DROUGHT ADAPTATION STRATEGIES OF FOUR GRAPEVINE CULTIVARS (*Vitis vinifera* L.): MODIFICATION OF THE PROPERTIES OF THE LEAF AREA

STRATÉGIES D'ADAPTATION À LA SÉCHERESSE DE QUATRE CÉPAGES DE VIGNE (*Vitis vinifera* L.) : MODIFICATION DES CARACTÉRISTIQUES DE LA SURFACE FOLIAIRE

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Summary : This essay studies the morphological and anatomical properties of the leaves of Garnacha tinta, Tempranillo, Chardonnay and Airén grapevines in order to discover the drought adaptation strategies present in *Vitis vinifera* L. The grapevines were grown under two water availability conditions: water limitation and non-water limitation. There was a significantly lower development of leaf area under conditions of water limitation compared to non-water limitation due to a reduction in the size of main and lateral shoot leaves, and a smaller number of leaves on lateral shoots. The development of the leaf area under water limitation conditions occurred on earlier dates than under non-water limitation conditions. Significantly lower stomatal density was observed under water limitation conditions rather than non-water limitation conditions exclusively in the Airén cultivar.

Résumé : Cette étude a analysé les caractéristiques morphologiques et anatomiques des feuilles de quatre cépages provenant de zones présentant des différences écologiques, afin de connaître les mécanismes d'adaptation à la sécheresse chez *Vitis vinifera* L. Garnacha tinta (Aragon, Espagne), Tempranillo (La Rioja, Espagne), Chardonnay (Bourgogne, France) et Airén (La Mancha, Espagne). Ces cépages ont poussé dans des lysimètres pesables dans wdeux conditions d'irrigation: contrainte hydrique ou traitement non contrainté. Le développement de la surface foliaire du cépage, le nombre de feuilles, la taille de la feuille, le poids spécifique des feuilles sur le rameau principal et sur les entre-cœurs ont été évalués. Nous avons observé différents mécanismes d'adaptation permettant aux cépages de lutter contre la contrainte hydrique: développement significativement moindre de la surface foliaire en conditions de contrainte hydrique par rapport à une irrigation non contrainte, dû à une nette réduction de la taille des feuilles du rameau principal et celles des entre-cœurs, ainsi qu'un nombre de feuilles inférieur sur les entre-cœurs, ce dernier paramètre étant le plus affecté par la contrainte hydrique. Le développement de la surface foliaire en conditions de déficit hydrique a été plus précoce que dans un régime de disponibilité totale d'eau. Une moindre densité stomatique significative en conditions de déficit hydrique par rapport à une irrigation normale a été observée uniquement sur le cépage Airén. Le contrainte hydrique a entraîné une nette réduction dans la production de matière sèche dans tous les cépages. Toutefois, en conditions de contrainte hydrique, la diminution de la production de matière sèche du Chardonnay et de l'Airén a été nettement moins importante que celle du Garnacha tinta. La production de matière sèche pendant la saison par unité de surface foliaire n'a pas été modifiée de façon significative par la contrainte hydrique, car il s'agit d'une caractéristique génétique. L'Airén a présenté des caractéristiques différentes par rapport aux autres cépages étudiés. Celui-ci a produit nettement plus de matière sèche au cours de la saison et ce, quelle que soit la disponibilité en eau. C'est aussi lui qui a développé le plus de surface foliaire. Toutefois, cette dernière a été nettement moins efficace au niveau de la production de matière sèche que les autres cépages. Il s'est distingué par le développement précoce de la surface foliaire dû au fort développement des entre-cœurs. Le poids spécifique des feuilles s'est avéré nettement inférieur aux autres cépages. La contrainte hydrique a réduit de façon significative la densité stomatique et il existe une relation entre la densité stomatique et l'activité physiologique de la feuille.

Key words: water stress, water limitation, adaptation leaf size, stomata, dry matter

Mots clés : contrainte hydrique, adaptation taille des feuilles, stomate, matière sèche

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INTRODUCTION

Drought is one of the main environmental causes of large reductions in production in the Mediterranean region. Plants have developed different adaptation mechanisms which enable them to avoid drought (that minimise the accurrence of damaging water deficit), to tolerate drought (those physiological adaptations that enable plants to continue functioning in spite of plant water deficits) or to optimise the utilisation of water (JONES, 1983). In some *Vitis vinifera* L. cultivars, strategies have been detected which, through the modification of the morphological and anatomical properties of the leaves, they succeed in avoiding water limitation effects. These strategies include a reduction in the leaf area (WINKEL and RAMBAL, 1993); high leaf water storage capacity (DÜRING and SCIENZA, 1980; SCHULTZ, 1996); and differences in stomatal density (FREGONI *et al.*, 1977; DÜRING and SCIENZA, 1980; SCIENZA and BOSELLI, 1981; KLIEWER *et al.*, 1985).

