

# REGULATING YIELD AND WINE QUALITY OF MINIMAL PRUNING SYSTEMS THROUGH THE APPLICATION OF GIBBERELIC ACID

## RÉGULATION DU RENDEMENT ET DE LA QUALITÉ DE VINS ISSUS DE SYSTÈMES DE TAILLE MINIMALE GRÂCE À L'APPLICATION DE L'ACIDE GIBBÉRELLIQUE

Karsten M. WEYAND<sup>1</sup> and Hans R. SCHULTZ<sup>1,2,\*</sup>

1: Institut für Weinbau und Rebenzüchtung, Fachgebiet Weinbau, Forschungsanstalt, von Lade Straße 1, 65366 Geisenheim, Germany

2: Fachbereich Geisenheim, Fachhochschule Wiesbaden, von Lade Straße 1, 65366 Geisenheim, Germany

**Abstract :** The use of gibberellic acid to increase berry size and to loosen up bunches in the production of seedless table grape varieties has a long history. The use of gibberellic acid on wine grapes, however, is very limited, mainly because some varieties can show substantial losses in bud fertility and thus in commercial yield. We tried to exploit the feature of decreases in bud fertility as a response to gibberellic acid applications (in the form of GA<sub>3</sub>) to regulate yield and quality aspects of minimally pruned (MP) White Riesling grapevines where other thinning methods have failed. Single applications of 50 mg L<sup>-1</sup> GA<sub>3</sub> at 500 L ha<sup>-1</sup> were used during full bloom in each year from 2002-2004 (MP GA<sub>3</sub>-02/03/04) in one MP treatment, while the second MP treatment received no GA<sub>3</sub> in 2003 (MP GA<sub>3</sub>-02/04). Treatments were compared with untreated MP vines and with a standard pruned, vertically shoot positioned system (VSP). GA<sub>3</sub> applications reduced inflorescence number by about 30-50% the year following treatment, while shoot number remained unchanged. GA<sub>3</sub> treated MP vines responded to this type of « thinning » by a 10-28 % increase in berry weight. Total yield of MP vines was reduced by 26-49% the year after first time application approaching VSP yield. Yield of the continuously treated vines (MP GA<sub>3</sub>-02/03/04) remained comparable to pruned VSP vines up to now (harvest 2005) (about 9-13 tons per ha). Discontinuing the GA<sub>3</sub> treatment for one year caused yield to re-approach the one of untreated MP vines the next year. Despite of the increase in berry weight, bunch structure remained less compact as compared to VSP fruit. Sugar levels at harvest of vines treated with GA<sub>3</sub> the previous year were similar to sugar levels of VSP vines and superior to untreated MP vines. We found no treatment effect on glycosyl-glucose (bound secondary metabolites, G-G) concentration but a slight increase in phenols and titratable acidity the year after GA<sub>3</sub> treatment. Sensory evaluation of the resulting wines revealed no or only minimal differences with a slight preference for the least yielding VSP and MP GA<sub>3</sub>-02/03/04 wines. The outlined strategy seems promising for the production of quality fruit in a highly economic viticultural system.

**Résumé :** L'utilisation de l'acide gibbéréllique pour augmenter la taille des raisins et favoriser l'aération des grappes de variétés de raisins de table sans pépins a une longue histoire. L'utilisation de l'acide gibbéréllique sur des grappes pour vinification est cependant très limitée parce que certaines variétés présentent des pertes substantielles de fertilité des bourgeons et ainsi des pertes de rendement commercial. Nous avons essayé d'exploiter cette faculté de décroître la fertilité des bourgeons en réponse à l'application de l'acide gibbéréllique (sous forme GA<sub>3</sub>) pour réguler le rendement et les paramètres qualitatifs de systèmes de taille minimale (MP) sur des grappes de Riesling car d'autres méthodes d'éclaircissage n'ont pas donné de résultat satisfaisants. 50 mg/L de GA<sub>3</sub> à 500 hl/ha ont été appliqués une fois en pleine floraison chaque année, de 2002 à 2004 (MP GA<sub>3</sub>-02/03/04) sur un traitement de MP, pour un second traitement de MP, l'application de GA<sub>3</sub> ne fut pas effectuée en 2003 (MP GA<sub>3</sub>-02/04). Ces traitements ont été comparés à des vignes de MP non traitées et à un système de taille standard (VSP). L'application de GA<sub>3</sub> provoqua une réduction du nombre d'inflorescences d'environ 30 % à 50 % l'année suivant le traitement alors que le nombre de sarments est demeuré inchangé. Les vignes de MP traitées ont présenté une augmentation de 10 % à 28 % du poids des baies en réponse à ce type « d'éclaircissage ». Le rendement total des vignes de MP a été réduit de 26 % à 49 % l'année suivant l'application en 2002, approchant le rendement du VSP. Le rendement de vignes continuellement traitées (MP GA<sub>3</sub>-02/03/04) demeura comparable à celui de vignes VSP jusqu'à présent (environ 9-13 t/ha). L'interruption du traitement de GA<sub>3</sub> pendant 1 an provoqua une augmentation du rendement se rapprochant à nouveau de celui de vignes de MP non traitées l'année suivante. Malgré l'augmentation du poids des baies, la structure des grappes est demeurée moins compacte comparée à des grappes de VSP. Le taux de sucre à la vendange pour des vignes traitées avec le GA<sub>3</sub> l'année précédente était similaire à ceux de vignes de VSP et supérieur à ceux de vignes de MP non traitées. Les différents traitements n'ont pas eu d'effet sur la concentration en glycosyl-glucose (composés secondaires liés, G-G), mais ont provoqué une légère augmentation des composés phénoliques et de l'acidité totale, l'année suivant le traitement de GA<sub>3</sub>. L'évaluation sensorielle des vins résultants n'a pas présenté de différence ou seulement minimale avec une légère préférence pour le VSP et les vins issus de MP GA<sub>3</sub>-02/03/04, avec ses rendements moins élevés. La stratégie présentée semble prometteuse pour une production de fruits de qualité dans un système de viticulture très économique.

**Key words :** minimal pruning (MP), vertically shoot positioned (VSP), gibberellic acid, thinning, bud fruitfulness, wine quality

**Mots clés :** taille minimale (MP), taille standard (VSP), acide gibbéréllique, éclaircissage, fertilité des bourgeons, vins de qualité

## INTRODUCTION

Minimal pruning (MP) can be a low cost, viable viticultural management technique to produce fruit of satisfactory quality for wine production with *Vitis vinifera* L. cultivars in warm but also in some cool climate areas of Australia (CLINGELEFFER, 1984; POSSINGHAM, 1996). In the cool climate areas of northern America (Northeast US, British Columbia), this technique has been used with *Vitis labruscana* cvs. and some hybrids for fruit juice and wine production (POOL, 1995; FENDINGER *et al.*, 1996; REYNOLDS and WARDLE, 2001). Despite of some promising results in various traditional wine growing regions of Europe with MP systems without crop control (IACONO *et al.*, 1998; MARTINEZ DE TODA and SANCHA, 1998; SCHULTZ *et al.*, 2000), other trials showed difficulties in adapting MP to climate and/or variety due to excessive yield and insufficient maturity (CARBONNEAU, 1991; OLLAT *et al.*, 1993; WEYAND and SCHULTZ, 2005). In cooler climates, MP vines are often less susceptible to fungal diseases such as *Botrytis cinerea* due to looser bunches (EMMETT *et al.*, 1994; SCHULTZ *et al.*, 2000) and with some cultivars, such as Riesling, exhibit more pronounced floral or fruity aromas if yield is not excessive (SCHULTZ, 2002). Thus, irrespective of *Vitis* species or cultivar, it is generally accepted that the use of MP in cool climates requires some type of crop control to achieve adequate maturity and wine quality levels (POSSINGHAM, 1996; REYNOLDS and WARDLE, 2001). In Europe an additional legal aspect comes into play, since yield may be beyond the contingents imposed by the European Community for quality wine production (SCHULTZ *et al.*, 2000).

