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Grapevine phylloxera, detection, chlorophyll, carotenoids, xanthophyll cycle pigments, β-carotene, Vitis vinifera

Disciplines
Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

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Preliminary Investigations of Pigment Responses to Phylloxera Infestation.

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Abstract

Early detection of grape phylloxera (*Daktulosphaira vitifoliae*) infestation is vital for the implementation of post-outbreak quarantine in Australia. Remote sensing systems exploit changes in leaf pigment content associated with plant stress and offer a real possibility of a phylloxera specific detection system. Pre-visual, symptomatic changes in the pigment content of phylloxera infested grapevine leaves were investigated using high performance liquid chromatography (HPLC) as a potential aid to improve current phylloxera detection methods. A glasshouse trial was established to characterise the response of two grapevine varieties, *Vitis vinifera* L. 'Cabernet Sauvignon' and 'Shiraz', to phylloxera infestation, in a controlled environment. Field trials were conducted on two grapevine varieties, *V. vinifera* L. 'Cabernet Sauvignon' and 'Pinot Noir', at two sites, to compare grapevine response to phylloxera infestation 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. With further investigation the identification of grapevine leaf pigment responses to phylloxera infestation may prove useful for the rapid, non-invasive, detection of phylloxera in commercial vineyards.

INTRODUCTION

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) is recognised as the most devastating insect pest to viticultural industries worldwide. In Australia early detection of phylloxera infestations remains vital for the implementation of post-outbreak quarantine and management.

Symptoms of phylloxera infestation can be difficult to differentiate from those of other stress conditions and in a field situation stressful conditions usually arise from a combination of environmental factors such as high temperature and irradiance. Although the response of whole plants to deficits in water and nitrogen supply has been studied, very little is known about the changes which occur in plant leaf pigments as a result of insect feeding damage to the roots. Leaf pigment content can provide valuable information about the physiological performance of a plant (Sims and Gamon, 2002) and

therefore can be utilised in stress detection methods. In Australia, multispectral remote sensing techniques have been employed for assisting phylloxera detection since the mid-1990's (Frazier et al., 2004) and the potential use of hyperspectral remote sensing systems is being investigated (Renzullo et al., 2004). However, detection still requires ground-based verification to confirm infestations (Herbert et al., 2003).

Pre-visual, symptomatic changes in the pigment content of phylloxera infested grapevine leaves (*Vitis vinifera* L.'Shiraz', 'Cabernet Sauvignon' and 'Pinot Noir') were investigated using high performance liquid chromatography (HPLC) as a potential aid to improving current phylloxera detection methods.

MATERIAL AND METHODS

Glasshouse Experiment

Potted, ungrafted *V. vinifera* L. 'Cabernet Sauvignon' and 'Shiraz' were infested with 20 phylloxera eggs, and grown under controlled glasshouse conditions during March – June 2004. Plant material, trial design and phylloxera infestation method are described in Blanchfield *et al*., 2006. The leaf pigment content of phylloxera infested and uninfested grapevines were compared at the end of a nine week period.

Field Experiment

Two ungrafted grapevine varieties, *V. vinifera* L. 'Cabernet Sauvignon' and 'Pinot Noir', were grown in commercial vineyards which allowed the comparison of phylloxera infested and uninfested vines, of the same variety, in adjacent blocks, under field conditions as described in Blanchfield *et al*., 2006. Sampling was conducted over two growing seasons (2004 and 2005) for the Cabernet Sauvignon variety and one growing season (2005) for the Pinot Noir variety from bunch closure (January) to just prior to harvest (March).

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) using a SphereClone 5u ODS1 column (Phenomenex, Sydney, Australia). Solvent A was adjusted to acetonitrile:methanol:Tris-HCL buffer 0.1M pH 8.0; 85:9:6. Solvent B remained methanol:hexane 80:20.

Statistical analysis

Differences in pigment content and pigment ratios of the glasshouse trial samples, as a function of variety, phylloxera infestation and treatment were analysed using ANOVA (Genstat, vers 8.1, Lawes Agricultural Trust, UK). Repeated measures ANOVA was used to investigate relationships in the pigment content and pigment ratios of the field collected samples as a function of variety, time and phylloxera infestation.