Grapevine cultivar can be considered to be adapted to drought in two different ways: when it reaches high production and quality even when conditions are not optimum (DÜRING and SCIENZA, 1980; ALBUQUERQUE-REGINA, 1993) or when it survive under drought conditions, because when a cultivar continues to transpire and photosynthesise when the water potentials of the leaf are very negative could endangers its survival (SCHULTZ, 1996).

The aim of the present paper is to study the effect of water limitation on the morphological and anatomical properties of the leaves of different genotypes of vine, and the repercussions that this has on the productive capacity of the grapevine. In order to do this, cultivars from ecologically different areas were chosen: Garnacha tinta is traditionally cultivated in the arid region of Aragón (Spain); Tempranillo in La Rioja; Chardonnay in the semi-humid and cool area of Burgundy (France); and Airén in the dry and warm region of La Mancha (Spain).

MATERIAL AND METHODS

This experiment was carried out in 1994 at the Universidad Politécnica de Madrid in Spain. Two-year-old grapevines were grown in 35-liter weighting lysimeters and were covered with a plastic film to avoid evaporation and infiltration of rainfall. Excess water from irrigation was allowed to drain into a separate container for quantification. The lysimeters were filled with a mixture of peat, sand, and organic soil (63:25:12). Each grapevine was restricted to one shoot. This experiment was completely randomized with 5 single grapevine repetitions. Two factors were controlled: cultivar and water availability. The cultivars used were Garnacha tinta, Tempranillo, Chardonnay and Airén grafted onto 1103 Paulsen. The water availability treatments were water limitation (L) and non-water limitation (NL).

NL grapevines were irrigated to maintain the potting medium close to field capacity by supplying the amount of water each week that the grapevines had consumed the previous week. Water consumption was determined gravimetrically, with allowances for drainage. L treatment consisted of grapevines receiving 50 % of the water consumption of NL grapevines for each cultivar, with corrections for the relationship of leaf area differences between treatments, calculated by the following formula:

$$W_L = 0.5 \cdot W_{NL} \cdot LA_L \cdot LA_{NL^{-1}}$$

where WL = water applied to L grapevines, W_{NL} = water applied to N_L grapevines, LA_L = leaf area of L grapevines and LA_{NL} = leaf area of NL grapevines.

Growing degree-days (GDDs), using a base temperature of 10 ºC, were calculated from budburst.

The cycle of vine growth was divided into three phases for the purpose of studying leaf area development. Shoot growth evolution of each experimental grapevine repetition was studied in order to determine these medium phases. Slow growth phase (phase S) was the time between budbreak (6 May, day of year [DOY] 126) and 5 July (DOY 186). Exponential growth phase (phase E) was between 5 July (DOY 186) and 16 August (DOY 228). Lastly, lag phase (phase L) was between 16 August and 20 September (DOY 263). Daily shoot growth for each phase was 1,5±0,2 cm; 2,2±0,2 cm and 0,5±0,1 cm respectively.

The leaf area of main and lateral shoots was measured by weekly using the non-destructive method of CARBONNEAU (1976 a and b). Leaf area was estimated by developing a second order polynomial equation, relating grapevine length to leaf area for each cultivar. The mean grapevine leaf area developed at each phase of the growth cycle and the whole cycle was calculated using this procedure. The percentage of leaf area developed in lateral shoots compared to the total leaf area developed was calculated in each phase. At the end of the experiment all leaves were removed, counted and measured with an area meter (LI-3100; LI-COR, Lincoln, Neb.).

The preparation of the samples for the counting of stomata was performed following the indications of CAPELLADES *et al.* (1990). On the reverse side of

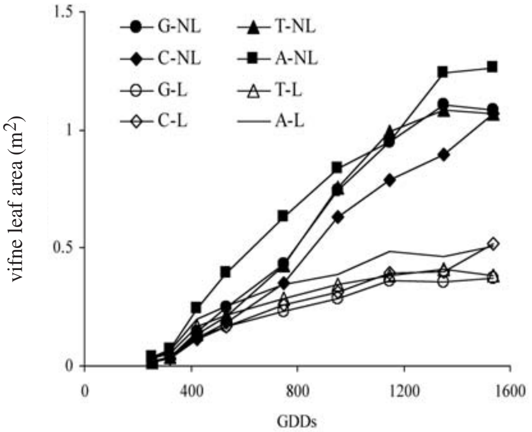

Fig. 1 - Leaf area development in two-year-old Garnacha tinta (G), Tempranillo (T), Chardonnay (C) and Airén (A) grapevines grown in 35-L lysimeters under water limitation (L) and non-water limitation conditions (NL) during the season.

