Mitteilungen Klosterneuburg 59 (2009): 129-133

Correspondence between gibberellin-sensitivity and pollen tube abundance in different seeded vine varieties

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During early berry development, the grapevine varieties 'Sylvaner' and 'Pinot gris' show an inverse relationship between endogenous gibberellin (GA) levels and sensitivity to exogenously applied GA_3. Moreover, endogenous GA levels correspond to the observed variety-dependent abundance of pollen tubes that might serve as a source for GAs. Pollen tube analyses of a broad spectrum of additional varieties with known GA sensitivity support this hypothesis.
Keywords: *Vitis*, endogenous gibberellin status, pollen tube abundance

Korrelation zwischen Pollenschlauchabundanz und Gibberellin-Sensitivität bei verschiedenen Rebsorten. *Während der frühen Beerenentwicklung zeigen die Rebsorten 'Sylvaner' und 'Grauburgunder' eine negative Korrelation zwischen den Konzentrationen endogener Gibberellinsäure (GA) und ihrer Sensitivität gegenüber exogenen GA_3-Applikationen. Darüber hinaus korreliert der endogene Gibberellinstatus mit der sortenspezifischen Anzahl keimender Pollenschläuche, die als GA-Quelle dienen könnten. Pollenschlauchanalysen eines breiten Spektrums von Ertragsrebsorten mit bekannter Gibberellinsensitivität unterstützen diese Hypothese.*
Schlagwörter: *Vitis*, endogener Gibberellinstatus, Pollenschlauchabundanz

Correspondence entre l'abondance en tubes polliniques et la sensitivité au gibberelline chez de différents cépage. *Pendant le prime développement des baies, les cépages 'Sylvaner' et 'Pinot gris' montrent une corrélation inverse entre la concentration endogène en acide gibbérellique (GA) et leur sensibilité à l'application exogène de GA_3. En plus, la concentration endogène en gibbérelline correspond à l'abondance en tubes polliniques qui est typique de ces cépages et qui pourrait, en même temps, servir de source de GA. Des analyses de tubes polliniques exercées avec un grand nombre d'autres cépages, dont la sensibilté au gibbérelline est bien connue, soutiennent cette hypothèse.*
Mots clés: *Vitis*, concentration endogène en gibbérelline, abondance en tubes polliniques

Gibberellic acids (GAs) regulate various developmental processes in adult plants, including stem elongation, leaf expansion, flower formation, sex expression, and fruit set (Pimenta Lange and Lange, 2006). In grapevine, application of gibberellic acid (GA_3) during full bloom usually induces reduced fruit set in seeded varieties (Alleweldt, 1967a; Hofmann, 2004; Weaver, 1972), leading to reduced infestation rates with bunch rots, such as *Botrytis cinerea* and sour rot (Hill et al., 2003; Petgen, 2004; Roschatt et al., 2003). But different seeded vine varieties react quite differently to GA_3 applications at full bloom: sensitive varieties, such as 'Sylvaner', respond with a much higher degree of cluster loosening compared to less sensitive ones, such as 'Pinot gris' (Böll et al., 2009; Hofmann, 2004; Julliard and Balthazard, 1965). Furthermore, sensitive varieties suffer from severe side effects in the following year showing reduced fertility in cluster numbers and weight as well as late bud break (Alleweldt, 1967a and b; Hill et al., 2003; Julliard and Balthazard, 1965; Wea-

VER, 1960; WEAVER and MCCUNE, 1961).
Early flowering and pollination processes are known to be directed by GA (PHARIS and KING, 1985; SINGH et al., 2002). High GA levels have been reported in pollen and anthers of several monocotyledonous and dicotyledonous species. These GAs might not only be important for the pollination process itself, but might also regulate the subsequent ovule development (IZHAKI et al., 2002; PHARIS and KING, 1985). Thus, differences in endogenous GA levels of different varieties could be the cause for a different sensitivity towards exogenous GA applications. To test this hypothesis, endogenous GA levels in ovaries of two seeded varieties with known differences in gibberellin-sensitivity, 'Pinot gris' and 'Sylvaner' (BÖLL et al., 2009), were determined by GC-MS analyses during pre- and post-bloom development. In addition, pollen tube germination of these varieties together with nine additional varieties was quantified, and their potential impact on endogenous GA levels during early berry development is discussed.