One major goal to convert MP into a feasible management system suitable for quality wine production under cool climate European growing conditions was therefore to develop an economic strategy for crop control to improve quality without losing the typical advantages of these systems such as loose bunches and smaller berries with a high skin to pulp ratio (SCHULTZ *et al.*, 2000). Previous machine thinning trials with standard or modified vertical cutter bars or mechanical harvesters (POOL *et al.*, 1993) were not successful because the first method reduced leaf area to fruit ratio (SCHULTZ *et al.*, 2000), while the second caused substantial damage to the remaining bunches (RÜHLING, 1999) with possible negative consequences for wine quality (PETRIE *et al.*, 2003). The latter response is, however, not necessarily common to all varieties and under all growing conditions (SOMMER, 1995).

Aside of hand and mechanical thinning, a large range of chemical compounds have been tested to modify grape cluster structure or thinning berries, bunch parts or entire

bunches albeit not on MP vines (WEAVER and POOL, 1971; ZABADAL *et al.*, 1996). Used agents belonged to diverse chemical groups such as fertilizers (e.g. ammonium-nitrate), plant hormones and growth regulators (e.g. CCC (2-chloroethyltrimethyl-ammonium chloride), abscisic acid, ethephon (2-chloroethyl-phosphonic-acid)) or phytosanitary products (e.g. copper sulfate, sulfur), but variability in response, increased environmental awareness, human health concerns, and difficulties in handling prevented commercial use. Gibberellic acid, which is widely used in the production of seedless table grapes (WEAVER and POOL, 1971; DOKOOZLIAN *et al.*, 2001; MAY, 2004), can also be used to thin grape clusters (WEAVER and MCCUNE, 1959a; WEAVER and POOL, 1971) but can cause reduced and delayed bud break (WEAVER and MCCUNE, 1961) and loss of fruitfulness in seeded varieties the year after application (WEAVER, 1960; BANGERTH and GÖTZ, 1975). The latter feature is related to the antagonistic effect between cytokinins and gibberellins, which play a key role in bud fertility and inflorescence and flower formation (SRINIVASAN and MULLINS, 1979; SRINIVASAN and MULLINS, 1980; SRINIVASAN and MULLINS, 1981). It was exactly this negative effect which we thought using to regulate yield and quality in MP vines under cool climate conditions.

The objectives of the present study were therefore to use bloom time applications of gibberellic acid in a long-term strategy to adjust yield of MP vines to similar levels as standard pruned Riesling grapevines, while improving grape quality for wine production, without losing the typical MP advantages of exposed and loose bunches and small berries.

## MATERIALS AND METHODS

### I - EXPERIMENTAL DESIGN

Field experiments were conducted with *Vitis vinifera* L. cv. Riesling (clone: Gm 198/ rootstock: 5C, planted in spring 1977) from 2002 to 2005 in Geisenheim (50° N, 8° E), Germany. The experimental vineyard had a South to South-West exposure with a slope of 15-20 % and a loam to clay-loam soil. The minimal pruned plots (MP) were converted from a Sylvoz-trellis during the winter 1995 -1996 and compared to a standard vertical shoot positioning system (VSP). MP had a row by vine spacing of 2.8 m x 0.85 m and the VSP system of 2.0 m x 1.2 m, respectively. Both the MP and VSP-system were arranged in three replicated randomized blocks of 2-5 rows each with a total of 144 vines per pruning system. The VSP was cane- pruned to 8 buds per m<sup>2</sup> (19 buds per vine).

### II - GIBBERELIC ACID APPLICATIONS AND TREATMENTS

The commercial products « Set Fruit » (Comercial Química Massó S.A., Barcelona, Spain) and 'Gibb3' (Globachem NV, Sint-Truiden, Belgium) were used for gibberellic acid treatments in 2002 and 2003 - 2004, respectively. The two products had similar formulations with gibberellic acid in the form of GA<sub>3</sub> with concentrations of 9 % w/w and 10% w/w, respectively, in 10 g tablets, with sodium-bi-carbonate as releasing substance. The tablets were dissolved in distilled water and GA<sub>3</sub> was applied to MP vines at a concentration of 50 mg L<sup>-1</sup> equivalent to a total volume of 500 L ha<sup>-1</sup> with a backpack sprayer (type: 423 Port, Solo Kleinmotoren GmbH, Sindelfingen, Germany). All applications were done on a total of 48 vines per treatment until the canopy and inflorescences were slightly wet and repeated a second time after about 30 minutes. One treatment received GA<sub>3</sub> at full bloom (phenological stage BBCH 65 according to EICHHORN and LORENZ (1977)) in all experimental years (MP GA<sub>3</sub>-02/03/04). Application dates were 16th June in 2002, 5th June in 2003, and 16th June in 2004.

The second GA<sub>3</sub> treatment was applied in 2002 and 2004, but not in 2003 (MP GA<sub>3</sub>-02/04) to evaluate possible long-term carry-over effects. GA<sub>3</sub> was applied at BBCH 68 (mid-end of bloom, 17th June) in 2002, and at BBCH 65 in 2004, analogous to MP GA<sub>3</sub>-02/03/04.

### III - SHOOT AND INFLORESCENCE NUMBERS

To estimate the carry-over effects of GA<sub>3</sub> applications, we counted shoot and inflorescence numbers in the period between bud break and bloom in each year from 2003 to 2005. For all MP treatments, 4 strips of a canopy length of 0.85 m each (= planting distance of MP) were chosen at random within the three blocks for measurements because it was impossible to separate individual plants. For VSP we used 10 randomized individual plants.

### IV - CLUSTER STRUCTURE

Cluster structure was assessed for by determining the ratio of cluster length between the berries most proximal and distal to the peduncle to overall cluster weight (including the peduncle) for 25 clusters of each treatment on two dates in 2002 and 2003.

### V - BERRY AND SEED DEVELOPMENT, YIELD AND QUALITY PARAMETERS

Three field replicates of populations of 100 berries per treatment were collected at intervals of 6 to 14 days in 2002 and 2003 and 20 to 35 days in 2004 to determine berry weight, total soluble solids (TSS), total titratable acidity (TA) and pH from veraison until harvest. Berries were collected from the top, the centre and the bottom part of clusters at approximate equal proportions.

After weighing the berry samples to determine the 100-berry-weight, they were crushed with a household fruit squeezer and filtered (type: 33/N - Ederol, Binzer & Munktell GmbH, Battenberg, Germany). TSS was determined with a handheld refractometer (type: 2556/93, Leo Kübler GmbH, Karlsruhe, Germany). A must sample of 10 mL was used to determine total acidity (expressed as tartaric acid) and pH with an automatic titrator that was coupled to an auto-sampler and a control unit (Titrimo 719S, Metrohm AG, Herisau, Switzerland). To assess for GA<sub>3</sub> effects on berry seeds, seed number per berry was counted on a separately collected sample of 100 berries per treatment in October 2002 and 2003.