RESULTS

Glasshouse Trial

The leaf pigment content of the two varieties were analysed independently as they had significantly different total chlorophyll and total carotenoid concentrations. Total chlorophyll and total carotenoid concentrations were significantly reduced in leaves of the phylloxera infested Shiraz group ($p=0.007$ and $p=0.004$ respectively) but not in the

Cabernet Sauvignon variety, although the chlorophyll and carotenoid content of the Cabernet Sauvignon leaf samples were also reduced by phylloxera infestation. The ratio of total carotenoid to total chlorophyll was not effected by phylloxera infestation in either variety, neither was the β-carotene to total chlorophyll ratio nor the pigments of the xanthophyll cycle, of either variety (Blanchfield et al., 2006).

The ratio of the individual carotenoid pigments relative to the total carotenoid concentration was not significantly affected by phylloxera infestation in either variety at the end of the nine-week experimental period (Fig.1).

Field Samples

Analysis of pigment content was performed on individual varieties as significant differences in the pigment concentrations between the two varieties were observed (tables 1 and 2). Leaf samples from phylloxera-infested vines of both varieties had reduced chlorophyll content and reduced carotenoid content. Chlorophyll content was significantly lower ($P<0.05$) in Pinot Noir infested samples compared to uninfested samples and this decline was more significant in the Cabernet Sauvignon samples ($P<0.001$). Figure 2 shows the chlorophyll content of Cabernet Sauvignon and Pinot Noir leaves. In Cabernet Sauvignon the ratio of chlorophyll a to chlorophyll b increased over time with a larger increase in samples from infested plants (time by treatment interaction 0.05; Fig. 4a).

Total carotenoid content was significantly lower in infested samples of both varieties (Cabernet Sauvignon, $P<0.05$; Pinot Noir, $P<0.1$) (Fig 2). There was also a significant overall time interaction with a decline in carotenoid content recorded over time for both varieties ($P<0.05$).

For infested samples of Cabernet Sauvignon the decline in chlorophyll was larger than the decline in carotenoid at each sampling date resulting in a significantly higher ratio of total carotenoid to total chlorophyll (Table 2). For the Pinot Noir samples there was a significant treatment x time interaction with infested vines showing a reduction in this ratio from January to March and healthy vines showing an increase in the ratio from January to March (Fig. 3a). The VAZ to total chlorophyll ratio in Pinot Noir showed a similar significant trend (Fig. 3b). For Cabernet Sauvignon the VAZ to total chlorophyll ratio was slightly higher in infested leaves (0.01) but increased from January to March 2004 in all treatments (Table 2).

Other clear differences in the response of the two varieties varieties were observed when comparing the ratios of the photoprotective pigments β-carotene and zeaxanthin, which varied significantly in Cabernet Sauvignon samples but not Pinot Noir samples. In Cabernet Sauvignon in phylloxera-infested samples the ratio of β-carotene to chlorophyll increased over the entire sampling time but this increase was delayed until the second year in uninfested plants (Fig.4b). The proportion of the xanthophyll cycle pigment pool in the heat dissipating form, zeaxanthin, remained steady in infested samples throughout 2004 but increased from 2004-2005. In uninfested samples the proportion of zeaxanthin declined from January to March 2004 before increasing again in January 2005 indicating a more dynamic ability to photoprotect in the uninfested vines (Fig.4c). Figure 5 shows the effect of phylloxera infestation on the carotenoid composition of leaves from field grown Cabernet Sauvignon and Pinot Noir grapevines.

DISCUSSION

The carotenoid pigments were one of the foci of this study because of their role in the protection of the pigment-protein complexes in relation to the effects of stress (Young and Britton, 1990). Increases in the ratio of the total xanthophyll cycle carotenoids to chlorophyll are also observed under stressful conditions (Demmig-Adams and Adams, 1996).

The reduction in leaf chlorophyll content as a result of phylloxera infestation showed similar trends in both the field and glasshouse grown grapevines. Changes in the leaf pigment composition, such as an increase in the total carotenoid to total chlorophyll ratio and an increase in the xanthophyll cycle pigment pool compared to total carotenoids was also observed, albeit not always significant. The trends observed are consistent with pigment changes associated with plant stress and suggest further research is warranted. The glasshouse trial was limited to a nine-week period, and although significant changes were only observed in the Shiraz variety, similar changes in the pigment content of the Cabernet Sauvignon variety were observed and suggest significant results would have been observed over an increased time period. The artificial lighting environment inside the glasshouse does not provide illumination as severe as would be experienced under field conditions. Grapevines are considered to be adapted to high irradiance (Flexas et al., 2001), pigment responses to stress caused by phylloxera infestation may therefore be more subtle in a glasshouse environment than under field conditions.

