; Chl, $P = 0.0022$; Tcar, $P = 0.0065$) but Tcar/Chl was unaffected by treatment. Chl a/b was higher in water deficient vines than in nitrogen deficient vines ($P = 0.014$), but neither of these treatments was significantly different from the control vines.

Chlorophyll concentration was reduced by phylloxera infestation in Shiraz but not in Cabernet Sauvignon leaves. Shiraz leaves from phylloxera infested vines had 28% less chlorophyll than control vines, and 46% less chlorophyll than water stressed vine leaves (Fig 1; variety * phylloxera interaction, $P = 0.036$). The total chlorophyll concentration in Shiraz leaves was similar in phylloxera infested and nitrogen treatments both with and without phylloxera, but significantly lower in the phylloxera infested/water deficient treatment. Carotenoid concentration was similarly reduced but the Tcar/Chl was not affected by infestation. None of the other pigments in Shiraz were significantly affected by phylloxera infestation. Pigment composition in Cabernet Sauvignon was unaffected by infestation over the course of the experiment.

Field experiment

The field trial consisted of two grapevine varieties, Cabernet Sauvignon and Pinot Noir, growing in different vineyards, and compared infested and uninfested vines, of the same variety, in adjacent blocks, within the same vineyard. Pinot Noir contained approximately twice as much chlorophyll, on a leaf mass basis, as Cabernet Sauvignon and the Chl a/b was also significantly higher in the former variety (Fig 2; $P < 0.001$). Phylloxera infested vines of both varieties had reduced chlorophyll content (12 - 17%, Fig 2a; $P=0.016$) but Chl a/b was unaffected by infestation.

Relative to chlorophyll, Cabernet Sauvignon leaves had higher concentrations of carotenoids than Pinot Noir leaves, particularly those carotenoids involved in photoprotection such as β -carotene and the xanthophyll cycle pigments (Fig 3; $P < 0.001$). In both varieties, more than 40% of the xanthophyll pool was in the photoprotective form (Fig 3d). However Cabernet Sauvignon leaves maintained a higher de-epoxidation status than Pinot Noir leaves ($P=0.023$).

Although the total carotenoid content declined in infested leaves, the decline was less pronounced than the reduction in chlorophyll and as a result the Tcar/Chl pool was significantly higher in leaves from infested plants ($P=0.044$). The xanthophyll cycle pigments were responsible for much of this increase, with the relative size of the VAZ/Chl pool increasing 22% and 13% in leaves of infested Cabernet Sauvignon and Pinot Noir respectively. The proportion of this pool in the photoprotective forms (AZ/VAZ) was also higher in leaves from infested vines, albeit not significantly ($P = 0.1$). Infested Pinot Noir leaves contained slightly more β -car/Chl than leaves from uninfested vines but there was no difference between phylloxera infested and uninfested Cabernet Sauvignon for this pigment (Fig 3b variety * phylloxera interaction, $P = 0.051$).

PRI measurements

The PRI was originally developed to estimate changes in concentrations of the photoprotective xanthophyll cycle pigments that are formed when plants are stressed (Gamon *et al* 1997) but has also been shown to respond to Tcar/Chl (Sims and Gamon, 2002). The best associations between PRI and carotenoids for both field and glasshouse grown vines are shown in Table 1. For both experiments the strongest associations ($r^2 = 42\%$ glasshouse-, 31% field-grown vines) were between PRI and Tcar/Chl (Fig 4, Table 1). In the field grown vines the associations between PRI and β -car/Chl, VAZ/Chl, AZ/Chl and Z/Chl were also significant and their r^2 values greater than 10% (Table 1). However for glasshouse samples the r^2 value was only 7% for Z/Chl and the regression for AZ/Chl was not significant.

Discussion

Phylloxera infestation reduces chlorophyll and increases investment in photoprotective pigments

The most notable trend from both the field and glasshouse experiments, was an overall reduction in chlorophyll content when vines were phylloxera infested. This decline was observed in the field for both Cabernet Sauvignon and Pinot Noir varieties and in the glasshouse for Shiraz (after only nine weeks of infestation). For glasshouse grown Cabernet Sauvignon the decline in chlorophyll was not significant after nine weeks and unfortunately the trial was stopped after 10 weeks. However, given the decline observed in the field grown vines of this variety it seems likely that a significant decline would be observed within months of infestation. The declines in chlorophyll in the field grown grapevines also illustrate that this reduction is not an artefact of glasshouse conditions. Phylloxera infestation reduced chlorophyll content in field grown vines by 12% in Pinot Noir and 17% in Cabernet Sauvignon. The more marked reduction in the Cabernet Sauvignon may reflect that these vines had been infested for longer but we cannot rule out varietal or phylloxera genotype

differences at this stage. The added effect of the above mentioned factors plus varying environmental conditions at the different vineyard locations should also be considered.