### VI - GLYCOSYL-GLUCOSE (GG) ASSAY AND TOTAL PHENOLS

The pool size of secondary metabolites was measured twice each year (at mid-ripening and at harvest) with a modified glycosyl-glucose-assay (GG) (WILLIAMS *et al.*, 1995; WERWITZKE, 2003). Three randomized samples per treatment of 50 berries each were collected from different portions of the clusters as described above. The samples were homogenized with an Ultra-Turrax (IKA-Werke, Staufen, Germany) at 24000 rpm in a centrifuge vial. Ten mL of a 50 % ethanol in water (v/v) extraction solution were added to an aliquot of 1 g of the homogenate; samples were gently shaken every 5 minutes and were centrifuged after 1hr (7 min, 3500 rpm). Four mL of the supernatant were transferred into a 125 mL Erlenmeyer vial adding 36 mL of distilled H<sub>2</sub>O and one drop of hydrochloric acid (HCl) (5M) to adjust to pH 2.25. C18 Sep Pack (solid phase extraction) cartridges (Waters, Eschborn, Germany) were pretreated with methanol (ca. 10 mL) followed by water (ca. 10 mL) and the sample was loaded onto the cartridge (at 10 kPa pressure). The cartridge was then washed with 50 mL of water and the glycosides were eluted in ethanol (1.5 mL), followed by water (3 mL). The glycoside eluat was adjusted to 5.0 mL with water and two aliquots of 1 mL of the eluat were taken. To one, 3 mL of distilled water were added and maintained at room temperature for 1 hr. The other was hydrolyzed with 2 mL 2.25 M sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) at 100°C for 1 hr. One mL of 8.8 M sodium hydroxide (NaOH) was added to 3 mL of the hydrolyzed sample and the pH maintained at 2.0-2.2 (WERWITZKE, 2003). Of both samples (hydrolyzed and non-hydrolyzed (control)) 0.9 mL were loaded onto the regenerated C18 Sep Pack cartridge for pre-conditioning. This was followed up by 3 mL of control and hydrolyzed samples. Of each, 1.5 mL was transferred to a cuvette (1 cm), 0.5 mL of distilled water and 1mL 1 mM triethanolamine buffer (pH 7.6) added, and absorbance measured at 340 nm. Glucose was determined enzymatically by adding 20 µL of Hexokinase/Glucose-6-phosphate-dehydrogenase (Roche Diagnostics, Mannheim,

Germany) and measuring again at 340 nm. The absorbance difference of the control before and after adding the enzyme was subtracted from the absorbance difference of the sample before and after adding the enzyme; and this value was used to determine the concentration of glucose from a standard curve prepared from calibration solutions. The glycoside eluat not processed during the GG-assay was used to determine total phenolics as catechin equivalents according to FOLIN and DENIS (1912).

## VII - WINEMAKING AND SENSORY EVALUATION

Harvesting and winemaking were standardized for all treatments. In brief, all vines per treatment were harvested manually. Grapes were kept separate for each experimental block, then transported in 700 L stainless steel containers to the experimental winery where yield was determined. Between 130 and 150 kg of grapes per treatment were then crushed (System Rauch, Rauch Landmaschinenfabrik GmbH, Sinzheim, Germany) and pressed using a standardized processing procedure with two 380 L content automated membrane presses (Europress EHP 380, Scharfenberger, Bad Dürkheim, Germany). The must was treated with 40 mg L<sup>-1</sup> of sulfurous acid H<sub>2</sub>SO<sub>3</sub> and settled for clarification over night (10 to 12 hrs) in 150 L polyethylene tanks. Clarified must was used to determine TSS, acidity and pH as described above. About 70 L of must were inoculated with 20 g hL<sup>-1</sup> Oenoferm® Klosterneuburg (*Saccharomyces cerevisiae*) (Erbslöh, Geisenheim, Germany) and racked into two 50 L and 25 L glass-balloons for fermentation under controlled conditions (20-22°C). After fermentation another 100 mg L<sup>-1</sup> H<sub>2</sub>SO<sub>3</sub> were added and the wines were racked into one 50 L glass-balloon. All wines were protein stabilized with calcium-bentonite (Ca-Granulat, Erbslöh,

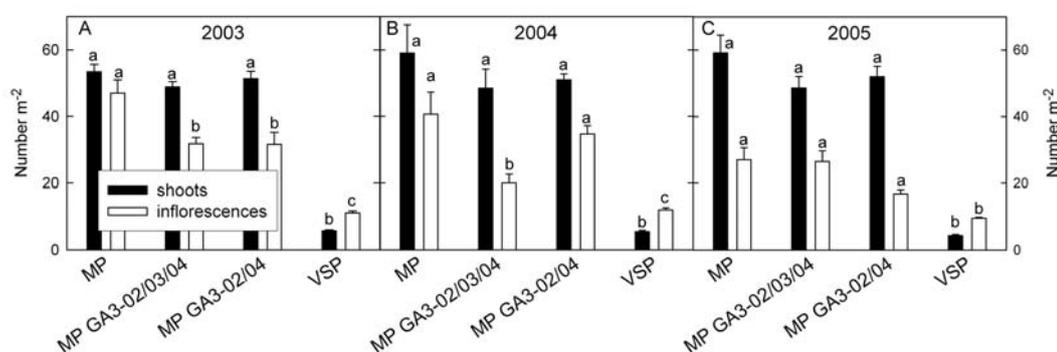
Geisenheim, Germany) using 300 g hL<sup>-1</sup> in 2002 and 200 g hL<sup>-1</sup> in 2003. Right before bottling, in March following the respective vintage, wines were cold filtered (Pilot-Z 20\*20 cm, Pall SeitzSchenk Filtersystems GmbH, Bad Kreuznach, Germany; filter-sheets: EK, Pall SeitzSchenk Filtersystems GmbH, Bad Kreuznach, Germany). Bottles were stored in the dark at 13 to 16°C.

Sensory evaluations were conducted with trained panelists of the Oenology Department. First, all wines were subjected to a repeated discriminating triangle test to detect perceptible differences between two wines. Prior to each test, two bottles of each wine were first homogenized and then separated again into their original bottles. Descriptive evaluations on a scale between 1 and 5 (5 was max.) for the attributes aroma-intensity, sweetness, bitterness, acidity, mouthfeeling, and aroma-quality were conducted in replicate with randomized wine pairs or triplets. Bottle coding, test sheet programming and statistical analyses of all sensory evaluations were done using the software Fizz® for Windows 2.00M (Biosystème, Couternon, France). For all sensory evaluations 'Sensus' wine tasting glasses (Schott Zwiesel Kristallglas AG, Zwiesel, Germany) were used, which were filled up to 0.1 L. Before every tasting session, wines were cool stored at 13 °C for one day and moved into the tasting room 15 minutes before tasting.

The 2002 vintage was evaluated in January/February and November 2004 and the 2003 vintage in October 2004 and a second time in July 2005.