In the field situation several variables could impact on the status of vine health and physiology when comparing pigment ratios or concentrations of phylloxera infested vines versus uninfested vines. These include date since infestation, phylloxera genetic status and level of abundance on grapevine, grapevine management (nutrient and water) and climate and root health. The phylloxera infestation at the field site where the Cabernet Sauvignon grapevines were located was first detected in 2000 and are known to have the phylloxera genotype G1 (Corrie et al., 2002). This genotype has been shown to have relatively high abundance levels at this site (Herbert et al., 2006). Climate data was not recorded, however, environmental conditions in combination with vineyard management procedures employed at the site may be masking any additional stress caused by the phylloxera population. However significant differences between the infested and uninfested grapevines were clearly observed from samples collected at this site. Infested samples showed an enhanced stress effect, with more pronounced changes in the chlorophyll a/b, the ratio of β-carotene to chlorophyll and the proportion of the xanthophyll cycle in the photoprotective form, zeaxanthin, than uninfested samples. In addition the results for zeaxanthin suggest that whereas uninfested leaves showed a typical dynamic seasonal response from January to March, the infested samples appear to maintain higher stress levels throughout the growing season.

The phylloxera infestation at the Pinot Noir field site was first detected in December 2003 and the phylloxera genotype has been identified as G20 (Corrie et al., 2002) which is known to be less virulent than G1 (Powell, 2006). Significant differences in pigment concentrations were observed in samples collected in 2005 and have been recorded at the same site in the previous season (Blanchfield et al., 2006). However, the differences are not as marked as in the Upton site samples, which may suggest the phylloxera population did not reach a critical size.

Overall changes in pigment content were detected prior to visible leaf changes developing, hence there is a real possibility they can be used to further enhance current detection methods used for phylloxera infestations. If changes in pigment composition, specific to phylloxera infestation, were identified and linked to high spectral resolution

reflectance spectrometry this technique could offer a faster, reliable alternative for regular testing of commercial vineyards for phylloxera infestations.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Blanchfield, A.L., Robinson, S.A., Renzullo, L.J. and Powell, K.S. (2006). Phylloxera-infested grapevines have reduced chlorophyll and increased photoprotective pigment content – can leaf pigment composition aid pest detection? Funct. Plant Biol. 33: 507-514.

Corrie, A.M., Crozier, R.H., van Heeswijck, R., Hoffman, A.A., 2002. Clonal reproduction and population genetic structure of grape phylloxera, *Daktulosphaira vitifoliae*, in Australia. Heredity 88, 203-211.

Demmig-Adams, B. and Adams, W.W. (1996). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci. 1: 21-26.

Dunn, J.L., Turnbull, J.D. and Robinson, S.A. (2004) Comparison of solvent regimes for the extraction of photosynthetic pigments from leaves of higher plants. Funct. Plant Biol. 31: 195-202.

Flexas, J., Hendrickson, L. and Chow, W.S. (2001). Photoinactivation of photosystem II in high light-acclimated grapevines. Aust. J. Plant Physiol. 28: 755-764.

Frazier, P., Whiting, J., Powell, K, and Lamb, D. (2004). Characterising the development of grape phylloxera infestation with multi-temporal near-infrared aerial photography. Aust. NZ. Grapegrower Winemaker. (Annual Technical Issue): 133-142.

Gilmore, A.M. and Yamamoto, H.Y. (1991). Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C18 high-performance liquid chromatographic column. J. Chromatography 543: 137-145.

Herbert, K., Powell, K.S., Hoffman, A., Parsons, Y., Ophel-Keller, K. and van Heeswijck, R. (2003). Early detection of phylloxera - present and future directions. Aust. NZ. Grapegrower Winemaker 473: 93-96.

Herbert, K. S., Hoffmann, A. A., Powell., K.S. 2006. Changes in grape phylloxera abundance in ungrafted vineyards from south-eastern Australia. J. Econ. Ento. (In press).

Sims, D.A. and Gamon, J.A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sensing Environ. 81: 337-354.

Powell, K.S., 2006. Sustainable long-term stratergies for phylloxera management under Australian conditions. GWRDC Final Report. pp 158.

Renzullo, L., Held, A., Powell, K.S. and Blanchfield, A. 2004. Remote sensing phylloxera infestation: current capabilities and future possibilities for early detection Aust. NZ. Grapegrower Winemaker. (32nd Annual Technical Issue):. 126-130.

