# Shoot Heterogeneity Effects on Shiraz/Richter 99 Grapevines. III. Leaf Chlorophyll Content

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**In this study, the leaf chlorophyll content of normally developed and underdeveloped shoots was compared in an attempt to quantify the effect of shoot heterogeneity in a Shiraz/Richter 99 vineyard, located in the Stellenbosch area of the Western Cape, South Africa. Comparisons are also made between normally developed and underdeveloped shoots from shaded and well-exposed canopies. No positive correlation was found between the photosynthetic activity and the chlorophyll concentration of the leaves at five weeks after véraison. Equal amounts of chlorophyll per cm$^2$ and a non-significant difference in the assimilation rate were calculated for the leaves of normally developed and underdeveloped shoots. No significant differences were found between the shaded and well-exposed canopies. It therefore appears that it is the effective surface area per leaf or per shoot rather than the chlorophyll concentration or activity that may be responsible for any apparent difference in the photosynthetic output of the leaves from normally developed and underdeveloped shoots in shaded or well-exposed canopies.**

In higher plants, the light-absorbing pigments embedded in specialised internal membranes (collectively called the thylakoid system) inside the chloroplasts consist largely of two kinds of chlorophylls, of which the content of chlorophyll *a* is usually two to three times that of chlorophyll *b* (Hunter, 2001, and references therein). Light promotes some reactions during chlorophyll synthesis, such as the production of delta-amino levulinic acid (ALA – the precursor of protochlorophyllide *a*) and the conversion of protochlorophyllide *a* to chlorophyll *a*. The conversion of chlorophyll *a* to chlorophyll *b*, however, is not dependent on light.

Although the synthesis of chlorophyll is dependent on light, higher chlorophyll concentrations were found in interior leaves than in peripheral, sun-exposed peach leaves (Kappel & Flore, 1983; Marini & Marini, 1983). For bean plants, Crookston *et al*. (1975) also found more light-harvesting centres in the plastids of shaded leaves than in exposed leaves. Regarding the grapevine, Hunter and Visser (1989) reported higher chlorophyll concentrations for the interior, recently matured basal leaves in the early stages of development, while maximum chlorophyll *a* and *b* concentrations were reached later during the growth season as the leaves were progressively situated towards the periphery of the canopy and more towards the apical part of the shoot. The variation in chlorophyll concentration of the different leaves during the growing season was ascribed to the differences in leaf age.

According to Hunter and Visser (1989), no consistent relationship exists between chlorophyll concentration and the photosynthetic activity of exterior leaves. A better relationship was found between the chlorophyll concentration and photosynthetic activity of mature, interior canopy leaves that were exposed to conditions of lower light. They further state that factors such as the source:sink relationship, feedback inhibition of photosynthesis by end products and internal resistance to $CO_2$ transfer within the leaf probably regulate photosynthetic activity to a greater extent than chlorophyll concentration and light intensity.

The purpose of this study was to quantify the effect of shoot heterogeneity in a Shiraz/Richter 99 vineyard on the leaf chlorophyll content of normally developed and underdeveloped shoots in shaded and well-exposed canopies. Possible relationships between chlorophyll content and photosynthetic activity, as well as the effect of chlorophyll content on the assimilation rate of the leaves on normally developed and underdeveloped shoots, were investigated.

## MATERIALS AND METHODS

### Vineyard

A vineyard containing seven-year-old *Vitis vinifera* L. cv. Shiraz, clone SH1A, grafted onto Richter 99 (*Vitis berlandieri* x *Vitis rupestris*), clone RY2A, was used for this study. The vineyard is

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situated on the experimental farm of the Agricultural Research Council (ARC) Infruitec-Nietvoorbij near Stellenbosch in the Western Cape (Mediterranean climate). The vines are spaced 2.75 m × 1.5 m on a Glenrosa soil with a western aspect (26° slope) and trained onto a seven-wire lengthened Perold trellising system with movable canopy wires (VSP). Rows were orientated in a north-south direction.

