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## Thinning intensity and water regime affect the impact cluster thinning has on grape quality

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### Summary

Late cluster thinning is a practice frequently used in the vineyards of semiarid regions, as it is claimed to increase total soluble solids and anthocyanin concentration. However, when performed in field conditions, it often leads to relatively inconclusive results: under some circumstances, it results in a noteworthy quality gain, whereas under other circumstances, it does not convey the improvement in quality desired.

The aim of this work was to evaluate the influence of thinning intensity and vine water status on the impact cluster thinning has on grape quality. Late cluster thinning (CT) was performed at four ‘Tempranillo’ vineyards during four consecutive years. The major effects of CT were an increase in berry soluble solid, anthocyanin and phenolics concentration. The impact CT had on these parameters was related to thinning intensity, although it was more closely related to vine water status of from veraison to harvest, particularly when compared to water status during the two weeks with the lowest water availability.

An estimation of the degree of water deficit that is likely to occur between veraison and harvest (considering water management guidelines of each winegrower and water reserves available in the soil or for later irrigation) has therefore to be considered in order to make proper decisions on cluster thinning, at least in the range of yield and water conditions included in this study. In rain fed or deficit irrigated vineyards water deficit alleviation might be one of the main mechanisms that make cluster thinning an effective technique to improve grape quality.

**Key words:** Yield control, leaf water potential, *Vitis vinifera* L.

**Abbreviations:** CT: cluster thinned; EAnt: extractable anthocyanins; FC: full crop; MalA: malic acid concentration; TSS: total soluble solids; TA: tritrate acidity; TAnt: total anthocyanins; TarA: tartaric acid concentration; TP: total phenolics;  $\Psi_{pd}$ : average pre-dawn leaf water potential between veraison and harvest;  $\Psi_{pd-min-2wk}$ : minimum average value of pre-dawn leaf water potential reached during two consecutive weeks; YL: yield loss.

### Introduction

Cluster thinning is a technique often implemented in field conditions, as it is claimed to improve grape quality,

through an increase in total soluble solids (GIL-MUÑOZ *et al.* 2009, VALDES *et al.* 2009) and in an enhanced anthocyanin and phenolics accumulation in red cultivars (GUIDONI *et al.* 2002, PEÑA-NEIRA *et al.* 2007, GIL-MUÑOZ *et al.* 2009). In vineyards from warm areas aiming at producing high quality red grapes, thinning is usually performed relatively late (around veraison), in order to maximize its benefits for sugar, anthocyanin and phenolics accumulation, and avoiding the increase in berry size that may occur if performed earlier (NAOR *et al.* 2002, KAPS and CAHOON 1989). Nevertheless, the qualitative response obtained after late thinning is quite variable as seen in Tab. 1, where earlier works reporting its effect on red grape quality have been summarized. As a consequence, its practicality is questioned by growers (PRESZLER *et al.* 2010), since the improvement of quality after cluster thinning needs to compensate in economical terms the cost of thinning itself [\$520 to \$650 by hand and \$220 mechanically according to TARDAGUILA *et al.* (2008)], and the decrease of yield per hectare it implies.

PRESZLER *et al.* (2010) have recently developed a model to establish the threshold that makes cluster thinning practices economically sustainable, which can be used to evaluate the suitability of this technique under different circumstances. However, to be fully operational, these models need a reliable prediction of the quality improvement that can be expected after cluster thinning.

There is not much information regarding what determines the quality changes observed in grapes after thinning. Thinning intensity itself does indeed condition it (NUZZO and MATTHEWS 2006, DIAGO *et al.* 2010) but, taking into account the lack of consistency between the results reported by earlier research (Tab. 1), there must be some other factors determining the effectiveness of this technique.

The aim of this work is to evaluate the role that thinning intensity and water availability play on the changes in composition observed in ‘Tempranillo’ vineyards after cluster thinning.

### Material and Methods

**Experimental sites and plant material:** Four ‘Tempranillo’ vineyards located in Southern Navarre (Spain) were used in this experiment from 2003 to 2006. Three of them (labeled TR1, TR2 and TR3) were located in Traibuenas (42°22′ N; 1°37′ W; WGS84 340 m asl), and a fourth one (CA) in Cascante (42°01′ N; 1°41′ W; WGS84 337 m asl).