Young, A. and Britton, G. (1990) Carotenoids and stress, in Stress responses in plants: adaptation and acclimation mechanisms (Alscher, R.G. and Cumming, J.R. ed.), pp 87-112, Wiley-Liss

Table 1: Summary of repeated measures ANOVA from Pinot Noir field collected grapevine leaf samples at Buckland Valley field site

	Tcarot	Tchl	AZ_VAZ	BC_Tchl	Tcarot_Tchl	VAZ_Tchl	Z_VAZ	chla_chlb
Treatment								
Infested	767.0	2255	0.792	0.108	0.347	0.127	0.672	3.464
Uninfested	907.0	2597	0.756	0.108	0.343	0.123	0.632	3.473
Time								
T1	791.0	2317	0.758	0.110	0.345	0.117	0.627	3.415
T2	883.0	2535	0.790	0.106	0.345	0.132	0.677	3.522
Treatment x Time								
Infested.T1	743.0	2053	0.787	0.112	0.363	0.130	0.642	3.513
Infested.T2	792.0	2457	0.797	0.104	0.330	0.123	0.703	3.415
Uninfested.T1	840.0	2581	0.729	0.109	0.327	0.105	0.611	3.317
Uninfested.T2	973.0	2613	0.782	0.107	0.360	0.141	0.652	3.629
LSD (Treatment)	151.9	317.5	0.058	0.002	0.009	0.010	0.081	0.161
LSD (Time)	75.3	229.4	0.058	0.006	0.024	0.019	0.097	0.352
LSD (Tr x Ti)	106.6	324.4	0.082	0.008	0.035	0.027	0.137	0.498

LSD = least significant difference at the 5% level of significance

Table 2: Summary of repeated measures ANOVA from Cabernet Sauvignon field collected grapevine leaf samples at Upton field site

	Tcarot	Tchl	AZ_VAZ	BC_Tchl	Tcarot_Tchl	VAZ_Tchl	Z_VAZ	chla_chlb
Treatment								
Infested	555.0	1306	0.634	0.119	0.438	0.178	0.393	2.52
Uninfested	640.0	1587	0.538	0.118	0.395	0.141	0.341	2.20
Time								
T1	641.0	1514	0.536	0.128	0.431	0.161	0.300	1.93
T2	585.0	1347	0.532	0.123	0.447	0.173	0.274	2.13
T3	567.0	1478	0.691	0.107	0.373	0.144	0.526	3.03
Treatment x Time								
Infested.T1	566.0	1282	0.552	0.125	0.449	0.175	0.294	1.98
Infested.T2	545.0	1237	0.571	0.123	0.468	0.189	0.297	2.11
Infested.T3	553.0	1398	0.780	0.110	0.398	0.170	0.587	3.46
Uninfested.T1	716.0	1747	0.520	0.130	0.413	0.148	0.306	1.87
Uninfested.T2	625.0	1456	0.493	0.122	0.426	0.157	0.252	2.14
Uninfested.T3	581.0	1558	0.602	0.103	0.348	0.119	0.466	2.60
LSD (Treatment)	65.1	107	0.059	0.011	0.043	0.041	0.121	0.17
LSD (Time)	68.9	192	0.030	0.005	0.038	0.023	0.050	0.19
LSD (Tr x Ti)	83.2	272	0.042	0.007	0.053	0.032	0.071	0.26

