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Response of Grape Leaf Spectra to Phylloxera Infestation

Lee F. Johnson

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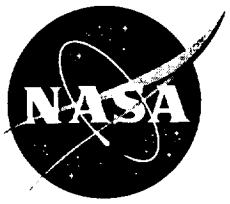

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RESPONSE OF GRAPE LEAF SPECTRA TO PHYLLOXERA INFESTATION

Lee F. Johnson

SUMMARY

During the 1993 growing season, leaf reflectance and chlorophyll concentrations were monitored with respect to phylloxera (root-louse) infestation in a Napa Valley (California) vineyard. Study plots were established in areas of severely infested, mildly infested, and uninfested sections of the vineyard. A hand-held chlorophyll meter, measuring leaf transmittance of near-infrared and red light, confirmed that reduced foliar chlorophyll concentrations were symptomatic of phylloxera stress in the sample vines. Bidirectional reflectance measurements of green and near-infrared light, taken on fresh leaves with a laboratory spectrophotometer, were related to chlorophyll concentration but did not allow discrimination of mildly infested from uninfested vines.

INTRODUCTION

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) infestation affects a number of California grape regions and in recent years devastated many Napa and Sonoma County vineyards (Granett *et al.*, 1991, 1996). The parasite damages the root system, depriving the vine of water and nutrients, thus posing immediate management problems in the form of reduced vine growth, decreased grape yield, retarded grape maturation, and lower wine quality. The infestation spreads rapidly throughout fields, and individual vines die within three to five years of initial infestation.

Pesticide application is ineffective for phylloxera control, due to the deep rooting zones characteristic of grapevines, and to the high rate of phylloxera reproduction. No effective biological control agent is known. Intervention practices (more severe pruning, additional irrigation, and fertilization) may serve to lessen phylloxera impact in the short term, but the only long-term solution is to remove the infested vines and replant with a more resistant rootstock. Replanting is generally done on a per-field basis, and is based on decisions concerning the economic viability of the field as a whole. Improved knowledge of the current and potential future extent of phylloxera infestation would enable growers to make more informed short- and long-term management decisions.

Phylloxera infestation is known to cause reductions in vine growth (Granett *et al.*, 1987; Wildman *et al.*, 1988). Johnson *et al.* (1996) and Lobitz *et al.* (1997) describe the relationship between crop canopy reflectance (measured by an airborne multispectral digital imaging system) and canopy density under various degrees of phylloxera stress. Decreased foliar nitrogen and chlorophyll concentrations are also known symptoms of phylloxera stress (Davidson and Nougaret, 1921). The current report describes the influence of phylloxera-related biochemical changes on spectra of individual leaves, and

examines the possibility of using leaf spectral analysis for "pre-visual" determination of phylloxera-induced stress. As leaf chlorosis is a common crop stress indicator, results here are of potential interest to the broader viticultural and agricultural communities.

METHODS

Study Site

Shortly before the 1993 growing season (early May), a partially phylloxera-infested field of Cabernet Sauvignon vines grafted to AxR#1 rootstock was chosen as the study site. The 12 acre field, located near Oakville CA (USA), was planted in 1981 in Clear Lake clay and Bale clay loams. The vines were trained on a standard two-wire trellis without shoot positioning. Rows were 3.65 m apart, oriented northeast to southwest, with a vine spacing within-row of 2.43 m. The site was clean cultivated to remove all vegetation except grapevines.

Determination of Infestation Level

Nine study plots were established at the study site, with each plot containing 40 vines (4 rows, 10 vines per row). Three plots were established in each of three infestation categories: infested/visually symptomatic, infested/visually asymptomatic ("pre-visual"), and uninfested. The plots were delimited on the basis of grower knowledge, a 1992 aerial infrared photograph, and a phylloxera survey. The phylloxera survey involved excavation of several shallow roots from beneath drip irrigation emitters to a depth of 18-45 cm, and use of a magnifying lens for visual examination for phylloxera presence. Vines received a rating based on the highest population found among the root pieces examined, according to the criteria of Table 1. Eight vines, four in each of the two middle rows of each plot, were designated as "data vines" for spectral analysis. To avoid damaging roots and possibly introducing additional stress on the data vines, phylloxera ratings were assigned as the mean rating of two immediately adjacent vines, one in the same row as the data vine and one in an adjoining row. Mean phylloxera ratings per category are shown in Table 2.

