RELATIONSHIPS BETWEEN CARBOHYDRATES AND REPRODUCTIVE DEVELOPMENT IN CHARDONNAY GRAPEVINE : IMPACT OF DEFOLIATION AND FRUIT REMOVAL TREATMENTS DURING FOUR SUCCESSIVE GROWING SEASONS

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Abstract

Aim : The present study was conducted in grapevines to evaluate the influence of source/sink disturbance on reserve restoration in the entire plant (trunk, roots) and the consequences on reproduction, yield, sugar yield, potential alcohol content and total acidity over four successive years of treatment.

Methods and results : At véraison, either total defoliation or total fruit removal was performed on Chardonnay plants grown in vineyards. The impact was measured each year on reproductive parameters and after four years on both plant weight and carbohydrate content. Defoliation induced an alteration in carbohydrate distribution in the entire plant, as revealed by decrease of starch content and parallel increase of soluble sugar content. These modifications affected the number of inflorescences/clusters per plant the year after. By contrast, fruit removal resulted in a significant increase of carbohydrate reserves in the whole plant, although the efficiency of sexual reproduction was not improved the year after.

Conclusion : Results confirm the close correlation between carbohydrate reserves and the efficiency of sexual reproduction. Moreover, grapevine is able to adapt the number of both inflorescences and flowers according to the amount of available carbohydrate reserves in perennial organs.

Significance and impact of the study : The quality of the crop is affected by defoliation, as shown by the decrease in yield and sugar yield. However, the impact on potential alcohol content and total acidity was less pronounced.

Key words : carbohydrate reserves, flowering, photosynthesis, source/sink ratio, *Vitis vinifera* L.

Résumé

Objectif : Chez la vigne, nous avons étudié l'effet d'une perturbation source/puits sur la restitution des réserves de la plante entière (tronc, racines), et les conséquences sur la reproduction, le rendement, le rendement en sucre, le degré alcoolique et l'acidité totale au cours de quatre années successives de traitement.

Méthodes et résultats : Un effeuillage (élimination des feuilles) ou un éclaircissage (élimination des grappes) a été effectué à la véraison sur des ceps de Chardonnay. Tous les ans, nous avons suivi des paramètres reproducteurs et après quatre ans de traitement, le poids des ceps ainsi que leur contenu en glucides a été déterminé. Nous pouvons conclure qu'il existe bien une corrélation entre le taux de réserves dans les parties pérennes de la plante et l'intensité de floraison l'année suivante. Une perturbation de la reconstitution des réserves dans les bois par suppression des feuilles, organes sources de nutriments, entraîne une réduction du nombre d'inflorescences par cep et du nombre de fleurs par inflorescence. Par contre, le taux de nouaison reste inchangé quel que soit le traitement, ce qui suggère fortement que la plante est capable d'appréhender la quantité de réserves dont elle dispose et de ne déclencher la formation que du nombre d'inflorescences et de fleurs dont elle pourra assumer le développement. A l'inverse, il ne semble pas possible d'augmenter le rendement de manière significative par ce type d'intervention.

Conclusion : L'éclaircissage n'a conduit ni à une augmentation significative du taux de réserves dans les bois ni à une floraison plus importante l'année suivante. Il semble donc que la plante, conduite selon les pratiques actuellement en vigueur, donne le maximum de son potentiel.

Signification et impact de l'étude : La qualité de la récolte est cependant affectée par l'effeuillage puisque le rendement et le rendement en sucre sont affectés. Cependant, l'impact sur le degré alcoolique et l'acidité totale est moins marqué.

Mot clés : réserves glucidiques, floraison, photosynthèse, relation source/puits, *Vitis vinifera* L.

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INTRODUCTION

In grapevine (*Vitis vinifera* L.) as in other woody plants of temperate climate, the inflorescence and leaf primordia are formed in the latent bud during spring and summer, after what, the bud enters winter dormancy during autumn (Srinivasan and Mullins, 1981; Morrison, 1991; Boss *et al.*, 2003; May, 2004). At budburst, the winter bud leaves its dormant state and continues its development during spring (Boss *et al.*, 2003). At that moment, preformed leaves and inflorescences grow rapidly and their needs in carbohydrates are considerable (Scholefield *et al.*, 1978). Carbohydrates required for annual development originate from both accumulated reserves in perennial woody organs the year before and photoassimilates synthesized in leaves (Loescher *et al.*, 1990; Mullins *et al.*, 1992; Zapata *et al.*, 2001; Lebon *et al.*, 2008).