LSD = least significant difference at the 5% level of significance

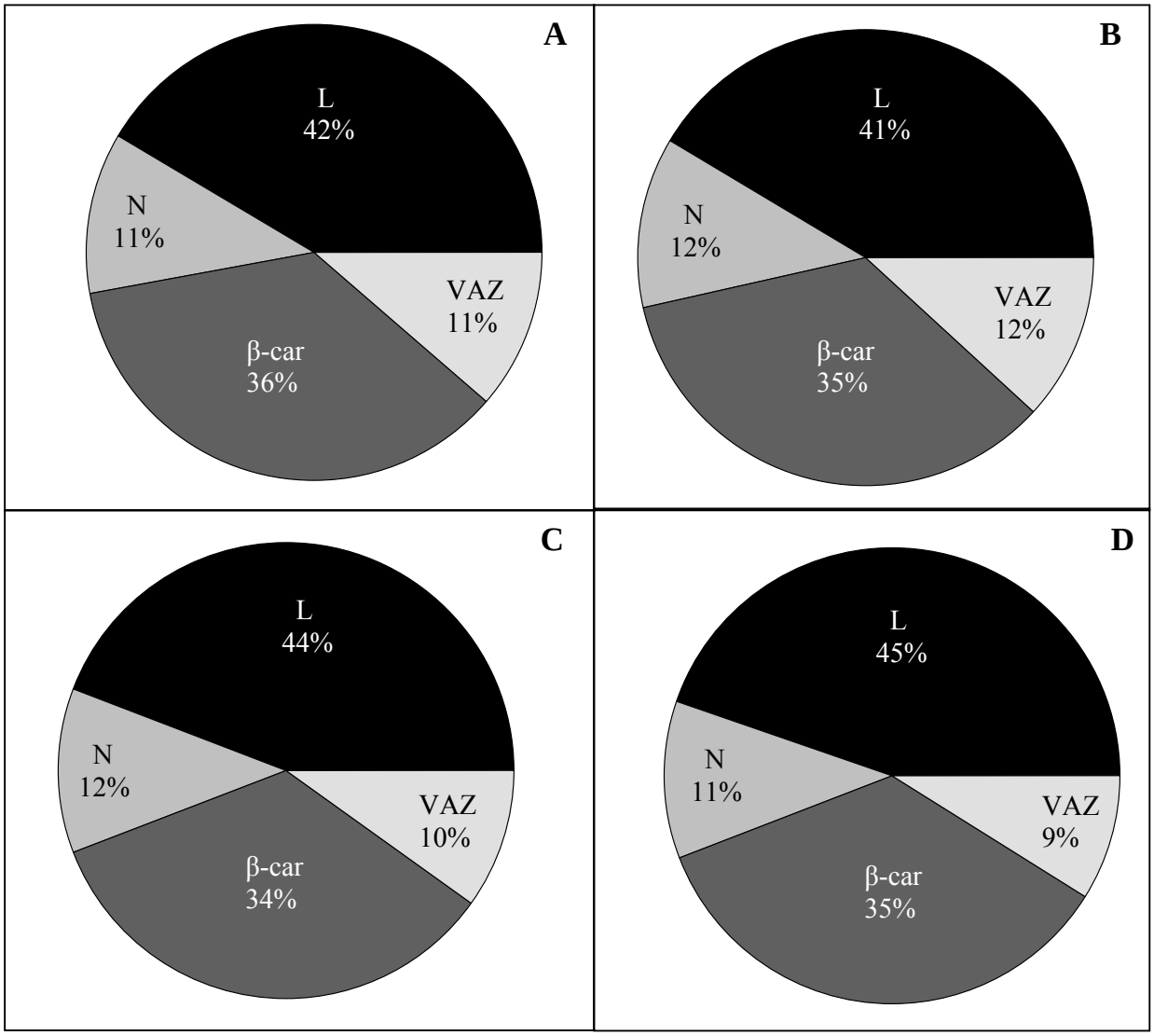
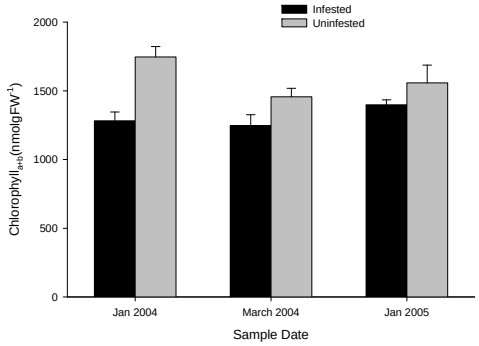
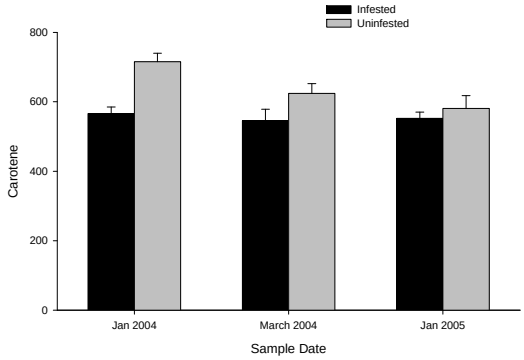


Figure 1. The effect of phylloxera infestation on the carotenoid pigments of Cabernet Sauvignon (healthy control (A), phylloxera infested treatment (B)) and Shiraz (healthy control (C), phylloxera infested treatment (D)) grapevine varieties grown for 9 weeks in a controlled glasshouse environment. The areas of the pie chart segments represent the concentration of lutein (L), neoxanthin (N), β-carotene (β-car) and xanthophyll cycle pigments (violaxanthin+antheraxanthin+zeaxanthin, VAZ) relative to total carotenoid concentration. Data represent mean, n=8.