In the glasshouse experiment, phylloxera infestation reduced leaf chlorophyll by 28% in control phylloxera infested Shiraz vines and 46% in water-deficient vines but only 2% in nitrogen-deficient vines. Shiraz vines responded less strongly to water-deficiency than nitrogen-deficiency when these stresses were imposed as single treatments, however when added to phylloxera infestation the water deficient plus phylloxera infestation treatment produced the largest decline in chlorophyll. This may indicate that phylloxera infestation interferes more strongly with water uptake and therefore exacerbates water stress but has a less significant effect on nitrogen deficiency. Further work is required to confirm these results since it was difficult to impose an adequate drought stress under glasshouse conditions and an open field experiment would provide a more realistic test of this hypothesis.

In other studies pigment composition of grapevines has been shown to be relatively resilient to drought and cold stress (Hendrickson *et al.* 2004; Medrano *et al.* 2002) although changes have been observed as a result of varying growth irradiance and virus infection (Bertamini *et al.* 2004; Bertamini and Nedunchezian 2004; Sampol *et al.* 2003). Our results are consistent with these findings and suggest that infestation of the roots imposes a considerable stress on the plants that is reflected in leaf pigments switching away from light harvesting towards a role in energy dissipation. This is particularly clear in the increase in xanthophyll cycle pigments and in the de-epoxidation status of these pigments. Grapevines are known to be highly resistant to photoinactivation of photosynthesis and to be able to dissipate a high proportion of absorbed light energy as heat. In previous studies cold- and water-stressed vines have been estimated to dissipate 64-92% of captured light energy via Δ pH dependant, xanthophyll-mediated nonphotochemical quenching (Hendrickson *et al.* 2004; Medrano *et al.*

2002). Phylloxera damage to the roots affecting water uptake would also explain higher levels of photoprotective pigments in infested vines in the field. Infestation increases the stress experienced at leaf level and leads to shifts in pigment composition to facilitate greater energy dissipation as heat. This response is similar to that shown in virus-infected plants including vines (Balachandran *et al.* 1997; Bertamini *et al.* 2004; Sampol *et al.* 2003).

High spectral resolution, reflectance spectrometry has been used to characterise the spectral response of vegetation to a number of stressors (Carter 1993; Gamon *et al.* 1997; Mohammed *et al.* 2000; Zarco-Tejada *et al.* 2003), including mite attack on apple trees (Penuelas and Filella 1998) and remote sensing is increasing in popularity as a tool for assessing plant physiological status. Individual leaf reflectance spectra taken from field sites and the glasshouse trial, simultaneously to the leaf sampling for HPLC analysis discussed in this paper, showed significant differences in key wavelength regions (Renzullo *et al.* 2004; 2006). The most commonly observed differences in the reflectance spectra of phylloxera infested and uninfested samples taken from the field occurred around the green peak (500 – 600 nm), the chlorophyll well (reflectances between 550nm – 700nm) and the red edge (660-760 nm). These regions are known to be affected by chlorophyll content in response to plant stress (Carter 1993; Zarco-Tejada *et al.* 2003). The phylloxera-infested leaves of both grapevine varieties taken from the glasshouse trial also possessed characteristic higher reflectances in the green peak and chlorophyll well compared to the unstressed controls. These results are consistent with the findings of lower chlorophyll levels detected in the leaves of phylloxera infested vines analysed by HPLC.