Développement de la surface foliaire sur des cépages de Garnacha tinta (G), Tempranillo (T), Chardonnay (C) et Airén (A) âgés de deux ans, poussant en lysimètres de 35-L en conditions de contrainte hydrique (S) ou non contrainte hydrique (NL) pendant la saison.

the limbo central intervein area, a surface area of 1 cm^2 was covered with nail polish. After drying, the nail polish was removed using transparent adhesive tape, and epidermal imprints were transferred onto a microscope slide for counting. A leaf from each experimental grapevine repetition was sampled and 5 counts were carried out in each leaf.

At the beginning of the experiment, five whole vine dry matter of each cultivar were determined after tissue was dried at 80 ºC in an oven and a constant weight was recorded. At the end of the season, dry matter was determined on five grapevines per treatment combination. Dry matter production was calculated as a difference between vine dry matter at the beginning and at the end of the experiment. Main and lateral leaves dry matter were determined separately. Specific leaf weight was calculated by dividing leaf dry matter and area.

RESULTS AND DISCUSSION

I - TOTAL LEAF AREA OF THE VINE

The leaf area developed by the grapevines (figure 1) under conditions of total water availability (greater than 1 m^2) was higher than that obtained by INTRIERI *et al.* (1992) under quite similar growing conditions. Therefore, the trial conditions and the cultural treatment performed on the trial grapevines contributed to excellent vegetative development.

During the 7 weeks following budbreak, the development of the leaf area occurred very slowly in all the cultivars (figure 1). KLIEWER *et al.* (1983) observed that this phase of slow development of the leaf area lasted some 4 weeks. Temperatures during this phase seemed to determine its duration. From the seventh week onwards (day 172), the development trend of the leaf area was linear compared to GDDs as was observed by WILLIAMS (1987). Under NL conditions, this trend was maintained until day 242 when it was observed that the development of the leaf area stopped in all the cultivars excepted Chardonnay, which continued to increase until the end of the trial. This was probably due to the fact that this cultivar, from a cold region, does stop its vegetative development only for temperatures lower than those recorded at the end of the trial. During this second phase, greater speed in the formation of leaf area was observed; in Tempranillo mean formation speed reached 170 cm^2 per day (table I). Under L conditions, the increase in the leaf area in this second phase was lower and growth cesation occurred 2 weeks before that in NL (day 228). In NL, as a mean value of the cultivars, 52 % of the total area formed developed in the second phase of shoot growth, 21 % in the first phase and 27 % in the last (figure 1). Nevertheless, when water availability was limited, the formation of the leaf area in the grapevines took place in the first phases with equal intensity (36 % was formed), while in the last phase, only 28 % of the leaf area was formed.

Water limitation causes a reduction in the development of the leaf area (MERIAUX *et al.*, 1974; KLIEWER *et al.*, 1983; FANIZZA and RICCIARDI, 1990). In our trial, the mean formation speed of leaf area in the two last phases and throughout the cycle was significantly influenced both by water availability and genotype, and the interaction between both factors was significant (table I). However, in the first phase of the cycle, the effect of water availability was not significant. This might have been due to the fact that the trial was started by irrigation of all the grapevines at field capacity and there is little transpiration during this phase due to low foliar development. In the second and third phases of the cycle, water limitation produced a mean reduction of 58 % and 60 %, respectively, in the formation speed of the leaf area. The differences in seasonal leaf area expansion between the irrigation treatments of all the cultivars were highly significant. Throughout the cycle, development of leaf area under L was reduced in 57 % compared to NL, as mean of the cultivar, the cultivar which most reduced the development of leaf area was Garnacha tinta (it decreased by 62 %), while Chardonnay reduced it by 49 %, mean reduction.

TABLE I

Expansion of leaf area (cm^2·day^{-1}) in two-year-old Garnacha tinta (G), Tempranillo (T), Chardonnay (C) and Airén (A) grapevines grown in 35-L lysimeters under water limitation (L) and non-water limitation conditions (NL).