Micro-sprinkler irrigation was applied at the pea-size berry and véraison stages. Pest and disease control was applied during the growth season according to the standard programme of the ARC.

**Experimental design**

The experiment comprised a completely randomised 2 × 2 factorial design. The two factors were: 1) the degree of canopy exposure (shaded and well exposed) and 2) the degree of shoot development (normal and underdeveloped).

Shaded and well-exposed canopies were created randomly in vines throughout the vineyard block. Only shoot positioning and topping were done to obtain shaded canopies, while additional suckering and leaf thinning (at berry set and pea-size berry on the basal half of the canopy/shoot) were applied to create well-exposed vines. Normally developed and underdeveloped shoots were selected on the basis of their comparative length and level of lignification at véraison. The average length of normally developed shoots was 105 cm to 115 cm, while the underdeveloped shoots were approximately 50 cm in length (Cloete *et al*., 2006). There were three replications for each of the four treatment combinations.

**Determinations of chlorophyll and photosynthesis**

The chlorophyll content of the leaves was determined according to the method described in Hunter and Visser (1989), using a LKB Biochrom Utrospec spectrophotometer (II E) and 2 mm quartz cells.

The equations used for calculating the chlorophyll concentration were as follows:

$$\text{Chlorophyll } a = (0.0127A_{663} - 0.00269A_{645}) \times 20\,000 \times 5 = \mu g/g \text{ fresh leaf mass}$$

$$\text{Chlorophyll } b = (0.0229A_{645} - 0.00468A_{663}) \times 20\,000 \times 5 = \mu g/g \text{ fresh leaf mass}$$

$$\text{Total chlorophyll} = (0.0202A_{645} + 0.00802A_{663}) \times 20\,000 \times 5 = \mu g/g \text{ fresh leaf mass}$$

The possible effect of leaf age on the leaf chlorophyll content was minimised by combining all the primary leaves on five shoots per treatment replicate when determining the chlorophyll concentration. Thus, it was primarily the effect of canopy exposure and shoot development on chlorophyll content that was determined.

The chlorophyll concentration (µg/g) of the fresh leaves was measured five weeks after véraison in 2002 and 2003. The chlorophyll content per unit leaf area (µg/cm$^2$) and the assimilation rate (µmol $CO_2$/µg chlorophyll/h) were only calculated in 2003. Leaf areas were measured with a LICOR LI-3100 area meter (Lincoln, Nebraska, USA). The assimilation rate was obtained by dividing the photosynthetic rate (measured with an ADC portable photosynthesis meter, Analytical Development Co., England, as described by Hunter & Visser, 1988) by the total chlorophyll per unit of leaf area. Photosynthetic measurements were taken at 10:00 on a scheduled day (five weeks after véraison). Sun leaves, situated basally on a shoot (first three leaves above the clusters), were measured in all cases. During the measurement, care was taken not to change the orientation of the leaves relative to the sun in order to maintain the exposure to photosynthetic photon flux density (PPFD). Three leaves were measured per replicate.

**Statistical analyses**

Non-parametric bootstrap analyses were used when they proved to be more practical than factorial ANOVA. The significance of the results was evaluated using 95% confidence intervals. During the interpretation of the figures, differences were considered significant when no overlapping of the 95% confidence intervals occurred.

RESULTS AND DISCUSSION

Higher concentrations of chlorophyll *a* (Fig. 1), chlorophyll *b* (Fig. 2) and total chlorophyll (µg.g$^{-1}$ fresh leaf mass) (Fig. 3) were found in the primary leaves of the underdeveloped shoots compared to the normal shoots in both years of study. This was attributed to the significantly lower PPFD received by the un-