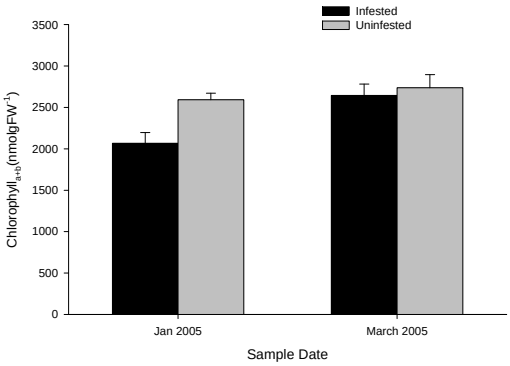
(a)


Infested
Uninfested
2000
1500
1000
500
0
Chlorophyll$_{a+b}$(nmolgFW^{-1})
Jan 2004
March 2004
Jan 2005
Sample Date

(b)


Infested
Uninfested
800
600
400
200
0
Carotene
Jan 2004
March 2004
Jan 2005
Sample Date

(c)


Infested
Uninfested
3500
3000
2500
2000
1500
1000
500
0
Chlorophyll$_{a+b}$ (nmolgFW^{-1})
Jan 2005
March 2005
Sample Date

(d)

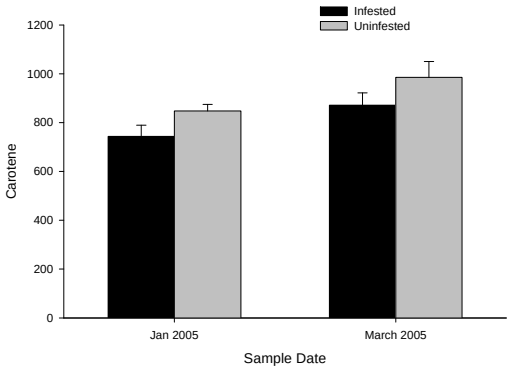
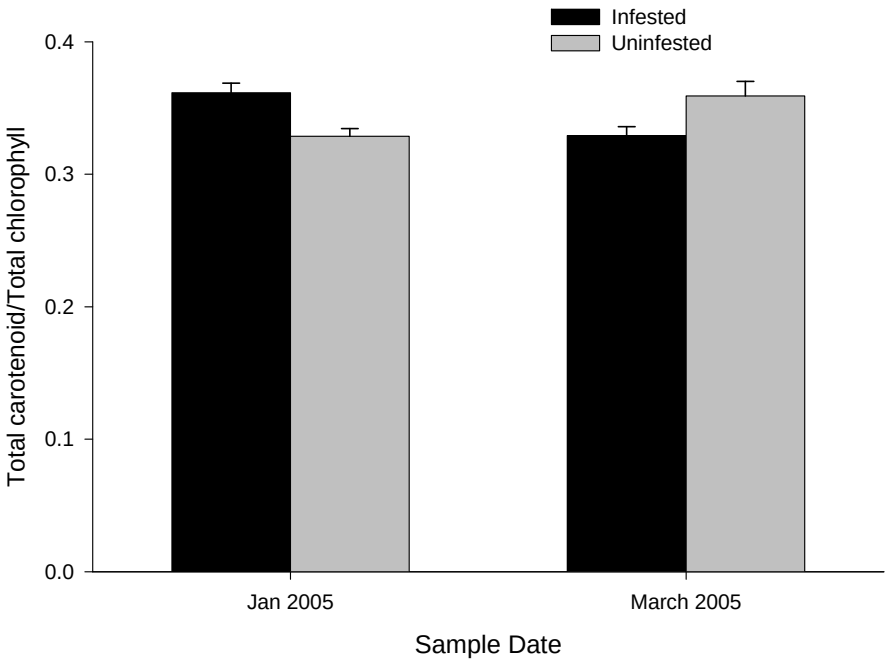

Infested
Uninfested
1200
1000
800
600
400
200
0
Carotene
Jan 2005
March 2005
Sample Date

Figure 2. The effect of phylloxera infestation on the chlorophyll and carotene content of field-grown *V. vinifera* (a, b) 'Cabernet Sauvignon', sampled in January 2004 and 2005 and March 2004 and (c, d) Pinot Noir, sampled in January 2005 and March 2005, grapevine varieties. Data represent mean ±SEM (Cabernet Sauvignon n=12; Pinot Noir n=8)

(a)



(b)