One specific trait of woody plants growing in temperate climate is the importance of carbohydrate reserves stored during the previous summer and autumn (Loescher *et al.*, 1990; Mooney and Gartner, 1991). In grapevine, starch is the major stored carbohydrate (Bouard, 1966; Mullins *et al.*, 1992), located in the wood-ray parenchyma of roots and trunk (Zapata *et al.*, 2004a). However, soluble carbohydrates (sucrose, glucose and fructose) are also present in perennial organs, trunk and root wood, during winter, providing tolerance to cold injury by decreasing the freezing temperature of the tissue sap (Glad *et al.*, 1992).

It has been shown that carbohydrates mobilized from reserves in perennial parts are used for rapid growth of annual shoots in early spring (Scholefield *et al.*, 1978; Weyand and Schultz, 2006), including stems, leaves and emerging inflorescences (Zapata *et al.*, 2004a). This pool of carbohydrates is restored in perennial organs by photosynthesis in the mature leaves, extending from flowering to leaf senescence, especially during berry ripening in warmer climate (Candolfi-Vasconcelos *et al.*, 1994a; Chaumont *et al.*, 1994; Miller *et al.*, 1997). Interruption of reserve replenishment has direct impact on reproduction the following year, both the number of inflorescences per plant and the number of flowers per inflorescence being affected (Duchêne *et al.*, 2003; Bennett *et al.*, 2005).

In grapevine, young developing leaves can perform photosynthesis in early spring (Lebon *et al.*, 2005), but they cannot be considered as photoassimilate exporters until they reach 50 % of their final size (Koblet, 1969; Alleweldt *et al.*, 1982; Petrie *et al.*,

2000). This means that carbohydrates accumulated in perennial organs during the previous year support the early development of annual organs. The opposite sink/source transition in leaves and roots occurs at the onset of flowering during female meiosis (Zapata *et al.*, 2004a; Lebon *et al.*, 2008). At that time, the sugars required for the developing inflorescences originate from three different sources including reserves and photosynthesis in mature leaves and young green inflorescences (Lebon *et al.*, 2005, 2008). The supply of carbohydrates during key steps of flower formation such as female meiosis is decisive in the achievement of functional flowers and in fruit set (Lebon *et al.*, 2004, 2008).

Thus, there is a subtle equilibrium between organs accumulating or releasing sugars and those assimilating carbon through photosynthesis during the annual cycle. In grapevine, the influence of the source/sink balance has been investigated (Scholefield *et al.*, 1978; Candolfi-Vasconcelos and Koblet, 1990, 1991; Candolfi-Vasconcelos *et al.*, 1994b; Chaumont *et al.*, 1994; Howell *et al.*, 1994; Koblet *et al.*, 1994; Bennett *et al.*, 2002, 2005; Smith and Holzapfel, 2009). Additionally, leaf area to fruit ratios required for adequate ripening under cool-climate conditions may be between 10 and 20 cm^2 leaf area/g of fruit (Kliewer and Dokoozlian, 2005). Murisier (1996) demonstrated a linear correlation between leaf area to fruit ratio and the formation of carbohydrate reserves in woody parts and roots. Moreover, few studies have focused on both source (defoliation) and sink (fruit removal) limitations on the same cultivar and on their impacts on reproductive parameters and physiological traits (Holzapfel *et al.*, 2006; Weyand and Schultz, 2006; Watt *et al.*, 2008; Smith and Holzapfel, 2009; Zufferey *et al.*, 2012)

The aim of this study was to investigate the influence of source/sink alteration at véraison, i.e. berry ripening, on reserve restoration in whole plant and, for the first time, its impact on flowering and fruit set during four successive growing seasons. In this view, the impact of total defoliation and fruit removal was estimated each year by gas exchange measurements, plant weight and non-structural carbohydrate content in entire plant (roots and trunk), and description of the reproductive development.

MATERIALS AND METHODS

1. Plant material

Experiments were performed on Chardonnay grapevines (*Vitis vinifera* L.) planted in 1986, grafted on 41B rootstock, and trained according to the

Chablis method which, consisted in maintaining five buds per cane after winter pruning. The vineyard was planted at a density of 7,575 plants/ha. The experimental site was located at the CIVC (Comité Interprofessionnel du Vin de Champagne) in Plumecoq, Chouilly (Marne, France), with an average annual temperature of 10,4 °C and annual precipitation of 634 mm. Plants under investigation were grown in the same location and were subject to rigorously similar cultural practices. The experimental design of the vineyard consisted of one Latin square (4 x 4 vines per treatment). Three replicates were carried out.