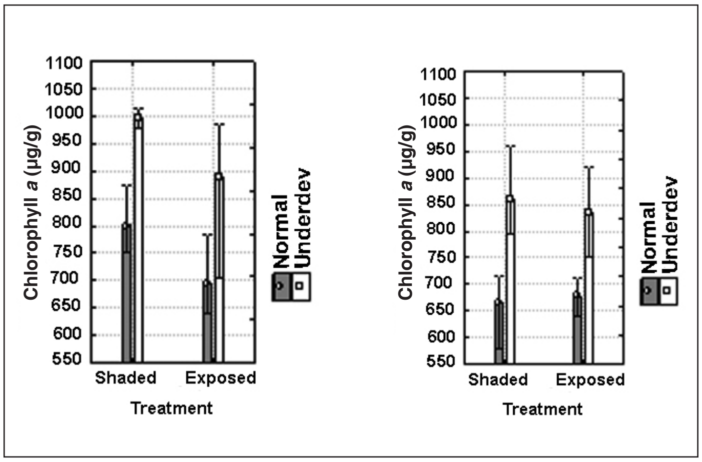


FIGURE 1

Chlorophyll *a* concentration of primary leaves from normally developed and underdeveloped shoots in shaded and well-exposed canopies measured five weeks after véraison. A: 2002, B: 2003. Error bars indicate 95% confidence intervals (bootstrap analysis).

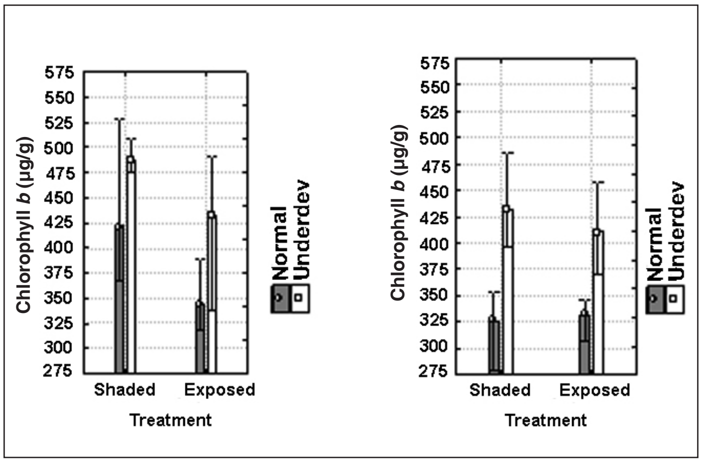


FIGURE 2

Chlorophyll *b* concentration of primary leaves from normally developed and underdeveloped shoots in shaded and well-exposed canopies measured five weeks after véraison. A: 2002, B: 2003. Error bars indicate 95% confidence intervals (bootstrap analysis).

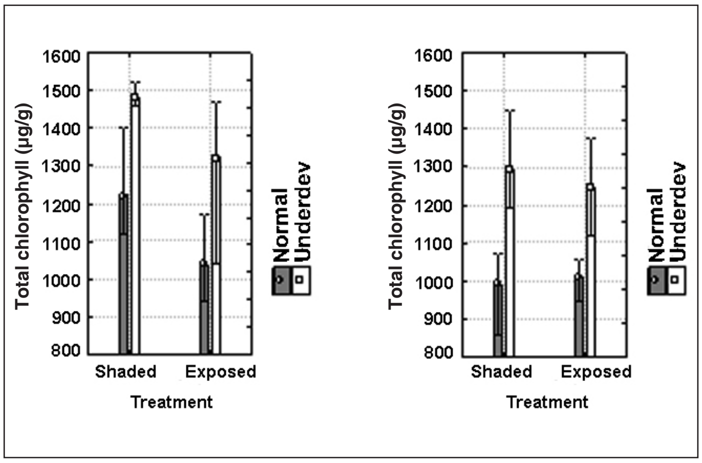


FIGURE 3

Total chlorophyll of primary leaves from normally developed and underdeveloped shoots in shaded and well-exposed canopies measured five weeks after véraison. A: 2002, B: 2003. Error bars indicate 95% confidence intervals (bootstrap analysis).