It is possible or even likely that as the growing season progressed, certain vines migrated from category 3 to category 2, and from category 2 to category 1 because of the progression of infestation and its effects. Thus the infestation categories are to be considered valid only at and near the time of designation (*i.e.*, May 1993). In this report categories will be discussed only in light of data collected in May 1993.

Chlorophyll Measurement

Monthly field measurements on all data vines were made with a Minolta SPAD-502 chlorophyll meter (Minolta Corp., Ramsey NJ) throughout the 1993 season. The meter operates by *in-vivo* measurement of light transmittance through the leaf in two spectral channels centered at 650 nm and 940 nm, and has been used to evaluate leaf chlorophyll concentration in several plant species (Yadava, 1986; Candolfi-Vasconcelos et al., 1994; Earl and Tollenaar, 1997).

Reported here are results from early- (18 May), mid- (26 July), and late-season (20 October). The measurements were made on one leaf per data vine, located two nodes above the second grape cluster on a vigorous shoot on the southeast side of the vine. Six Soil Plant Analysis Development (SPAD) readings were taken at various locations on the leaf surface and then averaged to represent the value of each data vine. Each average

SPAD reading was then converted to *in-vivo* chlorophyll concentration (mg/cm^2) based upon a regression relationship (CHL = 0.001605 * SPAD – 0.009951, $R^2 = 0.91$) reported by DeBenedictis *et al.* (1995) and Baldy *et al.* (1996).

Leaf Reflectance Measurement

Immediately after acquiring the SPAD readings, each sample leaf was clipped, placed in a freezer bag, and stored in a dark, chilled cooler chest for transport to the laboratory. Within 12 hours, spectral measurements were made on the leaves with an NIRSystems Model 6500 spectrophotometer (Silver Spring, MD). The NIRS6500 measured leaf bidirectional reflectance (%) throughout the 400-2500 nm region (bandwidth = 10 nm, sampling interval = 2 nm). Measurements were made of single leaf thickness against a white background. Two variables were extracted from the spectral dataset: (1) green peak (GP), defined as the reflectance amplitude (%) of green light (550 nm), and (2) red-edge inflection point (REIP). REIP is defined as the wavelength of maximum slope in the "red edge" spectral region, which is transitional between visible and near-infrared reflectance. In this study, REIPs occurred between 700-725 nm.

RESULTS

Chlorophyll Concentration vs. Infestation Category

Mean chlorophyll concentrations in May 1993 were 0.043, 0.051, and 0.055 mg/cm^2 for infestation categories 1, 2 and 3, respectively (Table 2). Single factor analysis of variance (ANOVA) showed that chlorophyll concentration varied significantly (0.01 level) among the May 1993 infestation categories (Table 3a). A means comparison showed that categories 2 and 3 differed significantly at the 0.025 level (Table 3a). These results confirm leaf chlorosis as a symptom of phylloxera-induced stress.

Leaf Reflectance vs. Infestation Category

Mean GP and REIP from May 1993 were calculated per infestation category (Table 2). Both spectral measures showed significant (.01 level) differences among infestation levels (Tables 3b, 3c). Overall, GP was positively associated with higher infestation levels, because of reduced chlorophyll concentrations and consequently reduced energy absorption in stressed vines. REIP was negatively associated with infestation level as chlorophyll reduction narrowed the chlorophyll absorption feature centered in the red. However, in both cases category 2 was not significantly different from category 3, suggesting that GP and REIP of individual leaves are not useful for identifying "pre-visual" phylloxera stress.