Since modifications of leaf area/crop level ratio at véraison are known to modify the level of reserves, treatments were applied at véraison (Candolfi-Vasconcelos and Koblet, 1990; Bennett *et al.*, 2005) and performed on the same plants during four successive growing seasons (2004-2008). Sixteen plants were defoliated except for two leaves (opposite to the cluster). The two maintained leaves were the two youngest fully expanded leaves, which were used to evaluate whether photosynthesis was mobilized to compensate the loss of other source leaves. On 16 other plants, all the clusters were removed to favour the restoration of reserves in woody organs. Only two clusters per plant were maintained at harvest where fertility (%) and fruit set were determined. As a control, 16 plants without treatment were also followed.

2. Gas exchange

Net photosynthesis (Pn), stomatal conductance (g_s) and internal CO_2 concentration (Ci) were measured with a portable infrared gas analysis system (Li-6400, Li-Cor Inc., Lincoln, NE). All measurements were performed between 9 am and 11 am (Chaumont *et al.*, 1994) with photosynthetically active radiation maintained artificially at 1 500 µmol. m^{-2}.s^{-1}, relative humidity around 30 %, temperature at 25 °C and constant CO_2 level of 360 µmol. l^{-1}.

Measurements were performed on leaves opposite to the cluster (the most important source) of eight different plants for each treatment. Measurements were performed one day before leaf or fruit removal (before treatment) and one week after treatment (after treatment).

Gas exchange measurements were performed in both the second and the third year. As results were similar during these two years, only results of the third year are presented.

3. Plant weight and non-structural carbohydrate content

Weight : At the end of the four-year study, three plants per treatment were collected by hand (with a shovel) in winter (February). The dry weight (g) was measured and then plants were separated in two (roots and trunk, respectively). Total non-structural carbohydrate (TNC) content including starch, sucrose, glucose and fructose was evaluated in each part.

Extraction : Samples were frozen in the field, in liquid nitrogen, and stored at -80 °C before use to avoid sugar hydrolysis. Afterwards, roots and trunk were lyophilized 72 h with a CS5L device (Serail Lyophilisateur®, France). Then, they were first ground with a heavy-duty cutting mill SM2000 blender (Retsch, Haan, Germany) and, thereafter, with a laboratory mortar grinder pulverisette 2 (Fritsch GmbH, Idar-Oberstein, Germany) to obtain a fine powder. Sugars were extracted from 100 mg of wood sample with 0.1 M of phosphate buffer (pH 7.5) according to Lebon *et al.*(2004). The mixture was centrifuged for 15 minutes at 10,000 g (at 4 °C). The pellet was used for starch determination and the supernatant for soluble sugar assay.

Starch content : The remaining pellet was suspended in dimethylsulfoxide/8 N hydrochloric acid (4:1, vol/vol) and starch was dissolved at 60 °C for 30 min under constant agitation. The extract was centrifuged for 10 min at 13,000 g (at room temperature). One hundred µl of the supernatant were mixed with 100 µl of iodine-hydrochloric acid (0.06 % KI and 0.03 % I_2 in 0.05 N HCl) and 1 ml of distilled water. After 15 minutes, the absorbance was read at 600 nm.

Soluble sugar content : The assay of soluble sugar content was performed using enzymatic kits (Scil Diagnostics GmbH, Viernheim, Germany) as described by Saladin *et al.* (2003).

All measurements were carried out in triplicate on both roots and trunk of the three plants collected in each treatment.

4. Reproductive development

The number of inflorescences per plant and the number of flowers per inflorescence were determined after each year of treatment, i.e. in year 2, 3 and 4. The number of flowers per inflorescence was determined according to Schneider (1992). Briefly, a linear correlation exists between the number of

flowers counted (NFC) on a photograph and the real number of flowers (RNF) per inflorescence. This correlation is independent of the year and the location on a cane but depends on cultivar. The regression equation used was: RNF = 8.63 + 1.537 x NFC for Chardonnay grapevines (Schneider, 1992). The counting was performed on 16 inflorescences per treatment.

At harvest, bunches used for flower counting were collected and the fruit set rate was determined after berry counting. Fifteen berries per bunch were randomly chosen to count seeds in order to estimate the fertilization rate as: fertilization rate = seed number x fruit set rate/4 (maximal number of fertilized ovaries).

5. Yield quantity and quality analysis

Each treatment plot was harvested individually and weighed. One hundred berries per treatment were crushed to determine potential alcohol content (% by volume), sugar yield (°Brix) by refractometry, and total acidity (g H_2SO_4/L) by titration with Bromothymol Blue and NaOH.