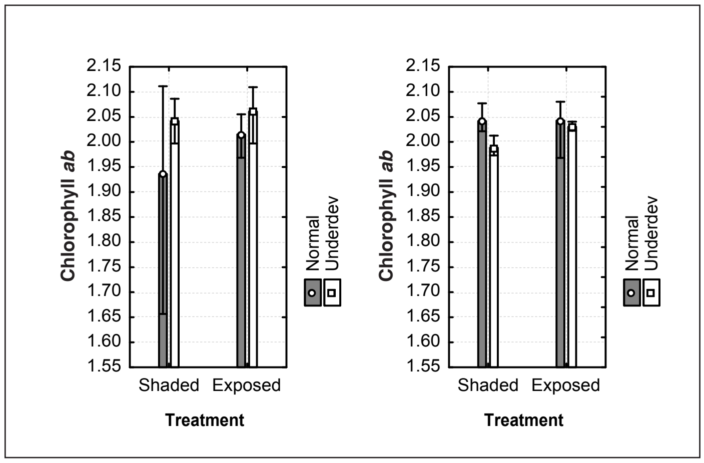


FIGURE 4

Ratio of chlorophyll *a* to chlorophyll *b* in primary leaves from normally developed and underdeveloped shoots in shaded and well-exposed canopies measured five weeks after véraison. A: 2002, B: 2003. Error bars indicate 95% confidence intervals (bootstrap analysis).

TABLE 1

Assimilation rate of primary leaves from normally developed and underdeveloped shoots in shaded and well-exposed canopies measured five weeks after véraison in 2003.

| Canopy exposure | Shoot development | Assimilation rate (µmol $CO_2$/µg chlorophyll/h) |
|---|---|---|
| Shaded | Normal | 0.093[a] |
| Shaded | Underdeveloped | 0.087[a] |
| Well-exposed | Normal | 0.093[a] |
| Well-exposed | Underdeveloped | 0.080[a] |

Values in a specific column designated by the same letter do not differ statistically on a 95 % confidence level.

derdeveloped shoots at five weeks after véraison (approximately 600 µmol/m$^2$/s, compared to 1200 µmol/m$^2$/s received by the normally developed shoots) (Cloete *et al*., 2008). Kappel and Flore (1983) and Marini and Marini (1983) also found that leaves under more shaded conditions tended to have higher chlorophyll per unit fresh leaf mass than those under sun-exposed conditions.

Interestingly, it seemed that the chlorophyll levels were affected differently by canopy exposure in the two years monitored. In 2002, noticeably higher levels of chlorophyll *a* (Fig. 1a), chlorophyll *b* (Fig. 2a) and total chlorophyll (Fig. 3a) were found in leaves from the shaded canopies, while very small differences were noticed in 2003 (Figs 1b to 3b). The ratio of chlorophyll *a* to chlorophyll *b* was not affected by the degree of canopy exposure or shoot development in 2002, although some differences were found in 2003 (Fig. 4). In the shaded canopies, this ratio was significantly higher in the leaves of normally developed shoots, while in the case of underdeveloped shoots the ratio was higher in the well-exposed than in the shaded canopies (Fig. 4b). Sestak, cited by Hunter and Visser (1989), states that the content of chlorophyll *a* is considered an exact characteristic of photosynthetic activity. It seems that normally developed shoots and well-exposed canopies are extremely important for the expression of the main light-absorbing pigments and the attainment of optimal photosynthetic activity in the canopy.