Material and Methods

Gibberellin extraction and GC-MS analysis

In 2006, berries of 'Sylvaner' and 'Pinot gris', grown in Franconian vineyards of the Bavarian State Institute for Viticulture and Horticulture, were collected and immediately frozen for quantitative determination of endogenous GAs. They were sampled at four developmental stages (pre-bloom (BBCH 61), post-bloom (BBCH 68), berries groat-sized (BBCH 73), berries begin to touch (BBCH 77) from seven to ten clusters to investigate GA levels of the two varieties over developmental time. Freeze-dried plant material (0.01 g and 0.1 g dry weight, respectively) was pulverized under liquid nitrogen and spiked with 17,17-d2-GA standards (1 ng each; purchased from Prof. L. Mander, Australian National University, Canberra, Australia). Samples were extracted, purified, derivatized, and analyzed by combined GC-MS using selected ion monitoring as described elsewhere (LANGE et al., 2005). Objective of this analysis were GA_{20}, GA_1 and GA_8, three successive GAs of the 13-hydroxylation gibberellin biosynthesis pathway, with GA_{20} being the inactive precursor and GA_8 the catabolic successor of the active plant hormone GA_1.

Pollen tube preparations

In 2006, at full bloom (BBCH 65), ovaries of several clusters of 'Pinot gris' and 'Sylvaner', in 2008, ovaries of 40 clusters of these varieties and nine additional vine varieties were collected in the field and fixed in FAA solution (50 % ethanol:acetic acid:formalin = 8:1:1 v/v/v). Additionally, for 'Sylvaner' and 'Pinot gris', ovaries from two different vineyards were collected to examine possible site effects.
In the laboratory 20 ovaries of each variety and site were divided into half longitudinally, rinsed with tap water and stained with aniline blue for 24 hours (2.3 g K_3PO_4 in 100 ml aqua dest. + 0.1 g aniline blue). Thereafter, the specimens were rinsed twice with tap water, mounted on microslides in glycerine and slightly squeezed. Pollen tubes in the styles of half ovaries were counted under a fluorescent microscope with blue light excitation. Data of the 2008 analyses were allotted to frequency categories and a category mean and standard error of the mean were calculated for each variety.

Results

GA analyses

The two varieties 'Pinot gris' and 'Sylvaner' expressed striking differences in their endogenous GA levels: endogenous GA levels were high in 'Pinot gris' before (BBCH 61) and particularly high shortly after fertilization (BBCH 68 and BBCH 73; Fig. 1). Especially the accumulation of GA_8, the catabolic product of the plant hormone GA_1, is indicating a long lasting GA synthesis and high turnover rates in 'Pinot gris'. In contrast, endogenous GA levels and turnover rates of 'Sylvaner' were much lower (Fig. 1).

Variety-dependent pollen tube abundance

Preliminary pollen tube analyses of 'Sylvaner' and 'Pinot gris' in 2006 had shown striking differences in pollen tube occurrence, with 'Pinot gris' ovaries displaying more than three times as many pollen tubes as ovaries of 'Sylvaner'. Comparable results were obtained for these varieties in 2008, without the vineyard site having any major effect. (Table 1). Altogether, the different studied varieties displayed a broad range of pollen tube abundance (Table 1, Fig. 2) that correlates negatively with their known sensitivity towards GA_3 applications (Table 1).