All the vineyards were grafted on 110 Richter, trained as a vertical shoot positioned bilateral cordon, and planting

Table 1

Summary of the effects reported for late cluster thinning in red grape varieties in earlier research. Number of cases for which thinning increased (+), did not change (=) or decreased (-) each grape quality parameter

Ref <sup>a</sup>	Var <sup>b</sup>	Range of cluster thinning intensity		Berry weight <sup>c</sup>			Total soluble solids			Titratable acidity			Total anthocyanins			Extractable anthocyanins			Total phenolics		
		t·ha <sup>-1</sup>	%	+	=	-	+	=	-	+	=	-	+	=	-	+	=	-	+	=	-
1	TE	4.6-9.8	34-41	0/2	2/2	0/2	2/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2	2/2	0/2	0/2
2	CS	2.3-4.4	25-41	1/3	2/3	0/3	2/3	1/3	0/3	0/3	2/3	1/3	0/2	2/2	0/2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	GR, TE	4.6-10.9	32-76	3/4	1/4	0/4	2/4	2/4	0/4	0/4	1/4	3/4	1/4	3/4	0/4	n.a.	n.a.	1/4	3/4	0/4	0/4
4	TE, SY	1.6-6.2	30-51	1/6	5/6	0/6	5/6	1/6	0/6	0/3	2/3	1/3	2/3	1/3	0/3	5/6	1/6	0/6	6/6	0/6	0/6
5	GR	n.a.	n.a.	0/2	2/2	0/2	1/2	1/2	0/2	0/2	2/2	0/2	0/2	2/2	0/2	n.a.	n.a.	2/2	0/2	0/2	0/2
6	SY	4	50	0/1	1/1	0/1	0/1	1/1	0/1	1/1	0/1	0/1	1/1	0/1	0/1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
7	CS	n.a.	n.a.	0/2	2/2	0/2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	2/2	0/2	0/2	0/2
8	CS	1.4-5.7	13-30	1/5	4/5	0/5	0/5	5/5	0/5	0/5	5/5	0/5	0/5	5/5	0/5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
9	CS	3.5-11.5	18-58	0/6	6/6	0/6	12/12	0/12	0/12	1/12	11/12	0/12	2/12	10/12	0/12	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
10	NB	n.a.	n.a.	0/3	3/3	0/3	2/3	1/3	0/3	n.a.	n.a.	n.a.	2/3	1/3	0/3	n.a.	n.a.	1/3	2/3	0/3	0/3

<sup>a</sup> References: 1: VALDES *et al.* 2009; 2: KELLER *et al.* 2008; 3: DIAGO *et al.* 2010; 4: GIL-MUÑOZ *et al.* 2009; 5: TARDAGUILA *et al.* 2008; 6: PEÑA-NEIRA *et al.* 2007; 7: ZHAO *et al.* 2006; 8: KELLER *et al.* 2005; 9: NUZZO and MATTHEWS 2006; 10: GUIDONI *et al.* 2002.

<sup>b</sup> Varieties: TE: 'Tempranillo'; CS: 'Cabernet Sauvignon'; GR: 'Grenache'; SY: 'Syrah'; NB: Nebbiolo.

<sup>c</sup> Number of situations (years, thinning intensities or varieties) for which thinning increased, did not change or decreased quality parameters with respect to the total number of situations studied. n.a., not available.

density was 3,333 plants per ha (3 x 1 m in Traibuenas, and 2.75 x 1.1 m in Cascante). In winter, bud number was fixed to 6, 2-bud spurs per plant and shoot number was manually maintained at 12 shoots per vine. At both sites drip irrigation system was available with two 1.8 L h<sup>-1</sup> emitters per vine. At the onset of veraison (5-15 % of colour change), two fruit levels were imposed: full crop (FC), where no thinning was performed; and cluster thinned (CT), leaving only the basal cluster of each shoot. At the beginning of the experiment, 6 to 9 replications of 20 vines per treatment were chosen at each vineyard as representative of the average vine size at the vineyard and homogeneous, according to their trunk cross sectional area (TCSA) estimated after measuring two orthogonal diameters of the trunk. All

the sampling and all the experimental measurements were performed on those vines. Fruit load treatments were imposed at the four vineyards in years 2004 and 2005, whereas in 2003 the three vineyards located in Traibuenas were studied (TR1, TR2 and TR3), and in 2006 the experiment was only performed at TR1. Climatic conditions and irrigation doses applied are summarized in Tab. 2.