A higher leaf area:mass ratio and lower leaf mass were found in the underdeveloped than in the normally developed shoots (Cloete *et al*., 2006). Since the chlorophyll was determined on a per gram fresh leaf basis, a larger leaf area from the underdeveloped shoots than from the normally developed shoots was in fact used for each analysis. A higher leaf area:mass ratio was also found for the leaves from shaded canopies in comparison to those from well-exposed canopies (Cloete *et al*., 2006). Since the effective leaf area (and not the mass) in the canopy is considered an indication of the physiological potential of the canopy (Carbonneau *et al*., 1997), the chlorophyll content per unit leaf area was determined in 2003 (Fig. 5). Although no statistically significant difference was found between the normally developed and underdeveloped shoots or between the shaded and well-exposed canopies, it seems that the chlorophyll content per unit leaf area (and thus the light interception ability) of the leaves tended to be higher in the better exposed canopies.

The light interception, and thus the assumed physiological potential, per unit leaf area did not seem to differ between the normally developed and underdeveloped shoots. However, according to Hunter and Visser (1989) the ability of a vine leaf to intercept light is not necessarily closely related to the $CO_2$-assimilating ability. Although the levels seemed lower in the underdeveloped shoots, no statistically significant differences in the assimilation rate (µmol $CO_2$/µg chlorophyll/h) of the leaves were found be-

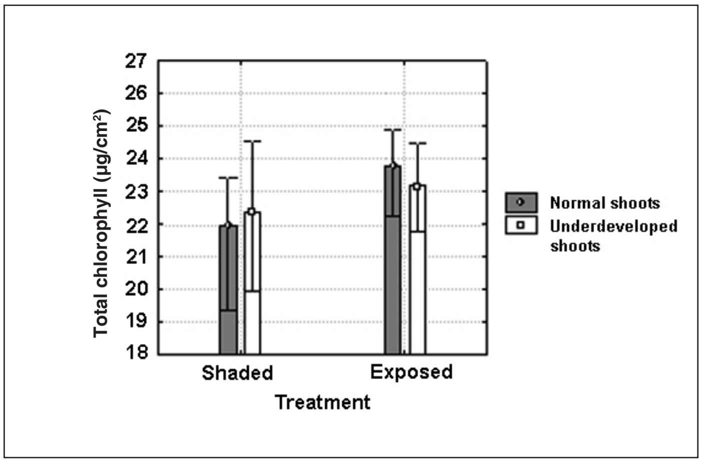


FIGURE 5
Total chlorophyll/cm² of primary leaves from normally developed and underdeveloped shoots in shaded and well-exposed canopies measured five weeks after véraison in 2003. Error bars indicate 95% confidence intervals (bootstrap analysis).

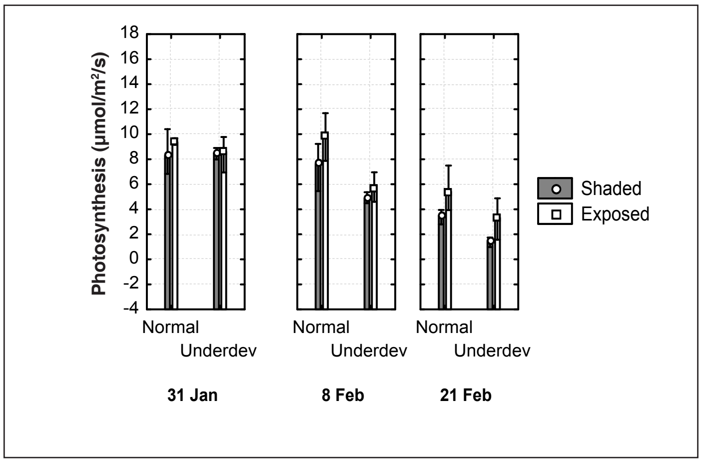


FIGURE 6
Photosynthetic rates of basal leaves from normally developed and underdeveloped shoots in shaded and well-exposed canopies measured in the second, third and fifth week after véraison. Error bars indicate 95% confidence intervals (bootstrap analysis).

tween the shaded and well-exposed canopies and between the shoot types in 2003 (Table 1).