## VIII - STATISTICS

Aside of the sensory statistics we used linear regressions and analyses of variances (ANOVA, Holm-Sidak-method) for the plant based data, which were calculated



**Figure 1 - Shoot and inflorescence number of MP, MP GA3-02/03/04, MP GA3-02/04 and VSP 'Riesling' grapevines for the seasons 2003 to 2005.**

Bars denote the average shoot and cluster numbers m<sup>-2</sup> of soil surface area ± SE from four replicated planting distances (0.85 m) for all MP treatments and ten entire vines for the VSP treatment, respectively. Bars with different letters indicate significant differences at the p < 0.01 level.

**Nombre de tiges et d'inflorescences des traitements MP, MP GA3-02/03/04, MP GA3-02/04 et VSP pour le cépage Riesling pour les millésimes 2003 à 2005.**

with SigmaStat 3.1 (Systat Software Inc., Point Richmond, CA, USA).

## RESULTS AND DISCUSSION

### I - SHOOT AND INFLORESCENCE NUMBER

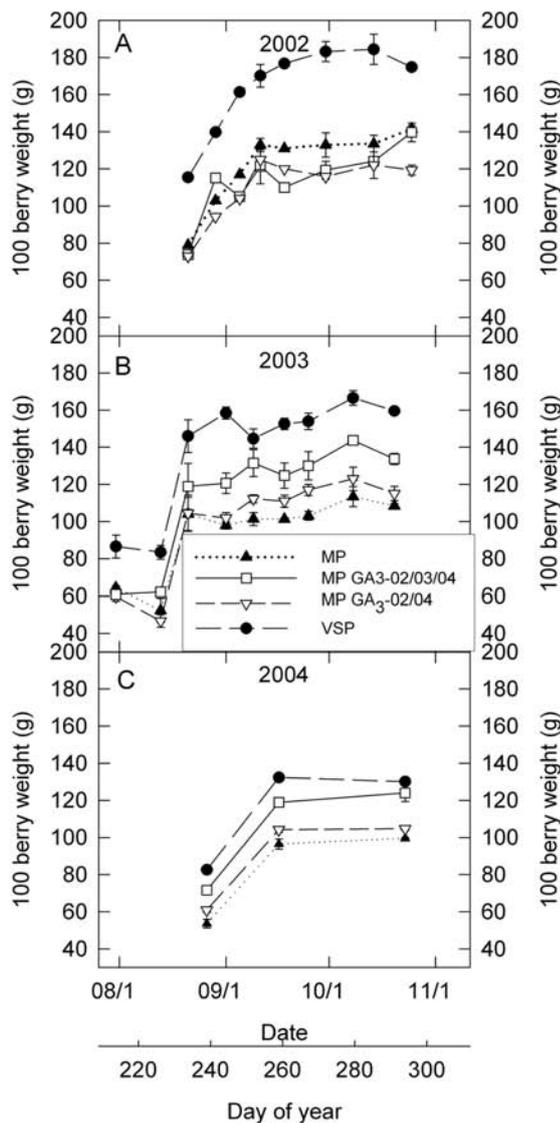
Shoot number recorded in spring was not significantly different between GA3 treated and non treated MP vines

(figure 1). MP vines had about 20 times as many shoots on a per m<sup>2</sup> soil surface basis than VSP vines (figure 1). Neither average shoot length nor leaf size were apparently affected by GA3 (data not shown). The yearly application of GA3 between 2002 and 2004 (MP GA3-02/03/04) caused the total number of inflorescences to decrease from about 46 m<sup>-2</sup> in 2002 (data not shown) to 30 m<sup>-2</sup> in 2003 (figure 1A) and further to between 21 and 25 m<sup>-2</sup> in 2004 and 2005 (figures 1B-C) indicating a strong effect on bud fertility, long recognized for pruned vines (WEAVER and McCUNE, 1961).

Untreated MP vines had much higher Inflorescence numbers in 2003 and 2004 (figure 1A, B) but had low inflorescence numbers in 2005. This may have been due to cool season conditions in 2004 (SRINIVASAN and MULLINS, 1980) and/or excessive yields from 2002 to 2004 (see figure 6), where the developing crop may have reduced carbohydrate and nitrogen reserves allocated to the buds and thus reduced bud fruitfulness the subsequent year (MAY, 2004).

When GA3 application was discontinued in 2003, inflorescence number of the MP GA3-02/04 treatment partly recovered and had significantly more inflorescences than MP GA3-02/03/04 in 2004, while numbers were still lower than those of untreated MP vines (figure 1B). Fruitfulness of individual VSP shoots was always highest with individual inflorescences being visually larger with a much higher number of individual flowers.

Additional to the reduction in total inflorescence number induced by the GA3 applications, we visually observed that many inflorescences were smaller, with single branches partly being more tendril-like and with a significantly lower dry weight (WEYAND and SCHULTZ, 2005). This is an indicator for the disrupted balance between cytokinins and gibberellic acid acting on the formation of inflorescence primordia (SRINIVASAN and MULLINS, 1979; SRINIVASAN and MULLINS, 1981). However, GA3 did not cause bud necrosis symptoms, uneven bud break or phytotoxic effects as observed in other experiments (WEAVER, 1960; ALLEWELDT, 1961; BRANAS and VERGNES, 1963; ZIV *et al.*, 1981). This may have been related to the concentration (50 ppm) and variety used (Riesling). WEAVER (1960), for example, found substantial losses in fertility and a high percentage of necrotic buds in five seeded varieties already at 25 ppm, whereas BLAHA (1963) did not find any negative effects with Riesling, Pinot blanc and Savagnin rose at a concentration of 100 ppm and seedless varieties such as Thompson Seedless and Black Corinth were even unaffected by concentrations as high as 1,000 ppm (WEAVER 1960). These discrepancies point directly to the problem, that every variety responds differently and that the



**Figure 2 - Changes in berry weight of MP, MP GA3-02/03/04, MP GA3-02/04, and VSP 'Riesling' grapevines during ripening for the seasons 2002 to 2004.**  
Data represent the means of three 100 berry populations  $\pm$  SE.

**Développement du poids de baies pour les traitements MP, MP GA3-02/03/04, MP GA3-02/04 et VSP du cépage Riesling pendant la maturation pour les millésimes 2002 à 2004.**

use of a GA3 strategy to control yield needs to be developed for each variety separately.

II - BERRY GROWTH AND CLUSTER STRUCTURE:

Individual berry weight, which is usually much lower for MP as compared to VSP vines (figure 2A), increased in the MP GA3-02/04 and MP GA3-02/03/04 treatments by 10 %, respectively 28 % after the first year of GA3 application (figure 2B). This increase was only significant for the MP GA3-02/03/04 treatment ( $p < 0.05$ ), but berries still remained significantly smaller ( $p < 0.05$ ) as compared to VSP. In 2004, average berry weight of VSP fruit was very low as compared to the previous years (figures 2A-C) and was not different to the MP GA3-02/03/04 berries, although their size had not increased as compared to 2003 (figures 2B, C). Both the MP and the MP GA3-02/04 berries were about 20 % smaller ( $p < 0.05$ ) (figure 2C). In the 2005 season (data not shown), all MP treatments (with or without GA3 application) had between 27-29 % smaller berries than VSP, suggesting that GA3 did not cause a strong long-term compensatory growth response for MP berries.