6. Statistical analysis

To determine whether values of control plants were significantly different from treated plants, analysis of variance (ANOVA) was used with EXCEL. Differences at $P<0.05$ were considered as significant.

RESULTS

1. Gas exchange

The results presented were obtained during the third year of treatment. Before defoliation at véraison, Pn was similar in treated and non treated plants, whereas a significant increase of g_s and Ci was noticed in vine to be defoliated or not (Figure 1). In contrast, defoliation led to a significant increase of Pn (Figure 1A), without modifications of g_s and Ci (Figures 1B, 1C). Following fruit removal, Pn, g_s and Ci were significantly higher in leaves of treated plants than in those of control plants, before treatment. A similar trend was observed after treatment except for Ci, which decreased.

2. Vegetative growth and carbohydrate content in whole plant after four successive treatments

Figure 2 shows that the dry weight of the whole plant (trunk + roots) increased significantly after four successive treatments, whatever the treatment. Defoliation did not change TNC content, whereas fruit removal induced a higher TNC content

(Figures 2B, 2D). After four successive defoliations, TNC was not modified in whole plant (trunk + roots) but sugar distribution was different. Starch decreased (Figures 3A, 3E) while both glucose and fructose concentration increased by 3- and 2-fold, respectively (Figures 3B, 3C, 3F, 3G). The same tendency was observed in both roots and trunk. For sucrose, trunk and roots showed opposite patterns since sucrose decreased in the former and increased in the latter (Figures 3D, 3H). Regarding fruit removal, the increase of sugar content was correlated to a significant increase of starch and soluble sugar
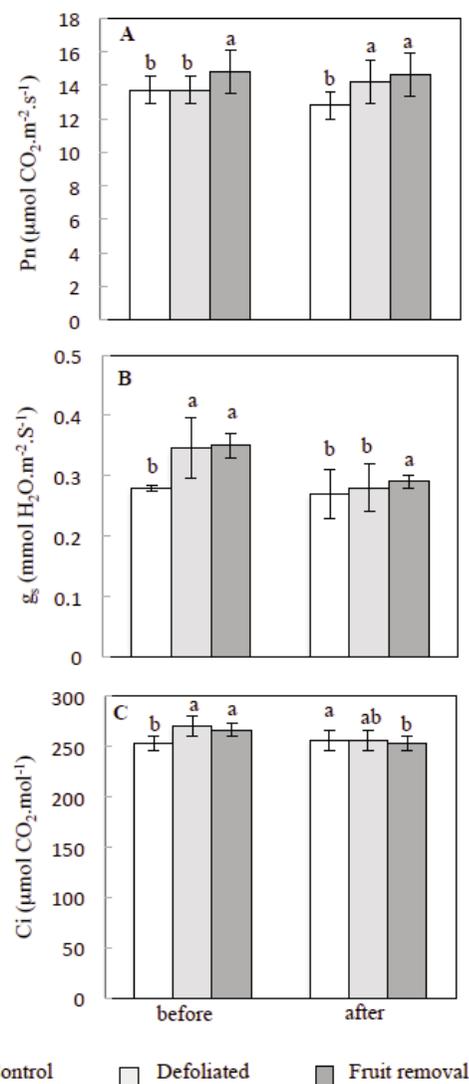

Figure 1. Effects of total defoliation and total fruit removal at véraison on gas exchange during the third year of treatment. Net photosynthesis (Pn, A), stomatal conductance (g_s, B) and internal CO_2 concentration (Ci, C) were measured before and after treatment.

Means (±SD) for a considered parameter before or after treatment were not significantly different from the control when marked by the same letter (P =0.05).

content in both roots and trunk except for sucrose in trunk, which remained stable (Figures 2, 3). The analysis of carbohydrate content was also performed on the different parts constituting the trunk and the root system. The same patterns of carbohydrate content were observed on one-year-old canes and 2- to 4-year-old trunk wood, and on fine (less than 5 mm), medium (between 5 and 10 mm) and thick (more than 10 mm) roots as in the root system (data not shown).

3. Reproductive development

The influence of source/sink disturbance on reproductive development was evaluated annually. The number of inflorescences per plant significantly decreased following the first two successive defoliations, while the number of flowers per inflorescence decreased in year 2 and increased in year 4 (Figures. 4A, 4B). Following fruit removal, these two parameters were not affected except the number of inflorescences per plant, which decreased in year 3 (Figure 4A), and the number of flowers per

inflorescence, which increased in year 4 (Figure 4B). Interestingly, after three successive treatments (year 4) of defoliation or fruit removal, the number of inflorescences per plant was similar between treated and non treated plants (Figure 4A).