Experimental measurements: a) Yield, berry size and grape composition: At harvest, cluster number was counted and yield was measured at 10 vines per replication. Berry size and grape quality parameters were measured at harvest, using one 300-berry sample at each replication. Samples were carried to the lab at low temperature for analysis,

Table 2

Irrigation volume and climatic conditions of the sites included in the study

Year	Site	Vineyard ID	Fruit set - veraison <sup>a</sup>				Veraison - harvest			
			Irr (mm)	T (°C)	R (mm)	ETP (mm)	Irr (mm)	T (°C)	R (mm)	ETP (mm)
2003	Traibuenas	TR1	50	24.3	30	379	0	22.4	151	183
		TR2	32				80			
		TR3	52				35			
2004	Traibuenas	TR1	28	22.7	53	317	0	21.4	40	203
		TR2	0				34			
		TR3	0				24			
	Cascante	CA	54	22.5	32	331	38	22.1	106	202
2005	Traibuenas	TR1	38	23.6	43	325	0	20.6	20	252
		TR2	24				34			
		TR3	54				43			
	Cascante	CA	96	23.4	36	360	60	21.8	33	224
2006	Traibuenas	TR1	9	24.0	145	319	8	20.6	157	194

<sup>a</sup> Irr: volume of irrigation water applied; T: mean temperature; R: rainfall; ETP: Evapotranspiration calculated according to Penman-Monteith.

weighted, and immediately homogenized with an LMU 9018 American blender (Man, México) for 10 seconds at speed #4. Part of this homogenate (100 g approx.) was filtered with a gauze tissue and used to measure TSS, tritatable acidity (TA) and malic (MalA) and tartaric (TarA) acid concentration. TSS was measured using a high precision temperature compensating refractometer (RFM840, Bellingham-Stanley Ltd, UK); 20 mL were titrated with NaOH 0.25M up to pH 8.1. with a pH-Burette 24 (Crison, Barcelona, Spain) to estimate acidity expressed as g TarA·L<sup>-1</sup>. MalA and TarA were determined using an EasyChem multiparametric autoanalyzer (Systea S.p.a., Italy) in 1.5 mL samples centrifuged 3 min at 4500 rpm ('Biofuge pico', Heraex Instrument) prior to analysis.

Total and extractable anthocyanins, and total phenolics were calculated according to the procedure described by GLORIES and AUGUSTIN (1993) and SAINT-CRICQ *et al.* (1998), a method widely used in wineries which has been proved to be a good tool to estimate wine color (KONTODAKIS *et al.* 2010).

b) Plant water status: Pre-dawn leaf water potential ( $\Psi_{pd}$ ) was measured twice a week from veraison to harvest in 4 replications per vineyard at FC vines, taking at least five young healthy leaves from five plants. Measurements were conducted using a Scholander pressure bomb (P3000, Soil Moisture Corp., Santa Barbara, CA, USA). When taking leaf samples and measurements, the precautions suggested by TURNER (1988) were considered. Water status of veraison to harvest period was characterized through two variables: the weighted average of pre-dawn leaf water potential [ $\Psi_{pd}^w$ , calculated as described in SANTESTEBAN and ROYO (2006)], and the minimum value reached, as an average, during two consecutive weeks ( $\Psi_{pd-min-2wk}$ ). Water status was not monitored in CT vines.

Data analysis: The effect of cluster thinning was analyzed separately for each vineyard and year through a one-way ANOVA. The effect thinning intensity and water status from veraison to harvest on the compositional changes of grapes from cluster thinned vines was analyzed by linear regression. Statistical analyses were performed

using PASW Statistics 18.0 software (SPSS Inc., Chicago, IL).