Phase S: 6 May to 5 July. Phase E: 5 July to 16 August. Phase L: 16 August to 20 September.
Factorial analysis of variance (CUL = cultivar, IT = irrigation treatment, CUL·IT = interaction).

Tableau I - Expansion de la surface foliaire (cm^2·jour^{-1}) pour les cépages Garnacha tinta (G), Tempranillo (T), Chardonnay (C) et Airén (A) âgés de deux ans ayant poussé dans des lysimètres de 35-L en conditions de contrainte hydrique (L) et en régime de non-contrainte hydrique (NL).

Phase S : du 6 mai au 5 juillet. Phase E : du 5 juillet au 16 août. Phase L : du 16 août au 20 septembre.
Analyse factorielle de la variation (CUL = cépage, IT = régime d'irrigation, CUL·IT = interaction).

	Phase S	Phase E	Phase L	Season
CUL	** x	**	**	**
IT	it	**	**	**
CUL·IT	*	**	ns	**
G-NL vs.G-L	ns	**	**	**
T-NL vs. T-L	ns	**	**	**
C-NL vs. C-L	ns	**	**	**
A-NL vs. A-L	ns	**	**	**
G-NL	3.6 b y	162.5 ab	105.6	94.2 b
T-NL	2.9 b	169.7 a	97.6	92.5 b
C-NL	2.9 b	134.6 c	117.1	89.2 b
A-NL	5.9 a	157.2 b	125.2	107.9 a
G-L	3.2 b	58.2 b	38.8 bc	35.6 b
T-L	5.0 a	58.7 b	32.0 c	37.9 b
C-L	3.0 b	64.7 b	61.8 a	45.5 a
A-L	5.0 a	74.8 a	45.9 b	46.0 a

x ns,*,**, non significant or significant at P = 0.05 or 0.01, respectively.
y Mean separation by Duncan's multiple range test at P = 0.05.

Genotype significantly influences the formation of leaf area when the grapevines were grown under the same conditions (ALBUQUERQUE-REGINA, 1993). Nevertheless, in our trial, the cultivars did not reaction in the same way throughout the whole cycle. Under NL conditions, Airén was characterised by speed values of leaf area formation which were significantly higher in the first phase of the cycle, in spite of being a late-budding cultivar. However, in the second phase, it was Tempranillo which presented significantly higher values, and in the last phase there were no significant differences among the cultivars. Taking into consideration the whole of the cycle, Airén developed a significantly larger leaf area than the other cultivars. Under L conditions, Airén developed notably more leaf area than the other cultivars in the two first phases, while in the last phase it was Chardonnay. Throughout the cycle, Airén and Chardonnay developed a significantly greater leaf area than Garnacha tinta and Tempranillo.

II - DEVELOPMENT OF LEAF AREA IN LATERAL SHOOTS

The values of the leaf area developed in the lateral shoots (table II) are higher than those recorded in the bibliography (HUGLIN, 1986; WILLIAMS, 1987; MULLINS *et al.*, 1992), due to the development of one single vine shoot with great vigour and without fruits. Under both conditions of water availability, the percentage of leaf area developed in the lateral shoots compared to the total leaf area formed increased throughout the season. Nevertheless, a greater increase was noticed when passing from the first to the second phase coincided with a greater expansion of leaf area development on the whole of the grapevine. (table I).

Both water availability and genotype had a significant influence on the leaf area developed in the lateral shoots, although the interaction between both factors was not significant (table II). In all the cultivars, water limitation caused a significantly lower development of leaf area in lateral shoots than in NL throughout the cycle and in the last two phases of the cycle, while in the first phase significant differences were only observed in the Airén cultivar. The mean reduction in the percentage of leaf area in lateral shoots caused by water limitation stood at 19 %. Although the development of leaf area in the lateral shoots is highly sensitive to water limitation (WILLIAMS and GRIMES, 1987), the effect of water limitation on the development of lateral shoots depends on the growing condition and the presence of clusters (GÓMEZ DEL CAMPO *et al.*, 2002).

The development of the lateral shoots was genetically controlled in most phases and throughout the cycle in this trial. The statistical differences among the cultivars were more noticeable under conditions of NL than of L. Under both conditions of water availability, Airén presented values of leaf area development in lateral shoots which could be found in the significantly higher group, compared to Chardonnay and Tempranillo which presented lower values.