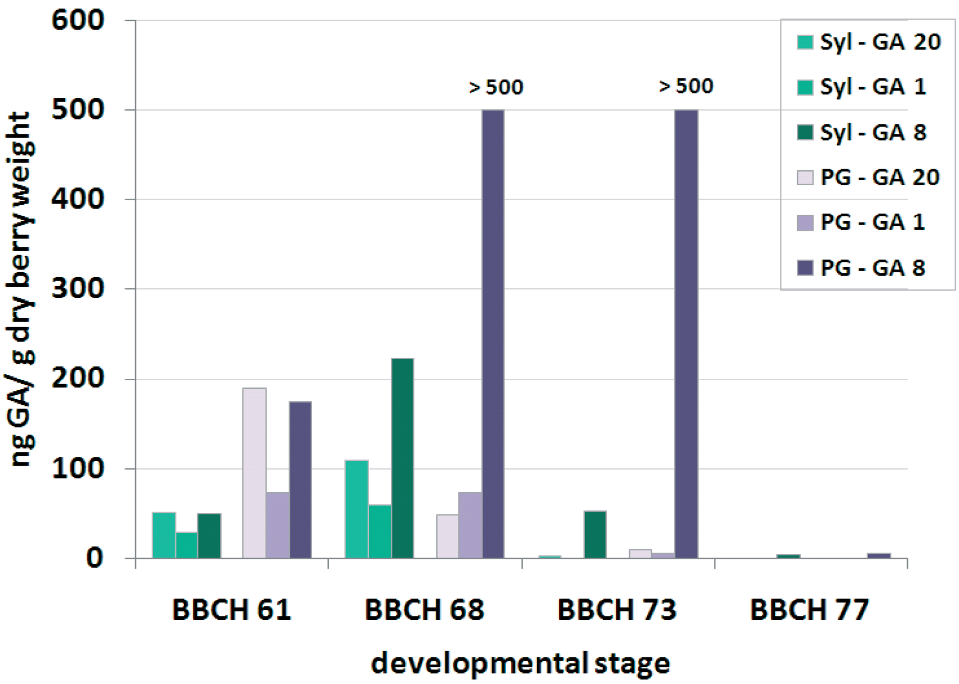

Figure 1: Endogenous gibberellin concentrations of the three consecutively metabolized gibberellic acids GA_{20}, GA_1, GA_8 of the 13-hydroxylation GA-pathway of gibberellin biosynthesis during early berry development of the varieties 'Sylvaner' (Syl) and 'Pinot gris' (PG)

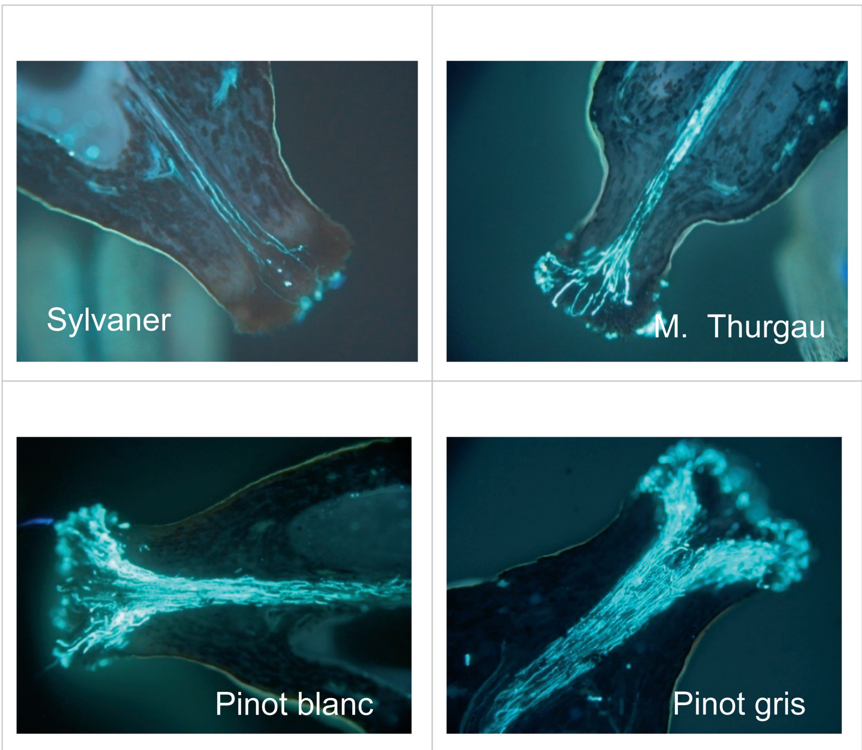

Figure 2: Pollen tube abundance in pistils of different varieties

Discussion

In the present study the known differential reactions of 'Sylvaner' and 'Pinot gris' to GA_3 application (Böll et al., 2009) correspond to the endogenous GA levels determined in these varieties: Compared to 'Sylvaner' GA levels and turnover rates were much higher and longer lasting in developing berries of 'Pinot gris' indicating that exogenous GA applications might only have a minor impact on the hormone balance of 'Pinot gris' in contrast to the more GA sensitive variety.