Given that changes in pigment composition can be detected prior to leaves showing visible changes, it is possible that they can be used to improve current methods for the early detection of phylloxera infestations. The PRI was originally developed to detect de-epoxidation status

of the xanthophyll cycle (Gamon *et al.* 1997), and although it performed adequately with these pigments in our field study, the correlation was much weaker in the glasshouse grown vines. However, as has recently been observed for a wide range of plant species and leaf structures (Sims and Gamon 2002) we found the best overall relationship was between PRI and Tcar/Chl (Table 1, Figure 4). The PRI is sensitive to both the Tcar/Chl and to changes in the relative xanthophyll cycle pigments, and generally correlates well with the latter in diurnal studies of the same leaves (Sims and Gamon 2002). However, the between treatment differences in Tcar/Chl in this study probably swamped the more subtle xanthophyll cycle pigment changes. However given that the relative increase in Tcar/Chl reflects increased vine stress this is still a promising index for future study. If such indices can be detected at a canopy level there is a real possibility that an appropriately-tuned airborne sensor could identify phylloxera infestation earlier than current methods.

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References

- Balachandran S, Hurry VM, Kelley SE, Osmond CB, Robinson SA, Rohozinski J, Seaton GGR, Sims DA (1997) Concepts of plant biotic stress. Some insights into the stress physiology of virus-infected plants, from the perspective of photosynthesis. *Physiologia Plantarum* **100**, 203-213.
- Bertamini M, Muthuchelian K, Nedunchezian N (2004) Effect of grapevine leafroll on the photosynthesis of field grown grapevine plants (*Vitis vinifera* L. Cv. Lagrein). *Journal Of Phytopathology* **152**, 145-152.
- Bertamini M, Muthuchelian K, Rubinigg M, Zorer R, Nedunchezian N (2005) Low-night temperature (LNT) induced changes of photosynthesis in grapevine (*Vitis vinifera* L.) plants. *Plant Physiology And Biochemistry* **43**, 693-699.
- Bertamini M, Nedunchezian N (2004) Photosynthetic responses for *Vitis vinifera* plants grown at different photon flux densities under field conditions. *Biologia Plantarum* **48**, 149-152.
- Burns AE, Powell KS (submitted) A novel approach for rapid screening of grapevine rootstocks for resistance to grape phylloxera. *Australian Journal of Grape and Wine Research*.
- Carter G (1993) Responses of leaf spectral reflectance to plant stress. *American Journal of Botany* **80**, 239-243.
- Carter G, Knapp A (2001) Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. *American Journal of Botany* **88**, 677-684.
- Chaumont M, Morot-Gaudry JF, Foyer CH (1995) Effects of photoinhibitory treatment on CO₂ assimilation, the quantum yield of CO₂ assimilation, D1 protein, ascorbate,

glutathione and xanthophyll contents and the electron transport rate in vine leaves.

Plant, Cell and Environment **18**, 1358.

Chaumont M, Osorio ML, Chaves MM, Vanacker H, Morot-Gaudry JF, Foyer CH (1997)

The absence of photoinhibition during the mid-morning depression of photosynthesis in *Vitis vinifera* grown in semi-arid and temperate climates. *Journal of Plant Physiology* **150**, 743.

Corrie AM, Kellow A, Buchanan GA, van Heeswijck R (1998) Phylloxera biotypes in

Australia. *Australian Grapegrower and Winemaker* **417**, 32-36.

de Benedictis JA, Granett J (1993) Laboratory evaluation of grape roots as hosts of

californian grape phylloxera biotypes. *American Journal of Enology and Viticulture* **44**, 285-291.

Demmig B, Winter K, Kruger A, Czygan F-C (1988) Zeaxanthin and the heat dissipation of

excess light energy in *Nerium oleander* exposed to a combination of high light and water stress. *Plant Physiology* **87**, 17-24.

Demmig-Adams B, Adams WW (1992) Carotenoid composition in sun and shade leaves of

plants with different life forms. *Plant Cell and Environment* **15**, 411-419.

Dunn JL, Turnbull JD, Robinson SA (2004) Comparison of solvent regimes for the extraction

of photosynthetic pigments from leaves of higher plants. *Functional Plant Biology* **31**, 195-202.

Edwards J, Lewis M, Powell K, Hackworth P, Lamb D (2004) Identification of phylloxera

from high resolution infrared aerial imagery: a comparative study between airborne imagery type. *Australian Grapegrower & Winemaker* **488**, 51-54.

Evain S, Flexas J, Moya I (2004) A new instrument for passive remote sensing: 2.