Leaf Reflectance vs. Chlorophyll Concentration

GP was negatively correlated with chlorophyll concentration throughout the season (r = -0.85, -0.70, and -0.80 for 18 May, 26 July, and 20 October, respectively) (Figures 1a, 2a, 3a). REIP was positively correlated with chlorophyll concentration throughout the season (r = 0.86, 0.71, and 0.86 for the same three dates, respectively) (Figures 1b, 2b, 3b).

CONCLUSIONS

Consistent with previous research and conventional thought, the current study found reduced foliar chlorophyll as a symptom of phylloxera-induced stress in the sampled vines. The study further found that grape leaf reflectance responded to phylloxera-induced stress with increased reflectance of green light and also by shifts toward shorter wavelengths of the red-edge inflection point. These expressions of leaf reflectance did not, however, provide a basis for discriminating "pre-visual" phylloxera stress from uninfested vines.

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Table 1. Criteria for phylloxera rating.

Rating	Observation
0	No phylloxera found
1	Phylloxera only on rootlets, or one or two individuals on older (than one year) roots
2	Individual phylloxera scattered among older roots, or one or two colonies on older roots
3	Several colonies established on older roots
4	Large populations of phylloxera present on older roots

Table 2. Per-category statistics for phylloxera ratings (unitless), leaf chlorophyll concentration (mg/cm^2), green peak reflectance (%) and red-edge inflection point (nm). Categories: 1 = infested/visually symptomatic (severe); 2 = infested/visually asymptomatic (mild); 3 = uninfested. All measurements May 1993.

	mean	std. dev.	std. error	min	max	n
Phlx ratings						
Cat 1	1.46	0.69	0.14	0.5	2.5	24
Cat 2	0.50	0.51	0.10	0.0	1.5	24
Cat 3	0.00	--	--	0.0	0.0	24
Chlorophyll						
Cat 1	0.043	0.004	0.0008	0.036	0.050	24
Cat 2	0.051	0.007	0.0015	0.040	0.062	24
Cat 3	0.055	0.005	0.001	0.043	0.064	24
Green peak refl.						
Cat 1	16.87	1.27	0.26	13.99	18.83	24
Cat 2	15.17	1.67	0.34	12.42	18.27	24
Cat 3	14.90	1.57	0.32	13.16	18.75	24
Red-edge infl. pt.						
Cat 1	719.0	2.3	0.47	715	723	24
Cat 2	721.4	2.4	0.50	717	725	24
Cat 3	722.3	2.5	0.51	715	725	24

Table 3. ANOVA results, 5/18/93 sampling.

a) Chlorophyll concentration

Source of variation	SS	df	MS	F_S	prob
Treatments	0.0017	2	0.00087	27.6	<.01
2 vs. 3	*0.00016*	*1*	*0.00016*	*5.3*	*<.025*
Within	0.0021	69	0.00003		
Total	0.0038	71			

b) Green peak

Source of variation	SS	df	MS	F_S	prob
Treatments	0.0054	2	0.0027	11.9	<.01
2 vs. 3	*0.00009*	*1*	*0.00009*	*0.45*	*ns*
Within	0.016	69	0.0002		
Total	0.0214	71			

c) Red-edge inflection point

Source of variation	SS	df	MS	F_S	prob
Treatments	142.3	2	71.2	12.3	<.01
2 vs. 3	*10.1*	*1*	*10.1*	*1.8*	*ns*
Within	397.2	69	5.75		
Total	539.5	71			

tmt:
1 = infested/visually symptomatic (severe)
2 = infested/visually asymptomatic (mild, or "pre-visual")
3 = control (uninfested)