We suggest that the observed difference in the endogenous GA levels of the two varieties might be related to the variety-dependent abundance of pollen tubes in their pistils during bloom that, again, was found to be much higher in 'Pinot gris' than in 'Sylvaner'. During pre-bloom, high GA concentrations are predominantly found in anthers and pollen, as studies of various plant species have shown, that have an influence on pollen germination as well as on pollen tube growth and possibly on the subsequent ovule development (Izhaki et al., 2002; Pharis and King,

Table 1: Mean pollen tube (pt) abundance of different vine varieties in relation to their GA_3 sensitivity

Variety	Mean pollen tube abundance ± SE (category values)	GA_3 sensitivity according to literature data	References
Traminer	1,8 ± 0,9	++	Theiler, 1977; Hill, 2003
Sylvaner I	1,9 ± 0,9	++	Hofmann, 2004; Julliard and Balthazard, 1965;
Sylvaner II	2,2 ± 0,8	++	Walg, 2005
Kerner	2,6 ± 1,0	+	unpublished results
Müller-Thurgau	2,7 ± 1,1	+	Siegfried and Jüstrich, 2008; Theiler, 1977
Riesling	2,7 ± 0,7	++, +/-	Hill et al., 2003; Julliard and Balthazard, 1965; Walg, 2005; Weyand and Schultz, 2006
Portugieser	2,9 ± 0,9	- -	Hill et al., 2003; Regner et al., 2002
Dornfelder	3,0 ± 1,2	- -	Hill, 2003
Pinot blanc	3,1 ± 1,1	- -	Julliard and Balthazard, 1965; Roschatt et al., 2003
Pinot meunier	3,4 ± 1,1	- -	Kast et al., 2005
Pinot gris I	3,7 ± 1,2	- -	Hofmann, 2004; Julliard and Balthazard, 1965;
Pinot gris II	4,1 ± 1,3	- -	Siegfried and Jüstrich, 2008
Pinot noir	4,1 ± 1,2	- -	Roschatt et al., 2003; Siegfried and Jüstrich, 2008; Theiler, 1977

Pollen tube frequency categories: 1 = 1-5 pt, 2 = 6-10 pt, 3 = 11-15 pt, 4 = 16-20 pt, 5 = >20 pt
GA_3 sensitivity: ++ = high, + = moderate, +/- = low, - - = none

1985; SINGH et al., 2002). The results of the pollen tube analyses of a broad spectrum of additional varieties with known GA sensitivity reported here strongly support our hypothesis: sensitive varieties such as 'Sylvaner', 'Traminer' and 'Kerner' were found to have a distinctly lower pollen tube abundance than the less sensitive Pinot varieties. Only 'Riesling' is difficult to classify along these lines, as it exhibits differential gibberellin sensitivities in different studies as well as at different sites (HILL et al., 2003; JULLIARD and BALTHAZARD, 1965; WALG, 2005), including moderate to strong side effects, that might depend on the cultivated clone. Furthermore, 'Riesling' responds to GA applications with an increase instead of a thinning of the developing berries that are, however, much smaller than untreated berries due to induced parthenocarpy.

Our results imply that a simple microscopic analysis of the pollen tube abundance of varieties with hitherto unknown gibberellin sensitivity might well indicate the endogenous GA status of a given variety which in turn is expected to predict the sensitivity to exogenous GA_3-treatment including possible adverse side effects.