Measurement of leaf and canopy reflectance changes at 531 nm and their relationship

- with photosynthesis and chlorophyll fluorescence. *Remote Sensing Of Environment* **91**, 175-185.
- Flexas J, Briantais JM, Cerovic Z, Medrano H, Moya I (2000) Steady-state and maximum chlorophyll fluorescence responses to water stress in grapevine leaves: A new remote sensing system. *Remote Sensing of Environment* **73**, 283.
- Flexas J, Hendrickson L, Wah Soon C (2001) Photoinactivation of photosystem II in high light-acclimated grapevines. *Australian Journal of Plant Physiology* **28**, 755.
- Frazier P, Whiting J, Powell K, Lamb D (2004) Characterising the development of grape phylloxera infestation with multi-temporal near-infrared aerial photography. *Australian Grapegrower & Winemaker 32nd Annual Technical Edition*, 133-136.
- Gamon JA, Serrano L, Surfus JS (1997) The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. *Oecologia* **112**, 492-501.
- Gilmore AM, Yamamoto HY (1991) Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C₁₈ high-performance liquid chromatographic column. *Journal of Chromatography* **543**, 137-145.
- Hall A, Lamb D, Holzapfel B, Louis J (2002) Optical remote sensing applications in viticulture: a review. *Australian Journal of Grape and Wine Research* **8**, 36-47.
- Held AA, Jupp DLB (1994) Use of the Compact Airborne Spectral Imager (CASI) for remote sensing of vegetation function and dynamics. In '7th Australasian Remote Sensing Conference'. Melbourne, Australia
- Hendrickson L, Chow WS, Forster B, Furbank RT (2004) Processes contributing to photoprotection of grapevine leaves illuminated at low temperature. *Physiologia Plantarum* **121**, 272.

- Herbert K, Powell KS, Hoffman A, Parsons Y, Ophel-Keller K, van Heeswijck R (2003) Early detection of phylloxera - present and future directions. *Australian & New Zealand Grapegrower and Winemaker* **473a**, 93-96.
- Lovelock CE, Robinson SA (2002) Surface reflectance properties of Antarctic moss and their relationship to plant species, pigment composition and photosynthetic function. *Plant Cell And Environment* **25**, 1239-1250.
- Maroco JP, Rodrigues ML, Lopes C, Chaves MM (2002) Limitations to leaf photosynthesis in field-grown grapevine under drought - Metabolic and modelling approaches. *Functional Plant Biology* **29**, 451.
- Medrano H, Bota J, Abadía A, Sampol B, Escalona JM, Flexas J (2002) Effects of drought on light-energy dissipation mechanisms in high-light-acclimated, field-grown grapevines. *Functional Plant Biology* **29**, 1197.
- Mohammed GH, Noland TL, Irving D, Sampson PH, Zarco-Tejada PJ, Miller JR (2000) 'Natural and stress-induced effects on leaf spectral reflectance in Ontario species.' Ministry of Natural Resources, Ontario, Canada.
- National Vine Health Steering Committee (NVHSC) (2003) National Phylloxera Management Protocol <http://www.gwrdc.com.au/nvhscphylloxera.htm>.
- Nichol CJ, Huemmrich KF, Black TA, Jarvis PG, Walthall CL, Grace J, Hall FG (2000) Remote sensing of photosynthetic-light-use efficiency of boreal forest. *Agricultural and Forest Meteorology* **101**, 131-142.
- Omer AD, Granett JD, De Benedictis JA, Walker MA (1995) Effects of fungal root infections on the vigour of grapevines infested by root-feeding grape phylloxera. *Vitis* **34**, 165-170.

- Penuelas J, Filella I (1998) Visible and near-infrared reflectance techniques for diagnosing plant physiological status. *Trends in Plant Science* **3**, 151-156.
- Renzullo L, Held A, Powell KS, Blanchfield AL (2004) Remote sensing phylloxera infestation: current capabilities and future possibilities for early detection. *The Australian and New Zealand Grapegrower and Winemaker 32nd Annual Technical Issue*, 126-130.
- Renzullo LJ, Blanchfield AL, Powell KS (2006) A method of wavelength selection and spectral discrimination of hyperspectral reflectance spectrometry. *IEEE Transactions on Geoscience and Remote Sensing*. in press.
- Sampol B, Bota J, Riera D, Medrano H, Flexas J (2003) Analysis of the virus-induced inhibition of photosynthesis in malmsey grapevines. *New Phytologist* **160**, 403.
- Sims DA, Gamon JA (2002) Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment* **81**, 337-354.
- Zarco-Tejada PJ, Pushnik JC, Dobrowski S, Ustin SL (2003) Steady-state chlorophyll a fluorescence detection from canopy derivative reflectance and double-peaked red-edge effects. *Remote Sensing of Environment* **84**, 283-294.