Seedless cultivars of *Vitis vinifera* generally respond to gibberellin application with an increase in berry size (WEAVER and McCUNE, 1959a; DASS and RANDHAWA, 1967; DOKOOZLIAN *et al.*, 2001), whereas seeded cultivars may not respond at all (WEAVER and McCUNE, 1959b; DASS and RANDHAWA, 1967;

REGNER *et al.*, 2002) or form larger (WEAVER and McCUNE, 1959b; DASS and RANDHAWA, 1967) and/or smaller berries (BLAHA, 1963; REGNER *et al.*, 2002). The variety Riesling typically responds to a bloom-time GA3 application with a large number of shot and small berries (BLAHA, 1963; HILL *et al.*, 2003; ZELT, 2005). Exogenous GA3 application at flowering has been shown to inhibit pollen germination and pollen growth

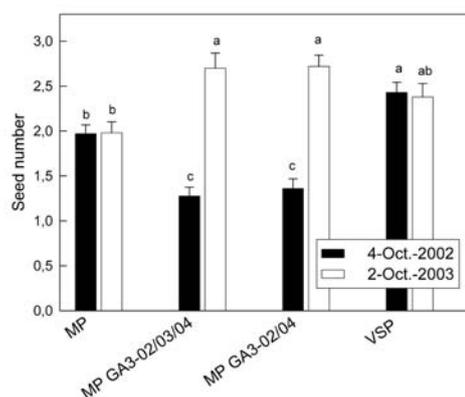


Figure 3 - Effect of pruning system and gibberellic acid on seed number per berry of randomized berry populations of MP, MP GA3-02/03/04, MP GA3-02/04, and VSP 'Riesling' grapevines for the seasons of 2002 and 2003.

Data are the mean  $\pm$  SE (n = 100).

Effet du système de taille et de l'acide gibbérellique sur le nombre de pépins par baies, mesuré sur une population randomisée de baies, cépage Riesling pour 2002 et 2003

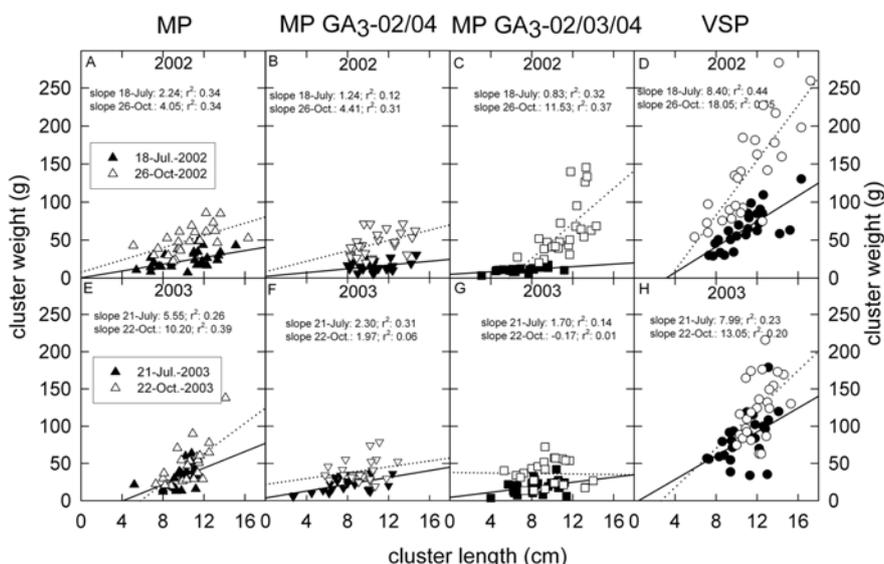


Figure 4 - Effect of pruning system and gibberellic acid on the cluster weight to cluster length ratio of randomized populations (n = 25) for clusters of MP, MP GA3-02/03/04, MP GA3-02/04, and VSP 'Riesling' grapevines collected in July and at harvest in October in 2002 (upper panel) and 2003 (lower panel).

The slopes of the linear regressions express the average degree of compactness for the cluster populations. Higher slopes denote a greater compactness.

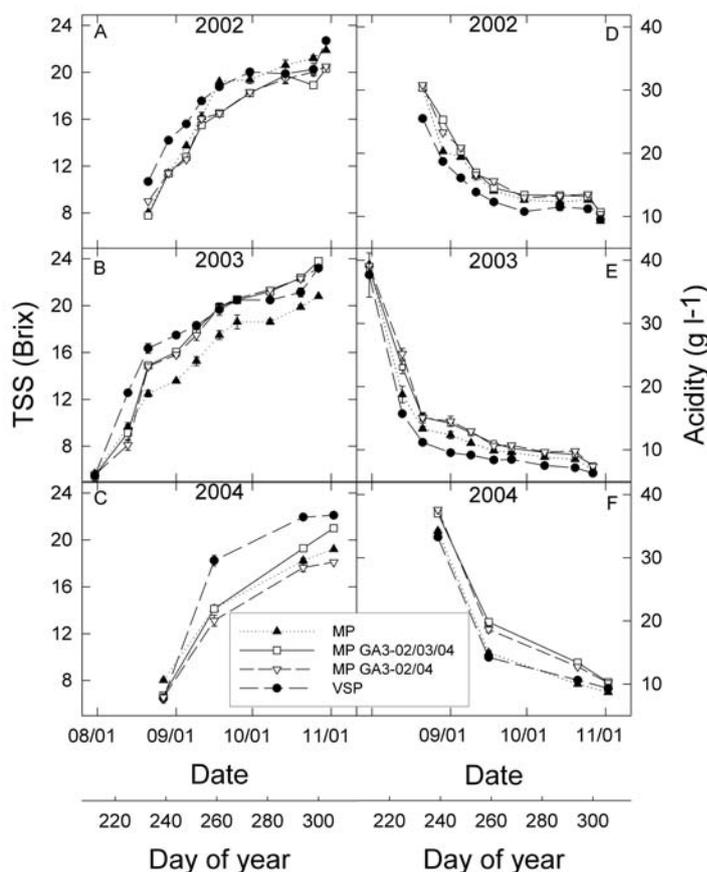
Effet du système de taille et de l'acide gibbérellique sur le rapport entre poids de grappe et longueur sur une population randomisée de grappes (n=25), cépage Riesling, mesuré en juillet et à la vendange en octobre 2002 (rang du haut) et 2003 (rang du bas).

and to reduce seed number in some varieties (KIMURA *et al.*, 1996). This may explain the significantly reduced seed numbers in berries during the first year of GA<sub>3</sub> application (2002) as compared to the VSP and MP treatments (figure 3). Smaller berries have been correlated with smaller seed numbers or size and thus reduced endogenous gibberellin-like activity during berry development (IWAHORI *et al.*, 1965; SCIENZA *et al.*, 1978). However, we observed only a very small and non-significant effect on berry size that year (figure 2A).

During the 2003 season, both MP GA<sub>3</sub>-02/04 and MP GA<sub>3</sub>-02/03/04 had seed numbers per berry which were higher than those from MP vines and similar to those from the VSP treatment, untypical for a GA<sub>3</sub> response of seeded varieties (DASS and RANDHAWA, 1967; BANGERTH and GÖTZ, 1975) (figure 3). Only the berry size of the MP GA<sub>3</sub>-02/03/04 treatment seemed to respond to the increase in seed number (figure 2B). Since MP GA<sub>3</sub>-02/04 did not receive a GA<sub>3</sub> application that year but had seed numbers similar to MP GA<sub>3</sub>-02/03/04, one explanation

for differences in berry development may have been differences in crop load (figure 6A). High crop loads induced substantially lower water potentials for the MP and MP GA<sub>3</sub>-02/04 treatments as compared to MP GA<sub>3</sub>-02/03/04 and VSP during the first period of berry development (WEYAND and SCHULTZ, 2005), when berry growth is particularly sensitive to water status (OJEDA *et al.*, 2001).