Acknowledgements

We would like to thank ANJA LIEBRANDT, MONIKA ADELHARDT and ASTRID BAUMANN for their valuable technical assistance. We would also like to thank THOMAS JARCHAU for constructive comments on the manuscript. This work was supported by the Forschungsring Deutscher Weinbau (grant 8503.187)

References

ALLEWELDT, G. 1967a: Physiologie der Rebe. Forschungsergebnisse der Jahre 1956-1960. Vitis 6: 48-62

ALLEWELDT, G. 1967b: Physiologie der Rebe. Forschungsergebnisse der Jahre 1961-1964. Vitis 6: 63-81

BÖLL, S., HOFMANN, H. und SCHWAPPACH, P. 2009: Einsatz der Wachstumsregulatoren Gibb3 und Regalis - warum Sorten unterschiedlich reagieren. Rebe und Wein (5): 24-27

HILL, G., HILL, M. und BUTTERFASS, J. 2003: Gibberelline - kleiner, weniger, besser? Dt. Weinmagazin (19): 32-35

HILL, M. B. 2003: Chemische Ausdünnung und deren Auswirkung auf die Rebe. Unpublished diploma thesis, Geisenheim 2003

HOFMANN, H. 2004: Traubendesign zur Fäulnisprävention. Rebe und Wein (5): 14-17

IZHAKI, A., BOROCHOV, A., ZAMSKI, E. and WEISS, D. 2002: Gibberellin regulates post-microsporogenesis processes in petunia anthers. Physiologia plantarum 115: 442-447

JULLIARD, B. et BALTHAZARD, J. 1965: Effets physiologiques de l'acide gibbérellique sur quelques variétés de vigne (*Vitis vinifera* L.). Ann. Amélior. Plantes 15: 61-78

KAST, W., FOX, R. und SCHIEFER, H.-C. 2005: Chancen und Risiken des Einsatzes von Gibb3. Rebe und Wein (5): 16-19

LANGE, T., KAPPLER, J., FISCHER, A., FRISSE, A., PADEFFKE, T., SCHMITTKE, S. and PIMENTA LANGE, M.J. 2005: Gibberellin biosynthesis in developing pumpkin seedlings. Plant Physiol. 139: 213-223

PETGEN, M. 2004: Was bringen Gibberelline? Dt. Weinmagazin (3): 28-32

PHARIS, R.P. and KING, R.W. 1985: Gibberellins and reproductive development in seed plants. Ann. Rev. Physiol. 36: 517-568

PIMENTA LANGE, M.J. and LANGE, T. 2006: Gibberellin biosynthesis and the regulation of plant development. Plant Biology 8: 281-290

REGNER, F., EISENHELD, C. und STADLBAUER, A. 2002: Versuche zur chemischen Beerenausdünnung bei Rebe. Mitt. Klosterneuburg 52: 3-9

ROSCHATT, C., HAAS, E. und PEDRI, U. 2003: Der Einsatz von Gibberellinen im Weinbau gegen Essigfäule. Obstbau Weinbau (4): 114-117

SIEGFRIED, W. und JÜSTRICH, H. 2008: Gibberellin-Versuche 2007 im Rebbau. Schweiz. Z. Obst- und Weinbau 144(10): 4-7

SINGH, D.P., JERMAKOV, A.M. and SWAIN, S.M. 2002: Gibberellins are required for seed development and pollen tube growth in Arabidosis. The Plant Cell 14: 1-15

THEILER, R. 1977: Einsatz von Wachstumsregulatoren zur Bekämpfung der Stiellähme. Mitt. Klosterneuburg 27: 197-204

WALG, O. 2005: Ertrag regulieren und Trauben gesund erhalten mit neuen Verfahren - Chance oder Risiko -. Dt. Weinbau-Jahrb. 56: 39-48

WEAVER, R.J. 1972: Plant growth substances in agriculture. - San Francisco: Freeman, 1972

WEAVER, R.J. 1960: Toxicitiy of gibberellin to seedless and seeded varieties of *Vitis vinifera.* Nature 187: 1135-1136

WEAVER, R.J. and McCune S.B. 1961: Effect of gibberellin on vine behavior and crop production in seeded and seedless *Vitis vinifera*. Hilgardia 30: 425-444

WEYAND, K. and SCHULTZ, H. R. 2006: Regulating yield and wine quality of minimal pruning systems through the application of gibberellic acid. J. Int. Sci. Vigne Vin 40(3): 151-163

Received August 13, 2009