Fruit set significantly decreased after one (year 2) or three (year 4) years of defoliation, whereas no significant effects were observed following fruit removal (Figure 4C). At maturity, the yield declined in the defoliated treatment with a decrease in bunch number (20 %) and bunch weight (45 %) (data not shown). Neither defoliation nor fruit removal during three successive years had any effect on fertility (Figure 4D).

Yield was affected after the second year of defoliation (52 % and 46 % of control), whereas sugar yield was lower from the first year with 82 % of control (Table 1). Sugar yield decreased as the defoliation number increased. Potential alcohol content and total acidity were decreased in year 2 and 3, but no modification was measured in year 4.
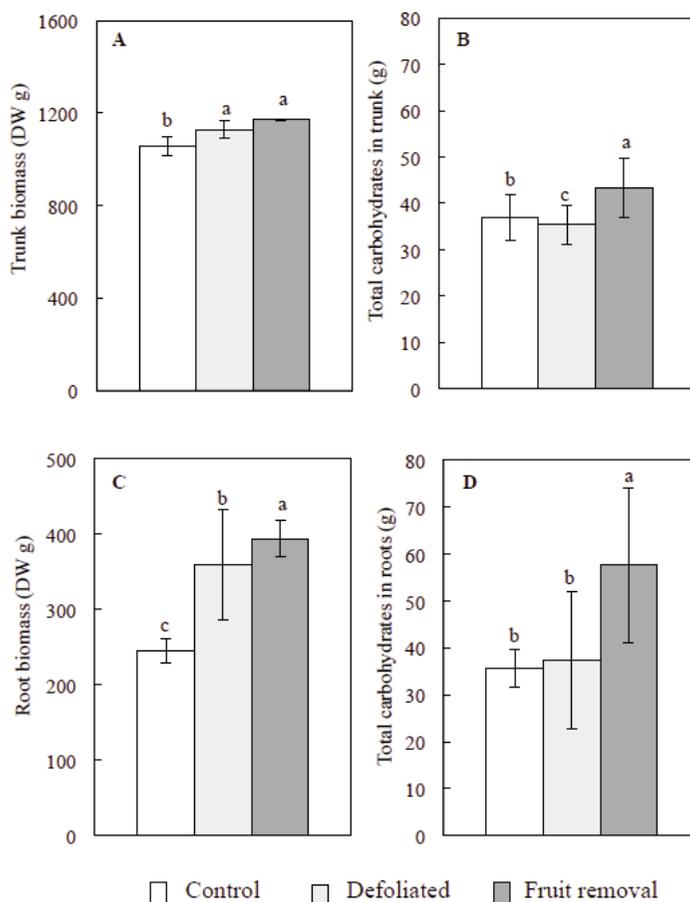