Despite the substantial reduction in inflorescence number and some increase in berry weight for GA<sub>3</sub> treated MP vines, the loose structure of the clusters of control MP vines was largely retained (figure 4). Cluster compactness was derived from the slope of the regression analysis of cluster weight as a function of cluster length in mid-July and prior to harvest in 2002 and 2003 (figure 4A-H). In both years and at both stages, clusters of VSP had the highest slope coefficients of all treatments. This indicated, that clusters were more compact than those of untreated and treated MP vines (figure 4A-H), which was related to larger individual berries and to a lar-



**Figure 5 - Total soluble solids, TSS (A-C), and total titratable acidity (D-F) of three 100 berry samples of MP, MP GA<sub>3</sub>-02/03/04, MP GA<sub>3</sub>-02/04, and VSP 'Riesling' grapevines during ripening for the seasons 2002 to 2004. Data represent means  $\pm$  SE.**

**Taux de sucres (TSS) (A-C) et acidité totale (D-F) de 100 échantillons de baies en 3 répétitions des traitements MP, MP GA<sub>3</sub>-02/03/04, MP GA<sub>3</sub>-02/04 et VSP pour le cépage Riesling pendant la maturation de 2002 à 2004.**

ger total number of berries per cluster (WEYAND and SCHULTZ, 2005). To achieve loose clusters for better control of bunch rot was one of the primary goals of early experiments with wine grapes of pruned vines (WEAVER and McCUNE, 1959b, WEAVER *et al.*, 1962), where pre-bloom applications proved to be most effective in elongating the rachis and changing grape structure (WEAVER and McCUNE, 1959b). However, in our study, clusters were not elongated (relative late application of GA3) and the loose cluster structure did not reduce bunch

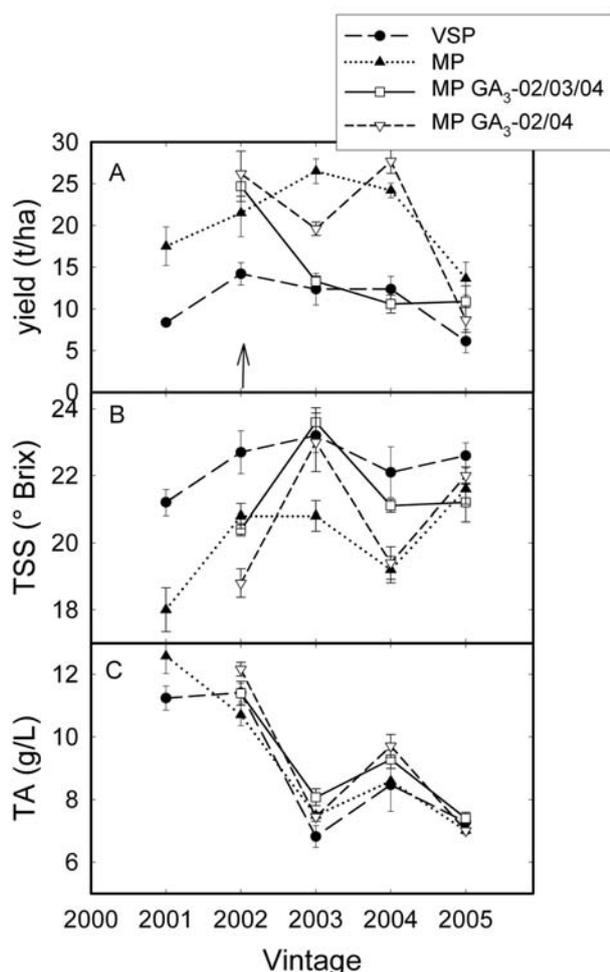
rot for untreated and treated MP vines as compared to VSP.

### III - BERRY RIPENING, YIELD AND MUST COMPOSITION

Sugar accumulation in the berries started latest in 2004 and earliest in 2003 due to seasonal differences (figure 5A-C). In 2002 (figure 5A) VSP had highest TSS early during ripening, but differences to the other treatments diminished towards harvest (figure 5A). There was no effect of GA3 on TSS and TA (figure 5D), likely because yield was comparable to untreated MP vines (figure 6A).

In 2003, both GA3 treatments had similar concentrations of TSS as VSP during the second half of the ripening period, with the MP control being retarded in sugar accumulation (significant at  $p < 0.05$ , figure 5B). There was some effect of GA3 on TA, which was increased in treated MP vines despite higher TSS values and lower yields (figures 5E and 6A). This effect was also present in 2004 (figure 5F), where TSS accumulation of all MP treatments was retarded as compared to VSP during most of the ripening phase (figure 5C). Only the MP GA3-02/03/04 treatment reached TSS levels comparable to VSP at harvest (VSP and MP GA3-02/03/04 were significantly different at  $p < 0.05$  as compared to MP and MP GA3-02/04).

In general TSS responded to crop load. Between 2001 and 2005, untreated MP vines yielded between  $13.6 \text{ t ha}^{-1}$  (2005) and  $26.5 \text{ t ha}^{-1}$  (2003) as compared to about  $7\text{-}13 \text{ t ha}^{-1}$  for pruned VSP vines. When yield was expressed in  $\text{hl ha}^{-1}$ , MP vines exceeded the crop size permitted by law for the variety Riesling ( $100 \text{ hl ha}^{-1}$  equivalent to about  $12.5 \text{ t ha}^{-1}$ ) in all years. During the first year of GA3 application, yield was unaffected (figure 6A, arrow) and TSS was lower both in berries and the resulting juice (figure 6B). For pruned vines of this cultivar, the GA3 concentration used (50 ppm) has been shown to be sufficient to reduce yield by about 20-30 % (AHMED MUSTAFA, 1989; ZELT, 2005). For MP vines, however, responsiveness may be much more dependent on yield potential and environmental conditions prevailing at the time of application (LOHNAU, 2003). Only the reduction in inflorescence number (and size, data not shown) in subsequent years caused a substantial reduction in yield of GA3 treated MP vines in our study and only then was TSS increased in berries and the final must (figure 5B, 6B). The later treatment date for MP GA3-02/04 in 2002 may have been responsible for the smaller losses in yield as compared to MP GA3-02/03/04 in 2003 (figure 6A) despite similar reductions in inflorescence numbers (figure 1A), since the degree of GA3 induced thinning (in the year of application), as well as possible reductions in bud fruitfulness the following year depend on the stage



**Figure 6 - Total yield (A), and juice TSS (B), and TA (C) of MP, MP GA3-02/03/04, MP GA3-02/04, and VSP 'Riesling' grapevines during the course of 5 seasons (2001-2005).**

Data represent means of three field blocks  $\pm$  SE. Arrow in panel A indicates the first application of GA3.