## Results and Discussion

Thinning resulted in a reduction of cluster number that ranged from 1.3 to 7.3, which in most cases resulted in a significant yield decrease (Tab. 3). The range of thinning intensities included in our study is relatively broad, and embraces most of the circumstances likely found at the vineyards of the Mediterranean area.

Berry mass was not affected by cluster thinning (Tab. 3). This lack of effect on berry size generally observed after late thinning (Tab. 1), and results from the fact when cluster thinning is performed near veraison, berry growth is not very sensitive to physiological changes (KELLER 2010); unlike early thinning, which often leads to a significant increase of berry size (NAOR *et al.* 2002, KAPS and CAHOON 1989) as it is performed pre-veraison, the most critical phase for the determination of berry size at harvest (KELLER 2010).

Concerning berry composition, a greatly variable response was found (Tabs 4 and 5). TSS was increased after thinning for 6 out of the 12 cases analyzed, no changes being observed for the remaining ones (Tab. 4). TSS increase is considered to occur as a consequence of a shift of the source:sink ratio, that allows a greater allocation of photosynthates into the remaining clusters (DI LORENZO *et al.* 2002). In warm areas, where the length of the season guarantees that TSS specifications required by the wineries are easily reached, provided no great water deficits occur, this gain is considered not to be very relevant in practical terms, as it partly results from an earlier ripening (NUZZO and MATTHEWS 2006). In fact, although no great changes in acidity were found in our study, some of the thinned vineyards showed a decrease in acidity, which would support the hypothesis sugar increased, at least partly as a result of advanced ripening. Lastly, regarding phenolic compounds, CT showed to affect anthocyanin and phenolics concentra-

Table 3

Cluster thinning effects on fruit load, yield and berry mass

Year	Vineyard <sup>a</sup>	Cluster number <sup>b</sup>			Yield (kg·vine <sup>-1</sup> )			Cluster mass (g)			Berry mass (g)		
		FC	CT	P	FC	CT	P	FC	CT	P	FC	CT	P
2003	TR1	18.9	13.2	0.000	4.4	2.6	0.000	229.1	195.6	0.326	1.46	1.45	0.953
	TR2	19.8	13.5	0.006	1.9	1.2	0.011	96.4	87.8	0.748	1.11	1.20	0.378
	TR3	20.7	13.4	0.002	2.9	1.7	0.000	127.1	136.4	0.562	1.02	1.15	0.462
2004	TR1	16.1	14.0	0.020	5.2	4.1	0.001	322.0	294.1	0.321	2.11	2.01	0.238
	TR2	13.6	11.1	0.038	2.9	2.2	0.003	215.0	198.2	0.515	1.55	1.58	0.818
	TR3	9.9	8.6	0.066	2.0	2.1	0.455	190.2	249.7	0.112	1.72	1.87	0.315
	CA	21.6	14.4	0.000	3.8	2.7	0.000	176.3	150.2	0.222	1.37	1.24	0.163
2005	TR1	16.9	8.5	0.000	4.2	1.9	0.000	247.3	218.3	0.203	1.54	1.43	0.051
	TR2	17.4	10.8	0.005	3.5	1.4	0.005	191.2	137.0	0.047	1.29	1.09	0.106
	TR3	11.3	9.4	0.031	2.1	1.8	0.074	212.3	197.4	0.647	1.36	1.27	0.532
	CA	16.9	10.0	0.000	3.2	2.3	0.006	189.4	226.0	0.236	1.20	1.41	0.023
2006	TR1	17.8	13.7	0.001	3.5	2.7	0.025	194.5	202.9	0.877	1.55	1.65	0.554

<sup>a</sup> Vineyard denomination following Tab. 2.

<sup>b</sup> Thinning treatments: FC, full crop; CT, cluster thinned vines.