Figure 2. Effects of four successive total defoliation and total fruit removal at véraison on biomass (dry weight per vine, DW) (A, C) and total non-structural carbohydrate content (B, D) in both root and aerial organs. Means (±SD) for a considered parameter were not significantly different from the control when marked by the same letter (P =0.05).
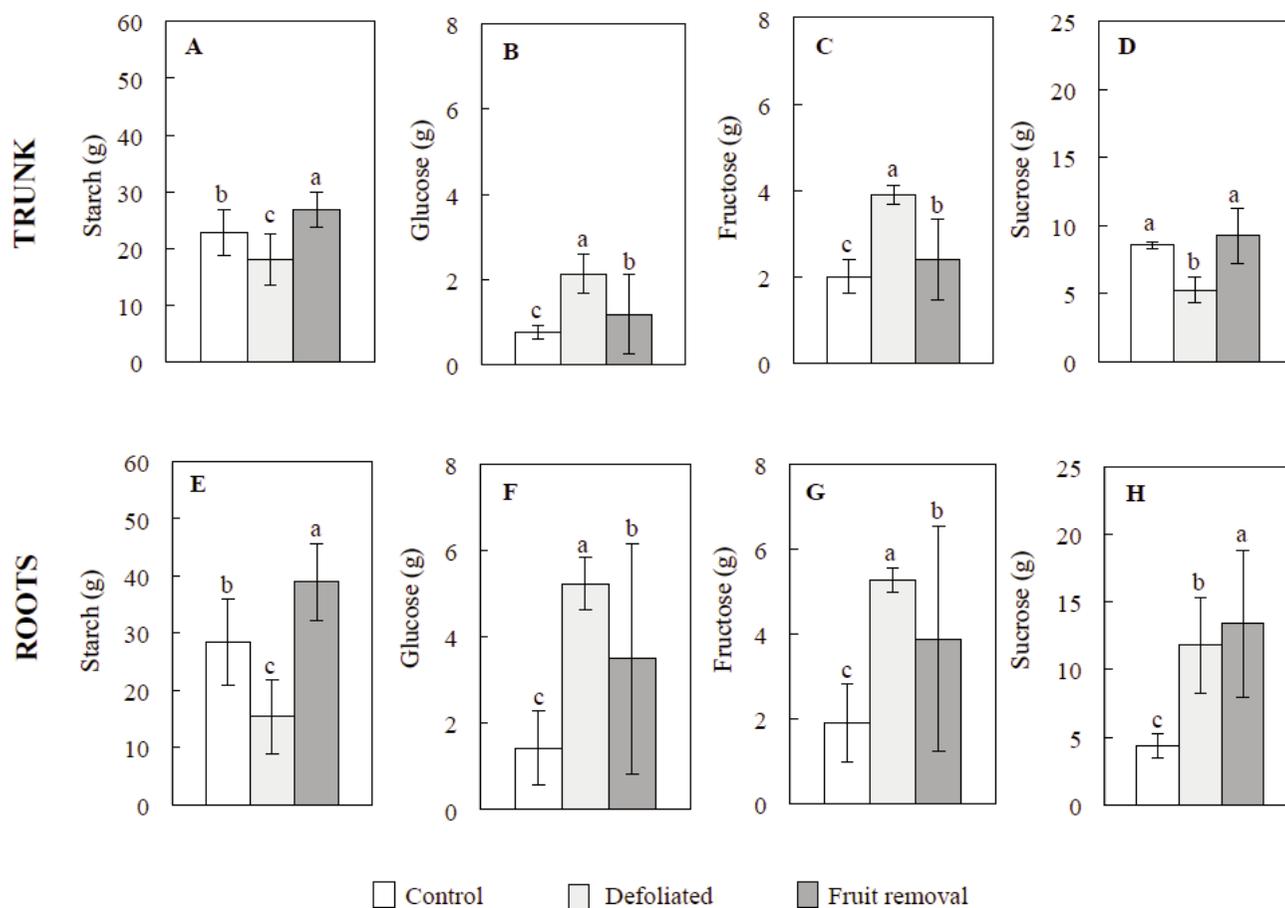
Figure 3. Effects of four successive total defoliation and total fruit removal at véraison on starch (A, E), glucose (B, F), fructose (C, G) and sucrose (D, H) in roots and trunk, respectively (g per organ). Means (±SD) for a considered parameter were not significantly different from the control when marked by the same letter (P =0.05).

	Control	Defoliated
Fruit Yield (kg/ha)		
Year 2	10226 (100%)[a]	11353 (111%) [a]
Year 3	21037 (100%) [a]	10877 (52%) [b]
Year 4	31848 (100%) [a]	14749 (46%) [b]
Sugar yield (kg/ha)		
Year 2	1330 (100%) [a]	1089 (82%) [b]
Year 3	2420 (100%) [a]	1182 (49%) [b]
Year 4	3511 (100%) [a]	1637 (47%) [b]
Potential alcohol content (% vol)		
Year 2	10.3 (100%) [a]	7.6 (74%) [b]
Year 3	9.5 (100%) [a]	8.3 (87%) [b]
Year 4	8.8 (100%) [a]	8.8 (100%) [a]
Total acidity (g H$_2$SO$_4$/L)		
Year 2	8.3 (100%) [a]	8.2 (99%) [b]
Year 3	8.4 (100%) [a]	7.8 (93%) [b]
Year 4	7.8 (100%) [a]	8.6 (110%) [a]