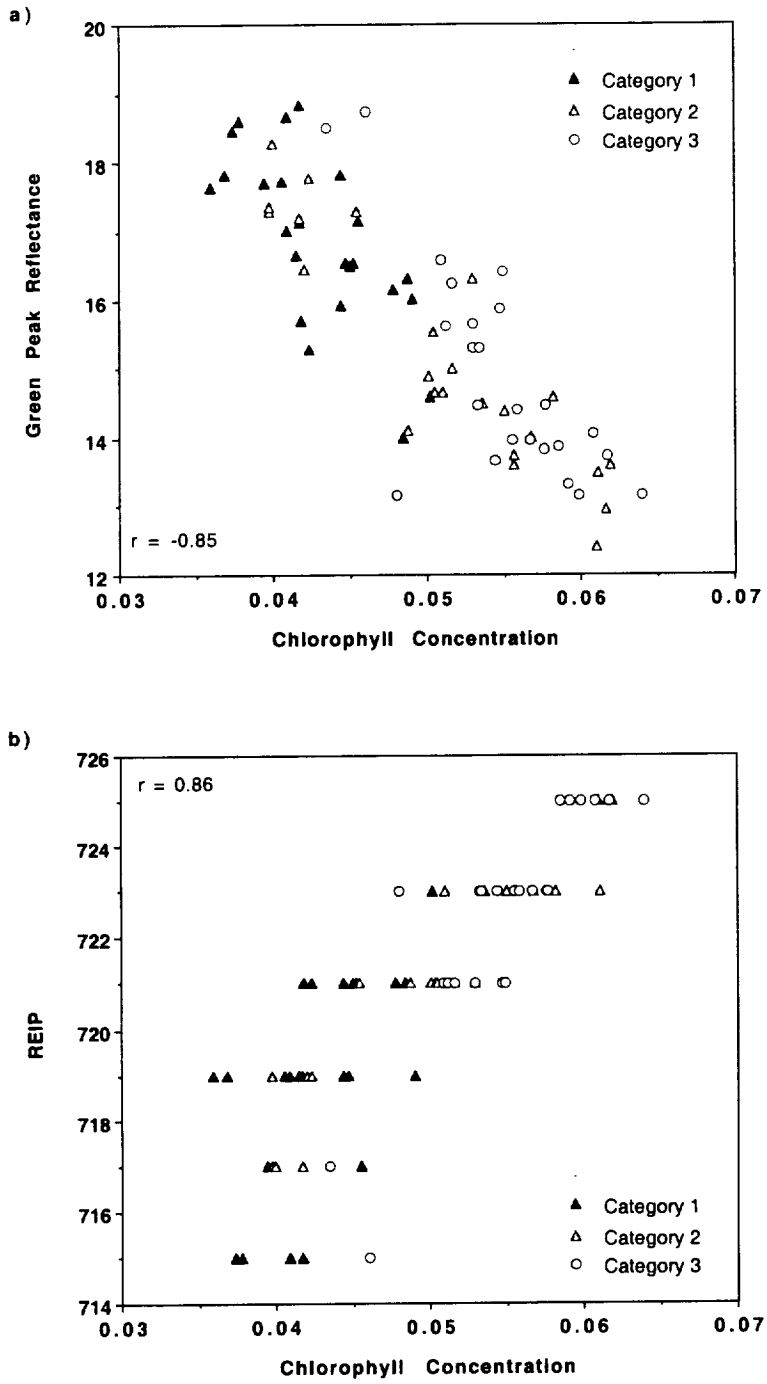

Figure 1. Leaf chlorophyll concentration (mg/cm^2) vs. leaf reflectance, 18 May 1993, severely infested (category 1), mildly infested (category 2) and uninfested (category 3) vines. a) Green peak reflectance (%), b) position of red-edge inflection point (nm).