Table 4

Cluster thinning effect on total soluble solids (TSS), pH, titratable acidity (TA), malic (MalA) and tartaric (TarA) acid concentration

Year	Vineyard <sup>a</sup>	TSS (°Brix) <sup>b</sup>			pH			TA (g·L <sup>-1</sup> )			MalA (g·L <sup>-1</sup> )			TarA (g·L <sup>-1</sup> )		
		FC	CT	<i>P</i>	FC	CT	<i>P</i>	FC	CT	<i>P</i>	FC	CT	<i>P</i>	FC	CT	<i>P</i>
2003	TR1	22.7	24.0	0.001	3.39	3.41	0.657	4.85	4.74	0.493	1.42	1.54	0.202	7.44	7.10	0.293
	TR2	22.8	22.9	0.599	3.49	3.41	0.219	3.46	3.39	0.355	1.01	1.04	0.783	5.70	5.27	0.268
	TR3	19.6	23.1	0.000	3.43	3.55	0.031	4.79	4.05	0.118	0.87	0.92	0.526	7.00	6.67	0.582
2004	TR1	22.2	22.2	0.896	3.38	3.36	0.456	5.94	5.94	0.990	1.23	1.37	0.679	6.39	6.89	0.070
	TR2	23.9	23.3	0.175	3.45	3.42	0.083	5.07	5.30	0.012	1.15	1.30	0.729	5.83	6.67	0.024
	TR3	23.5	22.4	0.118	3.49	3.48	0.850	5.45	5.34	0.715	2.06	1.83	0.185	7.33	8.00	0.374
	CA	21.6	22.3	0.004	3.54	3.57	0.549	5.52	5.27	0.296	1.13	1.15	0.879	8.06	7.67	0.204
2005	TR1	25.7	26.5	0.004	3.65	3.81	0.000	4.40	4.13	0.009	1.54	1.21	0.001	5.05	4.98	0.797
	TR2	24.2	23.7	0.035	3.59	3.82	0.020	4.22	4.00	0.330	1.56	1.19	0.079	4.57	5.05	0.132
	TR3	24.8	23.7	0.231	3.57	3.73	0.054	4.59	4.25	0.051	1.16	1.03	0.573	5.33	4.95	0.345
	CA	27.1	26.5	0.222	4.00	3.99	0.475	4.76	5.06	0.040	2.90	3.22	0.092	5.05	5.59	0.025
2006	TR1	22.0	22.7	0.046	3.23	3.31	0.089	5.38	5.34	0.823	0.70	0.85	0.512	8.90	8.70	0.743

<sup>a</sup> Vineyard denomination following Tab. 2.

<sup>b</sup> Thinning treatments: FC, full crop; CT, cluster thinned vines.

Table 5

Cluster thinning effect on total anthocyanins (TAnt), extractable anthocyanins (EAnt) and total phenolics (TP) concentration

Year	Vineyard <sup>a</sup>	TAnt (mg·L <sup>-1</sup> ) <sup>b</sup>			EAnt (mg·L <sup>-1</sup> )			TP (mg·L <sup>-1</sup> )		
		FC	CT	<i>P</i>	FC	CT	<i>P</i>	FC	CT	<i>P</i>
2003	TR1	654	968	0.002	273	389	0.000	913	1240	0.000
	TR2	923	1035	0.332	382	404	0.401	1249	1552	0.002
	TR3	555	884	0.000	232	327	0.003	872	1121	0.014
2004	TR1	611	706	0.013	276	314	0.013	837	870	0.492
	TR2	713	932	0.002	336	407	0.001	944	1100	0.011
	TR3	685	702	0.835	299	280	0.525	1017	1018	0.958
	CA	783	834	0.435	289	322	0.211	909	969	0.087
2005	TR1	701	689	0.824	397	368	0.189	719	889	0.031
	TR2	726	612	0.244	338	347	0.861	829	876	0.642
	TR3	602	568	0.748	296	290	0.907	824	760	0.365
	CA	811	835	0.608	389	446	0.010	935	935	0.997
2006	TR1	694	606	0.323	340	328	0.731	666	802	0.022

<sup>a</sup> Vineyard denomination following Tab. 2.

<sup>b</sup> Thinning treatments: FC, full crop; CT, cluster thinned vines.

tion only under some circumstances, as significant increases were observed for TAnt, EAnt and TP in, respectively, 4, 5 and 6 out of the 12 cases studied (Tab. 5).