**Rendement total (A), taux de sucres (B) et acidité totale (C) pour les traitements MP, MP GA3-02/03/04, MP GA3-02/04 et VSP, cépage Riesling à la vendange de 2001 à 2005.**

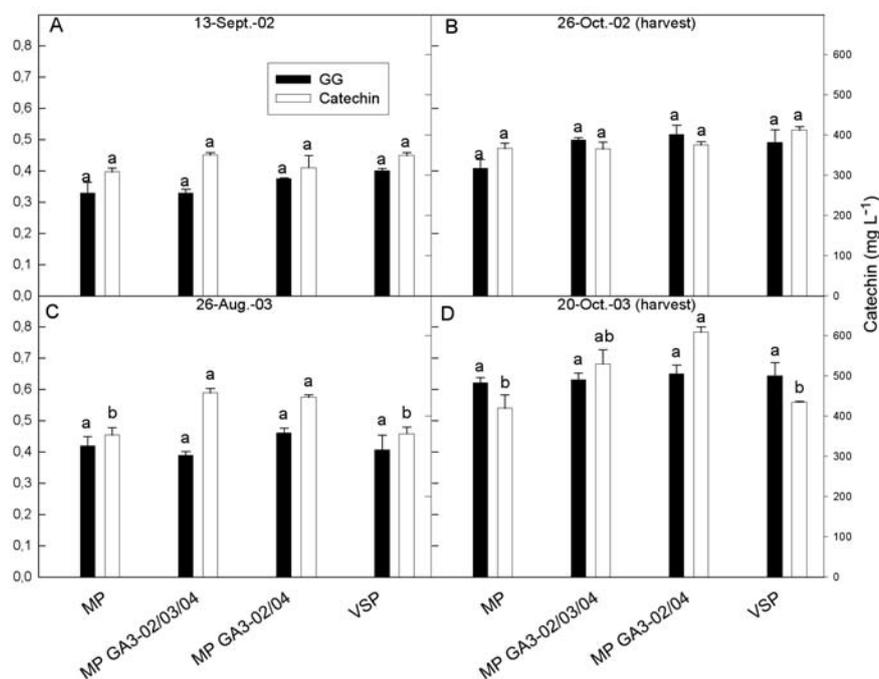
of flowering during which GA<sub>3</sub> is applied (WEAVER and MCCUNE, 1959b; AHMED MUSTAFA, 1989). Continuous GA<sub>3</sub> application brought crop size and TSS close to the level of pruned VSP vines (figures 6A, B) (- 55% in 2004 as compared to MP). Even slightly increased berry sizes could not compensate for this effect (see figure 2). Interrupting the GA<sub>3</sub> treatment for one year (MP GA<sub>3</sub>-02/04) caused yield to recover and TSS to drop to the level of untreated MP vines the next year (figure 6A, B), indicating that the effect on bud fruitfulness is rapidly reversible. However, due to the large differences in responsiveness to GA<sub>3</sub> between different cultivars, this is not necessarily to be expected with all seeded cultivars (ALLEWELDT, 1961; SRINIVASAN and MULLINS, 1981).

In most studies on seeded cultivars, TSS levels responded favorable to gibberellins if some control of crop size was achieved (WEAVER and McCUNE, 1959a, 1959b; BANGERTH and GÖTZ, 1975; AHMED MUSTAFA, 1989; HILL *et al.*, 2003). The response of TA, however, is less clear and seems to be variety specific. WEAVER and McCUNE (1959a) found decreasing and unchanged levels of TA, when the varieties Zinfandel and

Tokay were treated with a large range of gibberellic acid concentrations. For pruned Riesling vines, TA has also been reported to decrease with increasing concentrations of gibberellic acid (AHMED MUSTAFA, 1989; ZELT, 2005), while for MP vines there was no change (LOHNAU, 2003) or even an increase as in the present study. For untreated MP vines we often observed lower TA levels as compared to VSP even at higher yields, which has also been reported by others (IACONO *et al.*, 1998; REYNOLDS and WARDLE, 2001) and is usually attributed to a better exposure of MP grapes to sunlight (PRICE *et al.*, 1994).

In comparison to other studies on MP and pruned vines, our yield differences are much larger and support the opinion that MP systems need to be crop regulated in cooler climates (POSSINGHAM, 1996). One reason for these yield differences between studies may be related to the Riesling clone used, which has been shown to respond to MP much stronger in yield increase than other clones of the same variety (CLINGELEFFER, 1988).

#### IV - GLYCOSYL-GLUCOSE (G-G), TOTAL PHENOLS AND WINE SENSORY PROPERTIES



**Figure 7 - Glycosyl-glucose (G-G) per unit of berry fresh weight (BFW) and total phenols expressed as Catechin equivalents in berries from MP, MP GA<sub>3</sub>-02/03/04, MP GA<sub>3</sub>-02/04, and VSP 'Riesling' grapevines at mid-ripening and at harvest in 2002 (A - B) and 2003 (C - D).**

Data are the means of three field and two lab repetitions of randomized populations of 50 berries each ± SE. Bars with different letters indicate significant differences at the p < 0.05 level.

Glycosyl Glucose (G-G) par g de poids de baies fraîches (BFW) et composés phénoliques totaux exprimés en mg/L équivalent catéchine mesuré dans des baies du cépage Riesling pour les traitements MP, MP GA<sub>3</sub>-02/03/04, MP GA<sub>3</sub>-02/04 et VSP prélevées à mi-maturité et à la vendange en 2002 (A-B) et 2003 (C-D).

There is not much information available about the impact of either MP or gibberellic acid treatments on secondary plant metabolites, such as the pool of bound glycosides and phenols, and the possible consequences for the sensory properties of the resulting wines. In the present study, G-G concentration in the berries increased for all treatments during 2002 and 2003 between mid-ripening and harvest but showed no significant differences between treatments (figure 7). There was also no difference in the amount of total phenols (expressed as catechin equivalent) with the exception of 2003 at harvest, where both GA3 treated MP systems had higher values as compared to VSP and MP (figure 7D). Other studies have shown a tendency towards higher G-G levels when MP and VSP vines were compared (SCHAIBLE, 2002; WERWITZKE, 2003), but not for every year investigated. Application of gibberellic acid can increase G-G concentration in berries from pruned (SCHAIBLE, 2002) and MP vines (LOHNAU, 2003), but the response is not consistent for all varieties.

We found higher concentrations of phenolics in grapes of treated MP vines as compared to untreated MP and

VSP in 2003. This is consistent with observations of AHMED MUSTAFA (1989) and NIKFARDJAM *et al.* (2005) for several varieties including Riesling but the underlying mechanisms are still unclear.

A substantial portion of bound glycosides may be phenolic in nature and thus not relevant for the aromatic expression of wines (WERWITZKE, 2003). For Riesling this proportion has been estimated to be between 15 and 25 % (ZOECKLEIN *et al.*, 1998), which could indicate, that the increased phenol values at equal G-G concentrations found for GA3 treated MP vines may reduce overall aromatic expression.