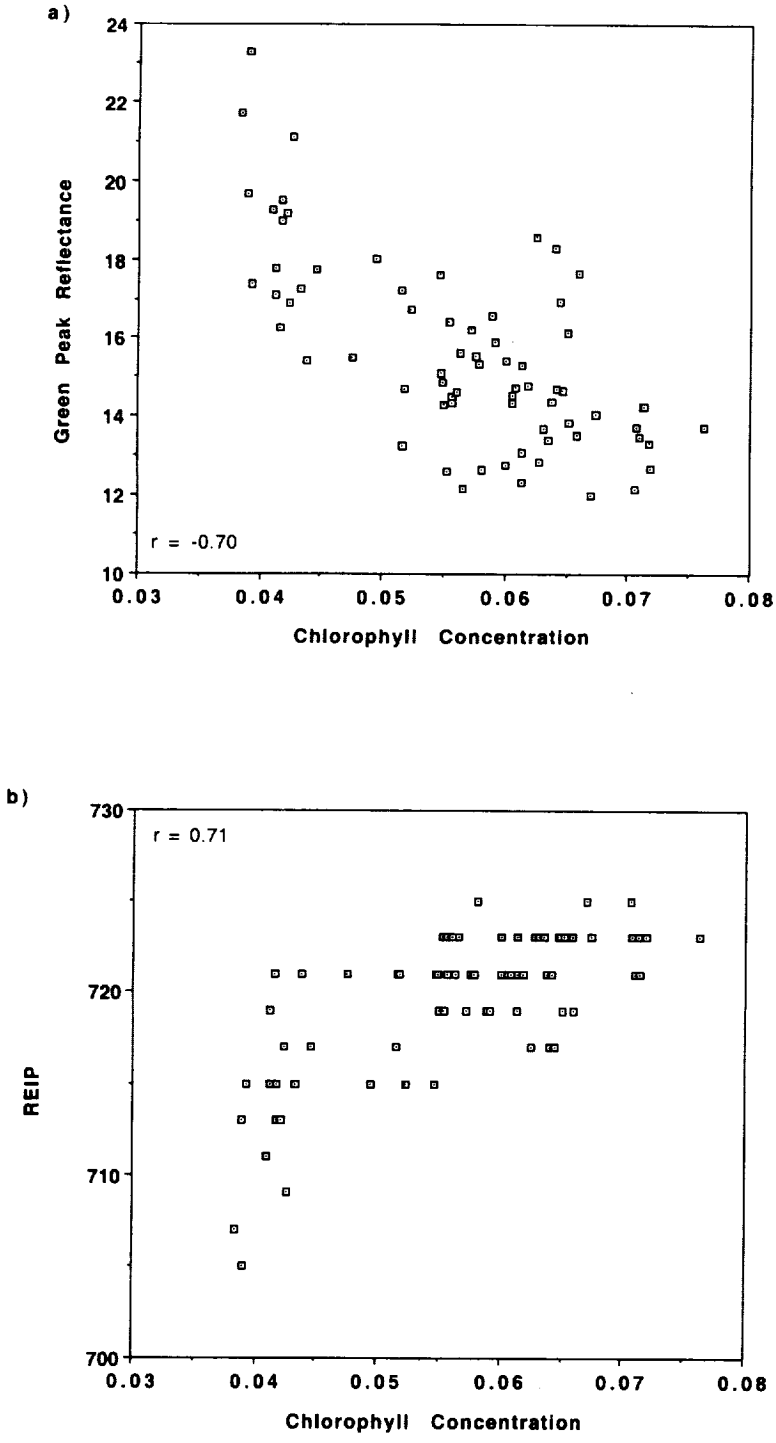

Figure 2. Leaf chlorophyll concentration (mg/cm^2) vs. leaf reflectance, 26 July 1993. a) Green peak reflectance (%), b) position of red-edge inflection point (nm).