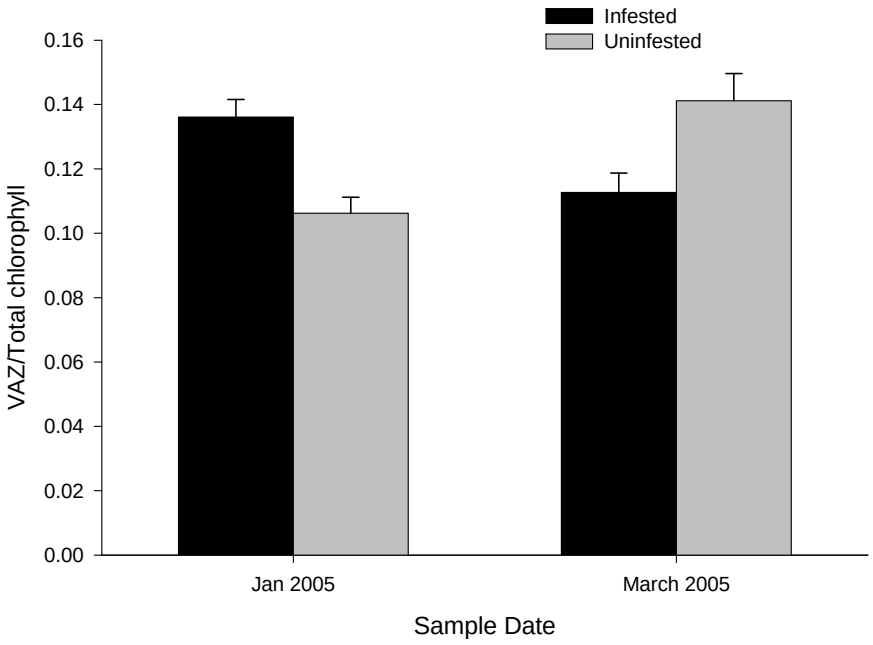
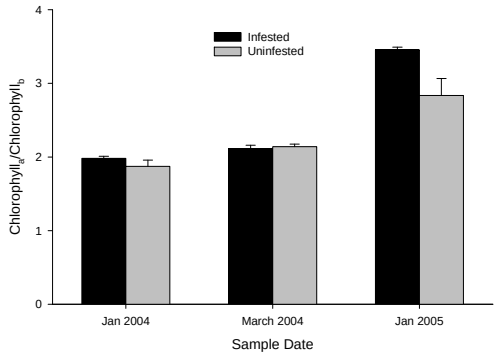


Figure 3. The effect of phylloxera infestation on (a) carotene/chlorophyll ratio and (b) VAZ/chlorophyll ratio of field-grown *V. vinifera* 'Pinot Noir' grapevine varieties, sampled in January 2005 and March 2005,. Data represent mean ±SEM where n=8.

(a)



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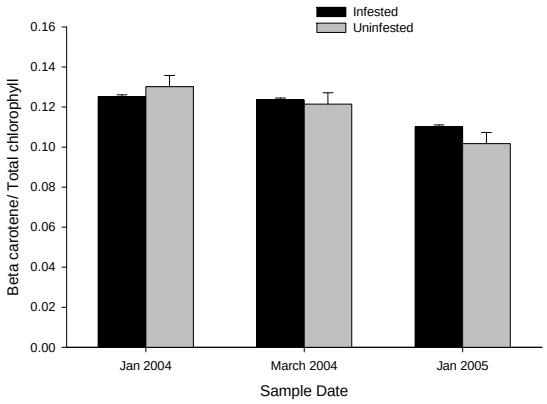

(c)

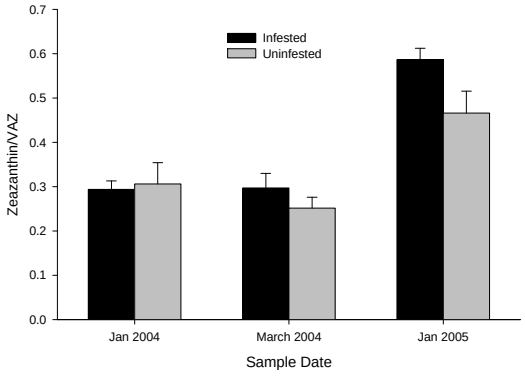

Figure 4. The effect of phylloxera infestation on (a) chlorophyll a/chlorophyll b ratio and (b) β-carotene/chlorophyll and (c) zeaxanthin/VAZ ratio of field-grown *V. vinifera* 'Cabernet Sauvignon' grapevine varieties., sampled in January 2004 and 2005 and March 2004, Data represent the mean ± SEM; where n=12; VAZ= (violaxanthin+antheraxanthin+zeaxanthin).