The lack of consistency between the result of the years and vineyards studied is similar to the one reported earlier (Tab. 1), which highlights there must be some other factors conditioning the effectiveness of late thinning. Thinning intensity and water status were identified as potential factors determining the relative impact cluster thinning had on TSS, TAnt, EAnt and TP, the four parameters more clearly modified. In the Figure, the relationship of the increase observed for these parameters and yield loss (YL), mean predawn leaf water potential from veraison to harvest ( $\Psi_{pd}$ ) and mean predawn leaf water potential during the two weeks with lowest water availability ( $\Psi_{pd-min-2wk}$ ) is presented. Data from TR3 in 2004 are not included in this

figure, as thinning did not decrease yield significantly and, therefore, cannot be considered as such.

The intensity of thinning, estimated as YL, has been shown to be significantly ( $P < 0.10$ ) related to the increase observed for TSS (Figure a), with a relatively low coefficient of determination ( $R^2 = 0.28$ ). Average vine water status between veraison and harvest estimated as  $\Psi_{pd}$  has shown a similar trend with respect to the increase in TSS but the observed relationship was not significant (Figure b). However,  $\Psi_{pd-min-2wk}$  showed a much closer relationship with TSS gain (Figure c,  $R^2 = 0.45$ ). A similar pattern was observed when the effect of YL,  $\Psi_{pd}$  and  $\Psi_{pd-min-2wk}$  was compared to the increase observed for TAnt, EAnt and TP after thinning (Figure d-l), the closest relationships being clearly found when these parameters were compared to  $\Psi_{pd-min-2wk}$  ( $R^2 = 0.39 - 0.45$ ). Although these coefficients