Sensory evaluation of wines by expert panelists using discriminatory triangle tests showed very few differences (table 1). This applied to wines with less than 12 and more than 16 months of bottle storage (bottling in March), respectively, of both observed vintages (2002 and 2003). Of the 2002 vintage, only VSP and MP GA3-02/04 could be discriminated after 21 months of bottle aging (significant at  $p < 0.01$ , table 1), while the panelists had no preference for either one of the wines. Similar to 2002, no significant differences could be detected for wines within

**Table I - Results of triangular tests of the experimental wines (Prefer.= preference).**

Résultats des tests triangulaires conduits sur les vins expérimentaux (Prefer. = préférence)

Tasting date	Vintage	Wine A	Wine B	Number of judges (total)	Number of correct judges	Prefer. Wine A	Prefer. Wine B
-	2002	VSP	MP	-	-	-	-
20.02.04	2002	VSP	MP GA <sub>3</sub> -02/03/04	16	6	3	3
20.02.04	2002	VSP	MP GA <sub>3</sub> -02/04	16	8	2	6
16.01.04	2002	MP	MP GA <sub>3</sub> -02/03/04	11	6	6	0
16.01.04	2002	MP	MP GA <sub>3</sub> -02/04	11	4	2	2
16.01.04	2002	MP GA <sub>3</sub> -02/03/04	MP GA <sub>3</sub> -02/04	11	6	4	2
19.11.04	2002	VSP	MP	11	6	3	3
19.11.04	2002	VSP	MP GA <sub>3</sub> -02/03/04	11	6	3	3
19.11.04	2002	VSP	MP GA <sub>3</sub> -02/04	11	9**	4	5
18.11.04	2002	MP	MP GA <sub>3</sub> -02/03/04	10	4	3	1
18.11.04	2002	MP	MP GA <sub>3</sub> -02/04	10	3	2	1
18.11.04	2002	MP GA <sub>3</sub> -02/03/04	MP GA <sub>3</sub> -02/04	10	1	0	1
07.10.04	2003	VSP	MP	13	6	2	4
07.10.04	2003	VSP	MP GA <sub>3</sub> -02/03/04	13	7	4	3
07.10.04	2003	VSP	MP GA <sub>3</sub> -02/04	13	5	4	1
07.10.04	2003	MP	MP GA <sub>3</sub> -02/03/04	13	6	4	2
07.10.04	2003	MP	MP GA <sub>3</sub> -02/04	13	6	5	1
07.10.04	2003	MP GA <sub>3</sub> -02/03/04	MP GA <sub>3</sub> -02/04	13	6	2	4
29.07.05	2003	VSP	MP	11	4	2	1
29.07.05	2003	VSP	MP GA <sub>3</sub> -02/03/04	11	2	1	1
29.07.05	2003	VSP	MP GA <sub>3</sub> -02/04	11	7*	4	3
29.07.05	2003	MP	MP GA <sub>3</sub> -02/03/04	11	8**	0	8
29.07.05	2003	MP	MP GA <sub>3</sub> -02/04	11	6	1	5
29.07.05	2003	MP GA <sub>3</sub> -02/03/04	MP GA <sub>3</sub> -02/04	11	7*	4	3

\*, \*\* = significant differences at  $p < 0.05$ ,  $p < 0.01$ , respectively.

the first 12 months of bottle aging for the 2003 season. However, wines of that vintage showed some differences after 16 months, when for example VSP and MP GA3-02/04 could be discriminated without preferences (table 1). Wines from the GA3 treatments were generally preferred to wines from the MP treatment indicating that the reduction in yield improved the sensory quality of fruit from MP vines.

Descriptive sensory evaluations were conducted after about 21 months of bottle aging for the 2002 vintage and 28 months of the 2003 vintage (figures 8A, B). Differences between the observed attributes were relatively small between all treatments for 2002 (fig. 8A), with the excep-

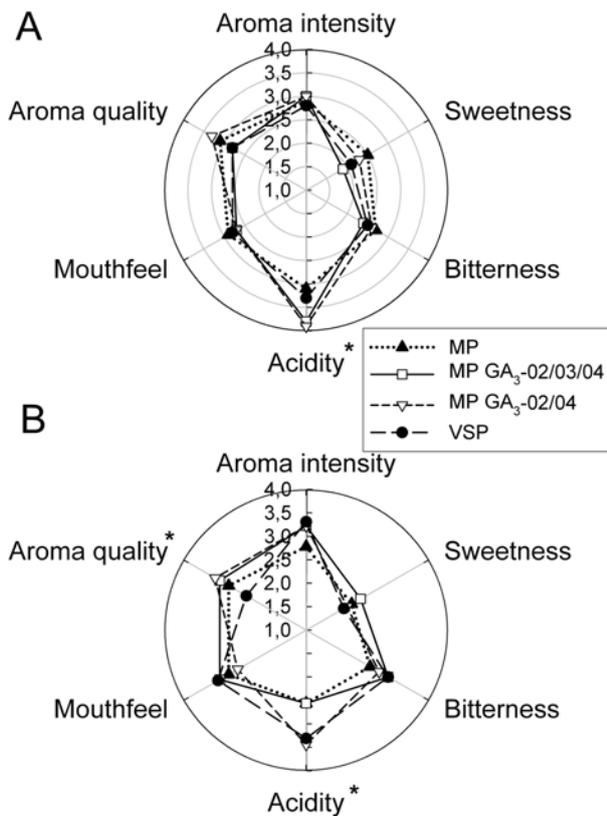
tion of a significantly higher ( $p < 0.05$ ) perception of acidity for the GA3 treated MP systems (figure 8A) which was consistent with the analytical data from the must (figure 6C).

When wines from the 2003 vintage were tasted, panelists judged the acidities of MP GA3-02/04 and VSP to be significantly more dominant than acidities of the MP and MP GA3-02/03/04 treatment, despite of the fact that VSP had the lowest acidity in the must (figure 6C). Aroma quality was significantly preferred for all GA3 treated MP systems as compared to VSP and MP (figure 8B). Simultaneously performed ranking of the wines did not give any significant results (data not shown). Recently, NIKFARDJAM *et al.* (2005) stated that wines from GA3 treated Riesling and Sauvignon blanc vines (pruned) were fresher, less oxidized and with more varietal character and related this to higher amounts of phenols. They argued, that polyphenols would not only act as antioxidants and thus protecting aroma compounds, but would contribute to mouthfeel and persistence. Although we did not analyze for phenolic composition of the finished wines, berry analyses showed higher amounts of phenols for the MP GA3-02/03/04 and MP GA3-02/04 treatments, which may have contributed to preserve varietal aroma during bottle storage.

## CONCLUSION

MP vines grown under cool climate and short season growing conditions are very economic to manage, but often yield far beyond standard VSP systems and permitted quantities for quality wine production. Some of the consequences are delayed ripening and insufficient maturity levels at harvest in some years. The application of gibberellic acid during bloom with the goal to reduce bud fruitfulness in the forthcoming season seems a promising tool to adjust yield and quality of MP vines to levels comparable to the standard VSP system, at least for the variety Riesling. This can be achieved without losing MP typical advantages such as loose clusters and small berries with a high skin to pulp ratio. Current knowledge suggests that applications during bloom every second year are not recommendable since strong alternate bearing effects may be induced. Further investigations should focus on the evaluation of differences in fruit quality within the huge canopy systems of MP vines in order to develop thinning strategies which can be targeted towards canopy zones exhibiting poor fruit quality.

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**Figure 8 - Descriptive analyses of wines elaborated from grapes of MP, MP GA3-02/03/04, MP GA3-02/04, and VSP 'Riesling' grapevines. A: vintage 2002, sensory evaluation on 18.-Nov.-2004 (n = 10); B: vintage 2003, sensory evaluation on 28.-Jul.-2005 (n = 11). \* denotes significant differences at the  $p < 0.05$  level (description in the text).**

**Analyse sensorielle descriptive des vins élaborés à partir des grappes des traitements MP, MP GA3-02/03/04, MP GA3-02/04 et VSP, cépage Riesling. A: millésime 2002, évaluation sensorielle du 18 nov. 2004; B: millésime 2003, évaluation sensorielle du 28 jul. 2004.**

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