Table 1. Influence of defoliation on fruit yield, sugar yield, potential alcohol content (% by volume) and total acidity. Means were not significantly different from the control when marked by the same letter (P =0.05).
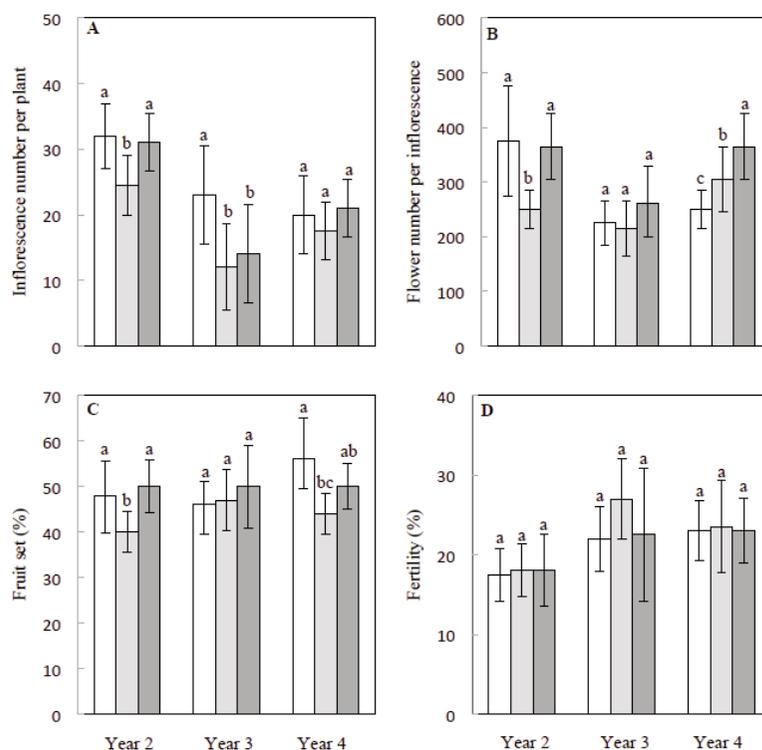
Figure 4. Effects of total defoliation and total fruit removal at véraison on inflorescence number per plant (A), flower number per inflorescence (B), fruit set (C) and fertility (D) during the second, third and fourth year of treatment. Means (±SD) for a considered parameter were not significantly different from the control of the same year when marked by the same letter (P =0.05).

DISCUSSION

Source/sink disturbance during four consecutive years at véraison in vines subjected to defoliation or fruit removal confirms the relationship between the carbohydrate reserves and the efficiency of sexual reproduction.

After fruit removal, the results show that carbohydrate reserves in the wood are elevated. A higher starch and soluble sugar accumulation was observed in whole plants. The increase of starch and soluble sugar content in the aerial organs could be explained by the lower needs of photoassimilates in the whole plant caused by sink fruit removal or by an increase of photoassimilate synthesis resulting from a stimulated photosynthesis following the wounding stress provoked by fruit removal. Considering the latter hypothesis, our results show that after fruit removal, photosynthetic activity increased with an increase of g_s and a decrease of Ci. Downton *et al.*, (1987) showed that during the period of rapid sugar accumulation in the fruit, Pn in leaves of plants with and without fruit were similar earlier in the morning, but a decline in Pn occurred earlier in the day for vines without fruit. Nevertheless, photosynthesis may be increased in Chardonnay by abiotic stresses such

as flumioxazin herbicide application in vineyard, as described by Saladin *et al.* (2003). In contrast, several reports indicated that fruit removal leads to lower rates of photosynthesis, as described in Cabernet Sauvignon (Iacono *et al.*, 1995 ; Quereix *et al.*, 2001). The Pn reduction may be due to a lack of photoassimilate requirement by bunch sink activity. This reduction may promote a feedback inhibition of photosynthesis since carbohydrates were accumulated in leaves (Iglesias *et al.*, 2002 ; Paul and Pellny, 2003).

After leaf removal, TNC content remained stable in whole plant, whereas sugar yield decreased. In vegetative organs, starch decrease should be entirely compensated by an increase of soluble sugar contents, a possible result of an interconversion between starch and soluble solids. The decrease of starch content may result from both the lack of leaves at véraison, which is a period of intense reserve restoration (Bennett *et al.*, 2002), and starch mobilization from woody parts for berry development (Stoev and Ivantchev, 1977). It was demonstrated that early defruiting over two consecutive seasons increased TNC in the roots, and to a lesser extent in the wood. In contrast, defoliation at harvest caused a decline in TNC concentrations

(Smith and Holzapfel, 2009). The required sugars for berry development in grapevine are provided by photosynthesis in leaves (Bennett *et al.*, 2002 ; Zapata *et al.*, 2004a, b). In absence of leaves, Candolfi-Vasconcelos *et al.* (1994a) have showed that berries mobilize sugars from the most proximal canes and next from other perennial organs. As a putative consequence of starch hydrolysis, soluble sugars could increase in whole plant. Berry development in defoliated plants needs less carbohydrates compared to control plants since the yield is reduced by 2-fold. In our defoliation treatment, two leaves (opposite to the cluster) were maintained on treated plants in order to determine whether they may compensate the loss of source organs by a higher photosynthetic activity. This assumption defined as 'compensatory photosyn-thesis' (Nowak and Caldwell, 1984) might be a general response of woody species, since it has been previously reported in lemon (Lenz, 1978 ; Syvertsen, 1994 ; Iglesias *et al.*, 2002) and in several other deciduous tree species (Ovaska *et al.*, 1993). Our data show that Pn increased in the remaining leaves. Measurements at véraison were performed on expanded leaves which had not yet reached their maturity stage with maximal photosynthetic activity. Indeed, defoliation performed on young grapevine leaves, at flowering (Candolfi-Vasconcelos and Koblet, 1991 ; Poni and Giachino, 2000) or at pea size (Petrie *et al.*, 2000), showed a large compen-sation capacity of remaining leaves by increasing their photosynthesis.