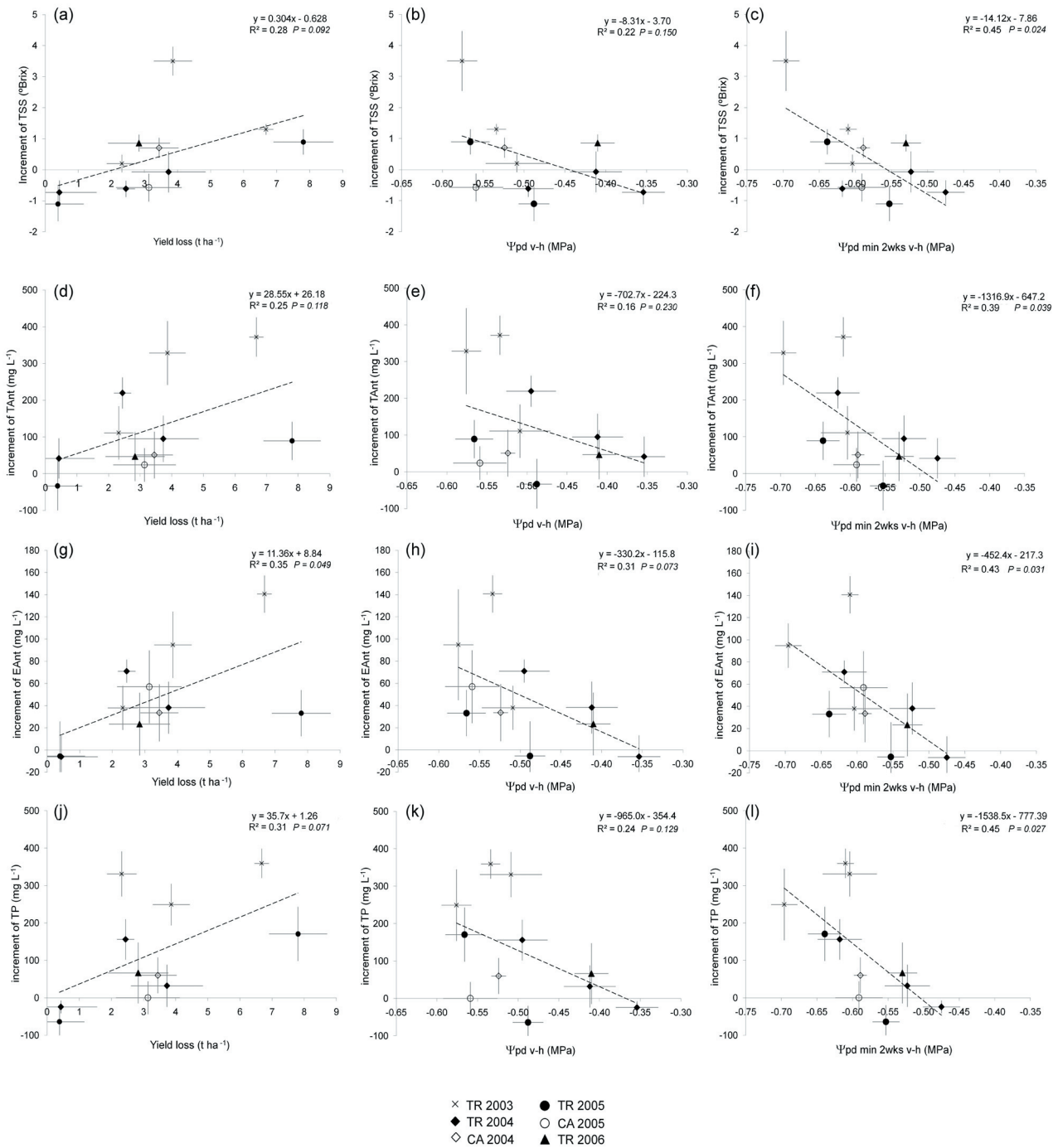


Figure: Influence of thinning intensity and water status on quality changes observed in berries after cluster thinning,  $\Psi_{pd}$ , average pre-dawn leaf water potential between veraison and harvest;  $\Psi_{pd-min-2wk}$ , minimum average value of pre-dawn leaf water potential reached during two consecutive weeks; YL = yield loss.

of determination are moderate, they are remarkable considering several vineyards and growing seasons were included in the study, which implies differences in weather conditions that would have probably conditioned cluster thinning effects too.

Thus, although thinning intensity has conditioned the impact cluster thinning has had on quality gain, which agrees with earlier research (NUZZO and MATTHEWS 2006, DIAGO *et al.* 2010), our results highlight the great role water deficit plays on the impact cluster thinning has on grape quality. The fact maximum water deficit has greatly deter-

mined the impact of cluster thinning on grape composition (and, as a consequence, on its profitability) is coherent with earlier reports for this variety that show that, under the standard growing conditions of this area (low to medium yields and deficit irrigation) leaf to fruit ratio plays only a moderate role in determining berry composition (SANTESTEBAN *et al.* 2010), more closely related to water status (SANTESTEBAN and ROYO 2006, SANTESTEBAN *et al.* 2011).

Although no measurements of water status were performed on cluster thinned vines, it is sensible to consider that thinning may have resulted in a decrease of water con-



sumption, since a reduction of sink size down-regulates leaf photosynthetic activity and water loss through transpiration (NAOR *et al.* 1997). This process has been proved to play a major role in other fruit crops (NASCHITZ and NAOR 2005, MARSAL *et al.* 2008, LOPEZ *et al.* 2010). In grapevines, this effect was already reported by BRAVDO *et al.* (1985) and by INTRIGLIOLO and CASTEL (2011) for early thinned 'Cabernet Sauvignon' and 'Tempranillo' vines respectively, although it is not always observed (KELLER *et al.* 2008).

Under the conditions of our study, when water availability is low, CT probably allows avoiding moderate to severe water deficit events between veraison and harvest, which results in a greater benefit associated to cluster thinning. Despite water deficit between veraison and harvest is known to promote anthocyanin synthesis (BUCCETTI *et al.* 2011, SALÓN *et al.* 2005, SANTESTEBAN *et al.* 2011), once deficit goes beyond a certain threshold, anthocyanin synthesis can be hindered (or at least delayed) by a decrease in photosynthetic activity (VAN LEEUWEN *et al.* 2009), which could explain that the greater effects of cluster thinning were observed under the most stressing conditions.

The results presented show the relevance of understanding the interaction between thinning intensity and water deficit. Our results suggest that (i) where no water deficit is expected from veraison to harvest, late cluster thinning will be effective only at moderate to heavily loaded vineyards, and, on the contrary, (ii) that if moderate to severe water deficit is expected, slight to moderate cluster thinning could result in a significant quality improvement.

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