Table 1. Summary of regression analysis of the photochemical reflectance index (PRI) against leaf carotenoids for glasshouse and field grown vines. Only significant regressions with r^2 values greater than 10% are shown, leaf carotenoids tested include total carotenoids/chlorophyll, β -carotene/chlorophyll, VAZ/chlorophyll, AZ/chlorophyll, Z/chlorophyll, AZ/VAZ, Z/VAZ.

Experiment	Carotenoid	Regression	r^2 (%)	P
Glasshouse	Total carotenoid/Chl	= 1.47 – 2.18PRI	42.6	<0.0001
Field	Total carotenoid/Chl	= 0.36 – 1.51PRI	31.0	<0.0001
	β -carotene/Chl	= 0.11 – 0.23PRI	25.4	<0.0001
	VAZ/Chl	= 0.13 – 0.95PRI	23.1	0.0002
	AZ/Chl	= 0.06 – 0.48PRI	16.9	0.002
	Z/Chl	= 0.03 – 0.22PRI	11.2	0.013

Figure 1. Effect of phylloxera infestation on chlorophyll (A, B) and carotenoid (C, D) concentration of leaves of Shiraz (A,C) and Cabernet Sauvignon (B,D) grapevine varieties grown for 9 weeks in a glasshouse trial. Vines were grown with optimum water and nutrients (Control) or subjected to water or nitrogen deficiency. Solid bars denote uninfested vines whilst open bars show phylloxera-infested vines. Chlorophyll a/b ratios are presented as text in appropriate chlorophyll bars (A, B). Data represent mean \pm SEM (n=8).