In addition to changes in reserve restoration, the loss of source organs at véraison affects the reproductive development by decreasing the number of inflorescences per plant. At véraison, an adequate supply of assimilates is essential for optimal inflorescence development (Candolfi-Vasconcelos and Koblet, 1990 ; Bennett *et al.*, 2005), while the development of uncommitted primordium into inflorescence primordium occurs in the latent bud (Srinivasan and Mullins, 1981, May, 2000 ; Boss *et al.*, 2003). Our results confirm that carbon availability at véraison is determinant for the quantitative transition from uncommitted to inflorescence primordium. Moreover, Loescher *et al.*, (1990) reported the importance that grape leaves remain active after harvest to establish carbohydrate reserves in root storage tissue for leaf growth and flower development the following spring. Since flower development occurs before canopy development, the early stages of reproductive growth are completely dependent on reserves. Thus, the lack of alteration of flower number over the years may be linked to the stability of TNC content in whole plant. Moreover, fruit set significantly decreased in

defoliated plants compared to controls. The modification in sugar distribution might explain the flower abscission. Indeed, Lebon *et al.* (2004) reported that inflorescences of Gewurztraminer, sensitive to flower abscission, exhibited higher concentrations of starch and sucrose, whereas those of Pinot noir, more resistant to flower abscission, presented higher levels of glucose and fructose.

The fertility of the remaining flowers in defoliated plants was comparable to the one in the control, as the fruit and seed number per berry was not significantly different in most cases. This suggests that the whole plant is able to integrate the amount of available reserves that may be devoted to reproduction and to adjust the number of initiated inflorescences and flowers accordingly. This conclusion was supported by previous assumption that flowering and fruit set were only dependent on carbohydrates originated from reserves in grapevine (Candolfi-Vasconcelos and Koblet, 1990), although others have demonstrated that fruit set could be correlated to carbohydrate source from current photosynthesis (Caspari *et al.*, 1998 ; Zapata *et al.*, 2004b ; Intrieri *et al.*, 2008). Altogether, these results clearly show the importance of reserve restoration during the post-bloom period on the reproductive process the following year in grapevine.

In our experiments, vine productivity was reduced by leaf removal. Yield, potential alcohol and total acidity decreased after defoliation (year 3 and 4). In year 4, potential alcohol and total acidity were equal to the control plant : could this indicate that the plant is adapted ? The cumulative effect of defoliation was previously demonstrated and was concomitant to reduction in starch reserves (Candolfi-Vasconcelos and Koblet, 1990 ; Holzapfel *et al.*, 2006). Leaf removal generally decreases cluster and berry numbers and cluster weight (Holzapfel *et al.*, 2006). However, the impact of leaf removal is variable and influenced by training system, fruit load, vine age, fertility, cultivar, rootstock, irrigation practices, and macroclimate. For example, leaf removal led to decreased titratable acidity, malic acid and pH in several *Vitis vinifera* cultivars (Main and Morris, 2004) but to decreased titratable acidity and increased pH in Semillon grapevine (Holzapfel *et al.*, 2006). Effects are more often attributed to reduced leaf shading (Smart *et al.*, 1985 ; Morrison, 1988 ; Morrison and Noble, 1990) than to reduced cluster shading (Koblet, 1987). The decrease in titratable acidity is attributed to a reduction in malic acid concentration (Wolf *et al.*, 1986 ; Bledsoe *et al.*, 1988).

CONCLUSION

Regarding the impact of successive treatments during four growing seasons, defoliation does not induce any decrease of TNC content but a modification of its distribution. The decrease of sucrose (transport carbohydrate) in aerial organs may be correlated to the alteration observed on the reproductive development (inflorescence number and fruit set). However, leaf removal led to a decrease of yield and sugar yield. Besides, a significant increase of carbohydrate reserves after fruit removal did not induce a subsequent increase of the number of inflorescences per plant or flowers per inflorescence and fruit set.

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