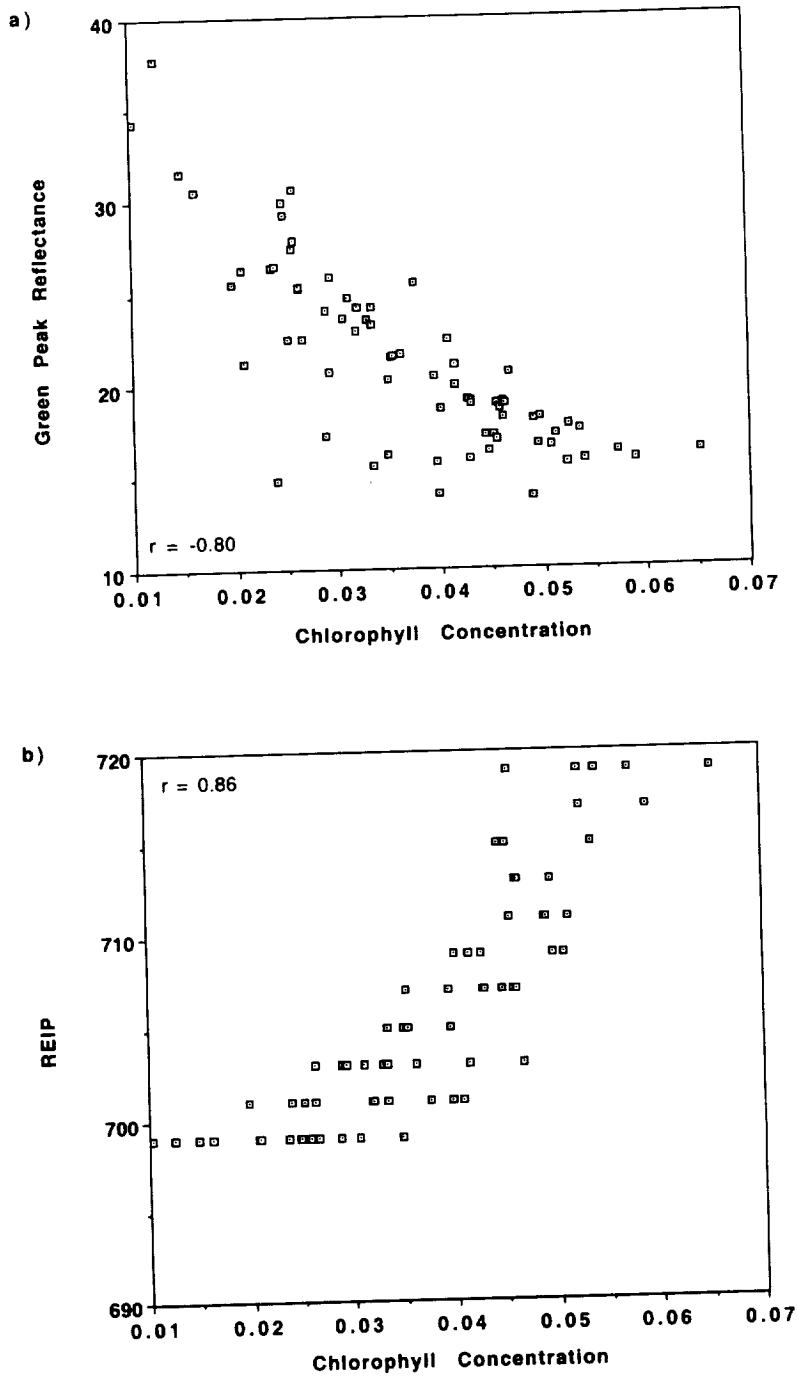

Figure 3. Leaf chlorophyll concentration (mg/cm^2) vs. leaf reflectance, 20 October 1993. a) Green peak reflectance (%), b) position of red-edge inflection point (nm).

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13. ABSTRACT *(Maximum 200 words)*

During the 1993 growing season, leaf reflectance and chlorophyll concentrations were monitored with respect to phylloxera (root-louse) infestation in a Napa Valley (California) vineyard. Study plots were established in areas of severely infested, mildly infested, and uninfested sections of the vineyard. A hand-held chlorophyll meter, measuring leaf transmittance of near-infrared and red light, confirmed that reduced foliar chlorophyll concentrations were symptomatic of phylloxera stress in the sample vines. Bidirectional reflectance measurements of green and near-infrared light, taken on fresh leaves with a laboratory spectrophotometer, were related to chlorophyll concentration but did not allow discrimination of mildly infested from uninfested vines.

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