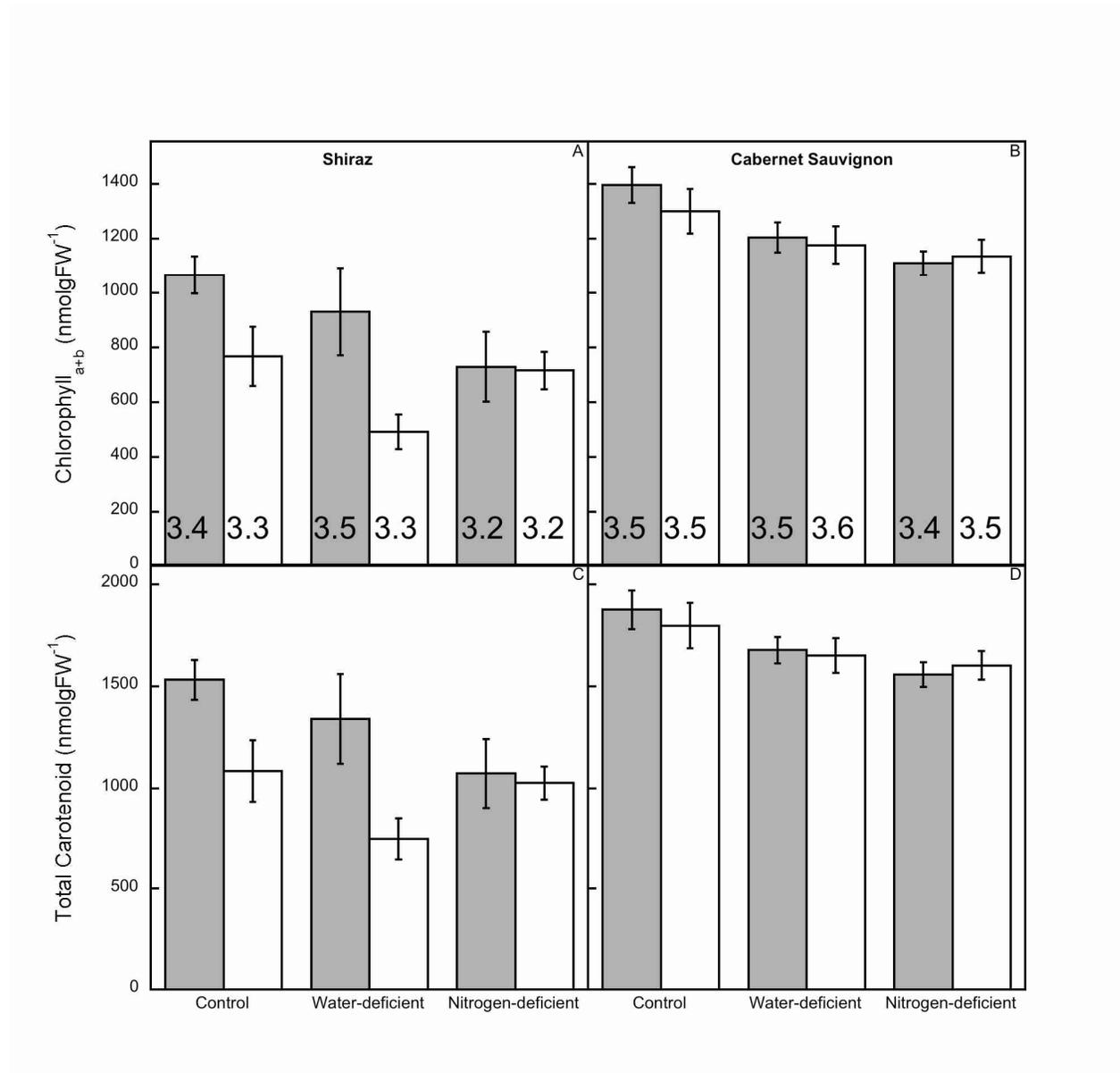


Figure 2. Effect of phylloxera infestation on chlorophyll concentration and Chl a/b of leaves of Cabernet Sauvignon and Pinot Noir grapevine varieties grown in the field. Solid bars denote uninfested vines whilst open bars show phylloxera infested vines. Data represent mean \pm SEM (CS n=20, PN n=8).