Although higher chlorophyll concentrations (µg/g) were found in the underdeveloped shoots and shaded canopies (compared to normal shoots and well-exposed canopies), higher photosynthetic rates were measured in the exposed canopies and for normally developed shoots (Cloete *et al*., 2008) (Fig. 6). These findings are in accordance with those of Kriedemann *et al*. (1970) and Hunter and Visser (1989), who found no consistent relationship between the chlorophyll concentration and the photosynthetic activity of vine leaves.

According to Cloete *et al*. (2006), the average area per leaf and per shoot is significantly lower for underdeveloped shoots than normally developed shoots. Although the assimilation rate did not differ between the shoot types, significantly higher total chlorophyll levels per leaf (and thus also per shoot) were evident for the normal shoots. An equal amount of chlorophyll per unit leaf area and similar assimilation rates would therefore result in higher photosynthetate production in normally developed shoots.

## CONCLUSIONS

Higher levels of chlorophyll *a*, chlorophyll *b* and total chlorophyll per fresh mass were found in leaves from underdeveloped shoots compared to those from normally developed shoots. The leaf chlorophyll content therefore appears to be higher in more shaded canopies compared to better exposed canopies. No positive relationship was found between the photosynthetic activity and the chlorophyll concentration of the leaves. The limiting value of chlorophyll with regard to optimal photosynthetic activity needs to be clarified in further studies, particularly in grapevine canopies that are complex and in which source:sink relationships are dynamic during the growth season.

No statistically significant differences were found in the chlorophyll concentration per unit leaf area and the assimilation rate between normally developed and underdeveloped shoots or between shaded and well-exposed canopies. It is argued that the effective area per leaf or per shoot probably plays a more important role than chlorophyll content *per se* in photosynthetate production by normally developed and underdeveloped shoots in both shaded and well-exposed canopies.

Therefore, based on the measured photosynthetic activity as well as the total expected photosynthetate production per leaf or shoot, canopy management practices that induce well-exposed canopies, together with normally developed, uniformly distributed shoots, appear very important in order to realise the full potential of grapevines.

## LITERATURE CITED

Carbonneau, A., Lebon, E., Mabrouk, H. & Sinoquet, H., 1997. Interactions: canopy shape × vigour level: consequences on architecture and microclimate of the grapevine. Acta Hort. 526, 91-105.

Cloete, H., Archer, E. & Hunter, J.J., 2006. Shoot heterogeneity effects on Shiraz/Richter 99 grapevines. I. Vegetative growth. S. Afr. J. Enol. Vitic. 27, 68-75.

Cloete, H., Archer, E., Novello, V. & Hunter, J.J., 2008. Shoot heterogeneity effects on Shiraz/Richter 99 grapevines. II. Physiological activity. S. Afr. J. Enol. Vitic. 29, 1-8.

Crookston, R.K., Treharne, K.J., Ludford, P. & Ozbun, J.L., 1975. Response of beans to shading. Crop Science 15, 412-416.

Hunter, J.J., 2001. Grapevine physiology. Private Bag X5026, Stellenbosch, 7599. E-mail hunterk@arc.agric.za.

Hunter, J.J. & Visser, J.H., 1989. The effect of partial defoliation, leaf position and developmental stage of the vine on leaf chlorophyll concentration in relation to the photosynthetic activity and light intensity in the canopy of *Vitis vinifera* L. cv. Cabernet Sauvignon. S. Afr. J. Enol. Vitic. 10, 67-73.

Kappel, F. & Flore, J.A., 1983. Effect of shade on photosynthesis, specific leaf weight, leaf chlorophyll content, and morphology of young peach trees. J. Amer. Soc. Hort. Sci. 108, 541-544.

Kriedemann, P.E., Kliewer, W.M. & Harris, J.M., 1970. Leaf age and photosynthesis in *Vitis vinifera* L. Vitis 9, 97-104.

Marini, R.P. & Marini, M.C., 1983. Seasonal changes in specific leaf weight, net photosynthesis, and chlorophyll content of peach leaves as affected by light penetration and canopy position. J. Amer. Soc. Hort. Sci. 108, 600-605.