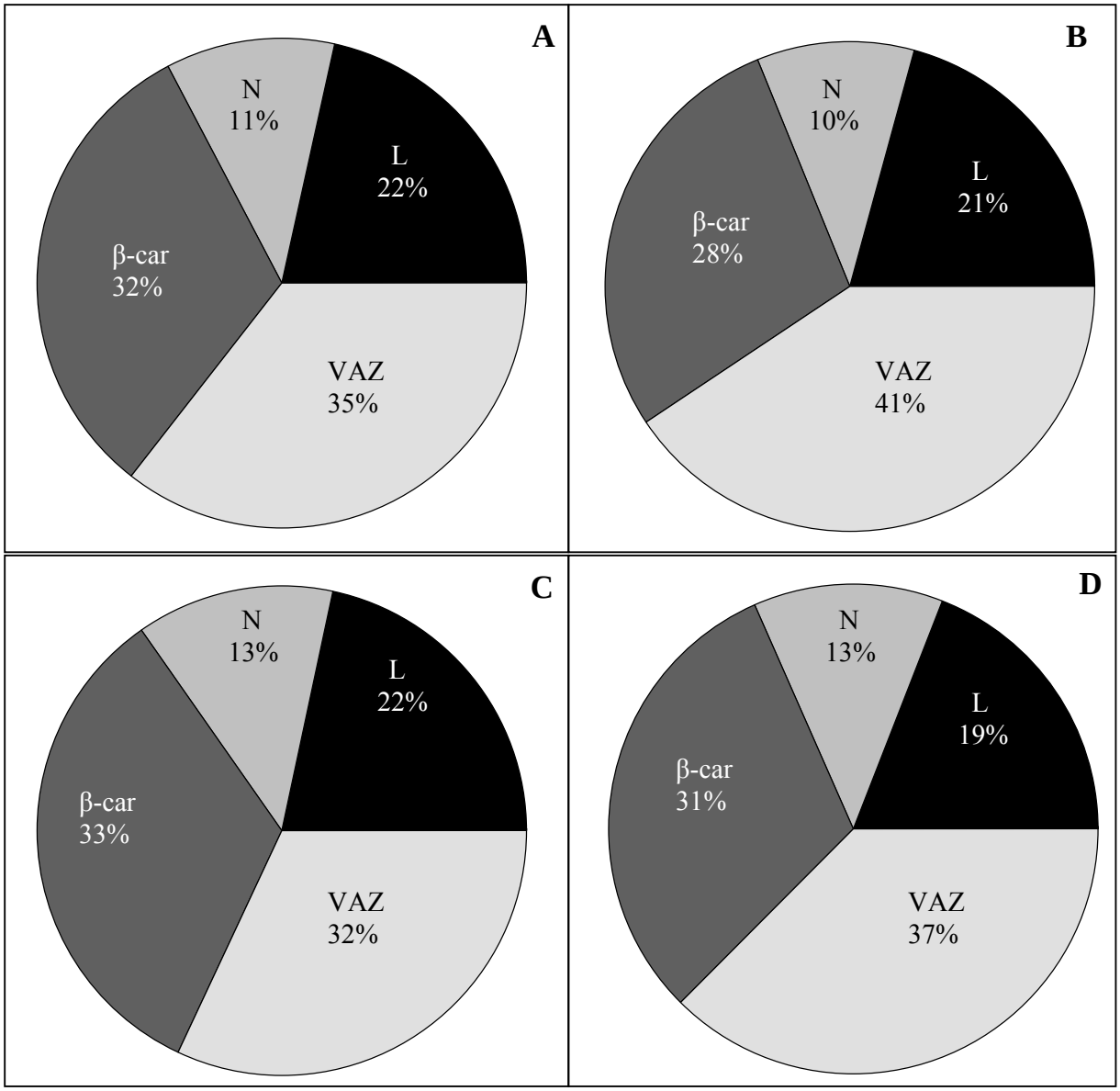

Figure 5. Effect of phylloxera infestation on carotenoid composition of leaves of field-grown Cabernet Sauvignon (A uninfested grapevines; B phylloxera infested grapevines) sampled in January 2004 and Pinot Noir (C uninfested grapevines; D infested grapevines) sampled in January 2005. The areas of the pie chart segments represent the concentration of lutein (L), neoxanthin (N), β-carotene (β-car) and xanthophyll cycle pigments (violaxanthin + antheraxanthin + zeaxanthin, VAZ) relative to total carotenoid concentration. Data represent mean, n=12.