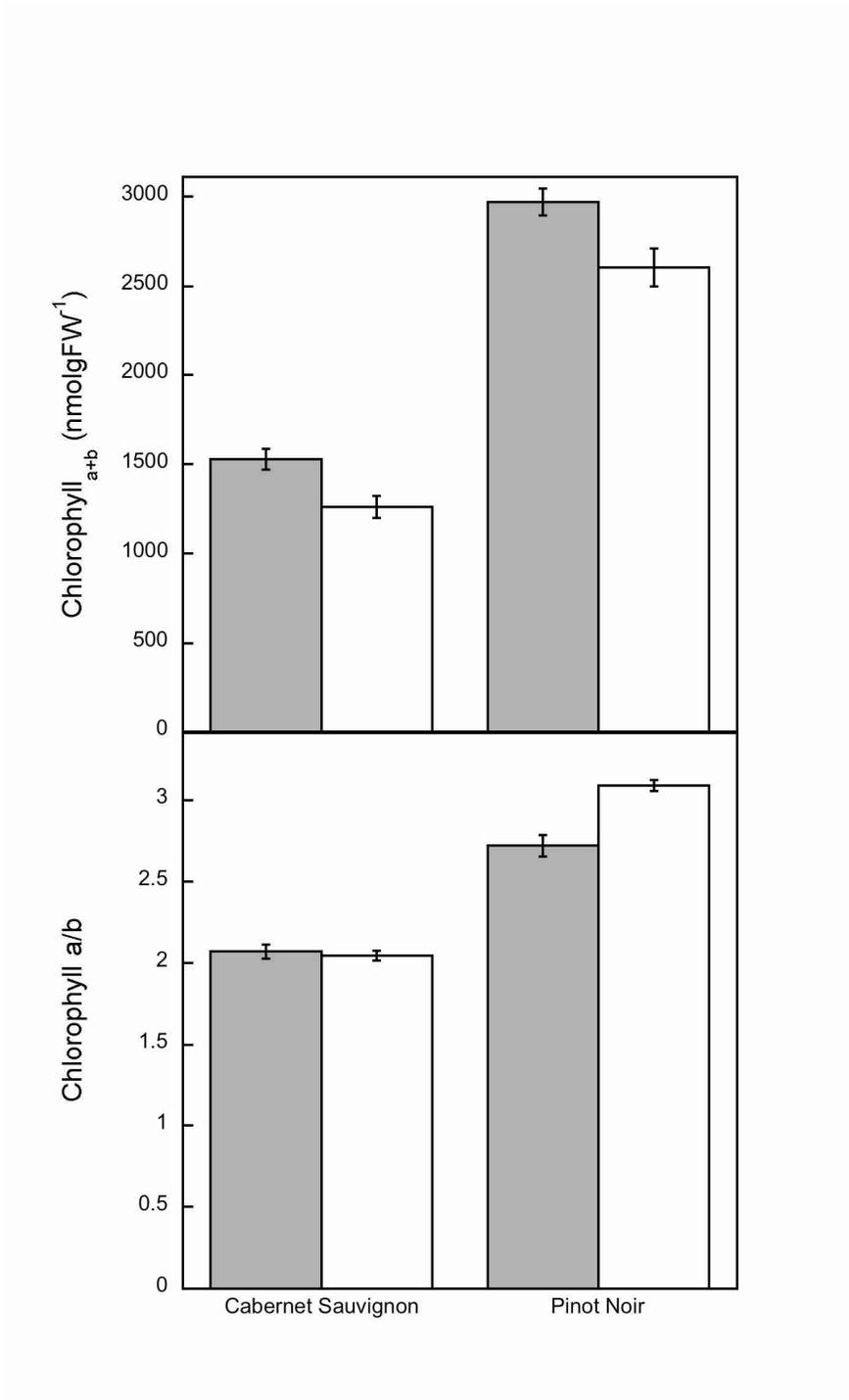


Figure 3. Effect of phylloxera infestation on carotenoid composition of leaves of Cabernet Sauvignon and Pinot Noir grapevine varieties grown in the field. Total carotenoid/chlorophyll (A), β -carotene/TChl (B), xanthophyll cycle (V+A+Z)/TChl (C) and portion of the xanthophyll cycle in the photoprotective forms (A+Z)/(V+A+Z). Solid bars denote uninfested vines whilst open bars show phylloxera infested vines. Data represent mean \pm SEM (CS n=20, PN n=8).

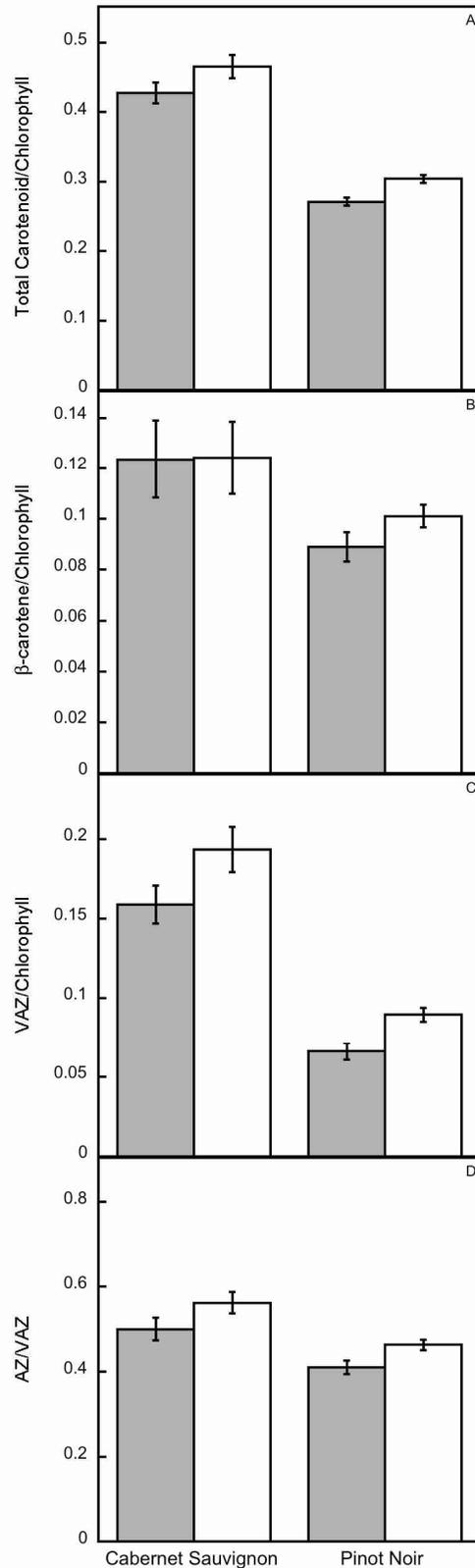


Figure 4. Total carotenoid/chlorophyll as a function of the photochemical reflectance index (PRI) for Cabernet Sauvignon (squares) and Pinot Noir (triangles) grapevine varieties grown in the field. Infested vines are denoted by open symbols, uninfested by filled symbols. Equation of regression line is given in Table 1.