III - LEAF NUMBER

The effect of the cultivar and water availability were significant in the number of leaves formed in the main shoot and in lateral shoots, and, in the whole of the grapevine, the interaction between both treatments was significant (table III). The total number of leaves on the grapevine was determined by the activity of the lateral shoots in the formation of new leaves, as, on average, in the trial 81 % of the leaves were on the lateral shoots.

In all the cultivars, water limitation caused a significant reduction in the formation of leaves on the whole of the grapevine. As a mean value of the cultivars, the grapevines under L conditions developed half the number of leaves as those under NL conditions. The most affected was Garnacha tinta (the number of leaves fell by 56 %) and Chardonnay was the least affected (the reduction was 44 %). In Tempranillo and Chardonnay, the number of leaves formed on the main vine shoot was not significantly affected by water availability, as was also observed in Carignan in the field (KLIEWER *et al.*, 1983). Water limitation affected activity to a greater extent in the meristems of the lateral shoots than in the main shoot, thereby causing a mean reduction of 57 % in the number of leaves on the lateral shoots, while on the main shoot, the number fell

TABLE II

Leaf area developed in lateral shoots (% compared to total leaf area) in two-year-old Garnacha tinta (G), Tempranillo (T), Chardonnay (C) and Airén (A) grapevines grown in 35-L lysimeters under water limitation (L) and non-water limitation conditions (NL).

Phase S: 6 May to 5 July. Phase E: 5 July to 16 August. Phase L: 16 August to 20 September.
Factorial analysis of variance (CUL = cultivar, IT = irrigation treatment, CUL·IT = interaction).

Surface foliaire développée sur les entre-cœurs (% par rapport à la surface foliaire totale) sur des cépages Garnacha tinta (G), Tempranillo (T), Chardonnay (C) et Airén (A) âgés de deux ans ayant poussé dans des lysimètres de 35-L en conditions de contrainte hydrique (L) et en régime de non-contrainte hydrique (NL).

Phase S : du 6 mai au 5 juillet. Phase E : du 5 juillet au 16 août. Phase L : du 16 août au 20 septembre.
Analyse factorielle de la variance (CUL = cépage, IT = régime d'irrigation, CUL·IT = interaction).

	Phase S	Phase E	Phase L	Season
CUL	* x	**	**	**
IT	ns	**	**	**
CUL·IT	ns	ns	ns	ns
G-NL vs.G-L	ns	**	**	**
T-NL vs. T-L	ns	**	**	**
C-NL vs. C-L	ns	*	**	*
A-NL vs. A-L	*	**	**	**
G-NL	11.63 ab y	61.61 ab	78.64	41.87 ab
T-NL	8.46 b	56.11 bc	77.01	38.92 b
C-NL	9.84 b	54.71 c	77.86	39.42 b
A-NL	14.54 a	64.59 a	80.52	44.49 a
G-L	10.06	48.13	67.43 ab	34.38
T-L	9.56	42.76	59.05 c	30.29
C-L	10.38	44.16	62.36 bc	32.22
A-L	11.43	50.02	70.07 a	36.25

x ns,*,**, non significant or significant at P = 0.05 or 0.01, respectively.

y Mean separation by Duncan's multiple range test at P = 0.05.

by 12 %. Therefore, the number of leaves on lateral shoots could be considered as an evaluation parameter of the level of water limitation suffered by a grapevine when there is no other limiting factor.

Genotype significantly determined the number of leaves formed on the main shoot, lateral shoots and on the vine as a whole. Nevertheless, under NL conditions, the number of leaves on the main shoot was not significantly influenced by the genotype. Under both water conditions the Chardonnay stood out for the greater total number of leaves it formed in comparison with other cultivars, and Tempranillo the lowest number.

IV - INDIVIDUAL LEAF SIZE

The leaves on the lateral shoots were smaller than those on the main vine shoot – they were practically half the size (table III). Similar differences between leaf size on main and lateral shoots were noticed in other experiments (PALLIOTTI *et al.*, 2000; WILLIAMS, 1987).

Water limitation had a significant effect on leaf size on main and lateral shoots and on the whole of the vine in all the cultivars. This reduction in leaf size seems to be mainly due to a reduction in length rather than in width (FANIZZA and RICIARDI, 1990). Water limitation caused a greater reduction in the leaf size of the lateral shoots than in that of the main vine shoot. As a mean of the cultivars, the leaf size of the laterals was 21 % smaller in the grapevines under L conditions than in NL, while in the leaves of the main shoot the reduction in size was 13 %. The greatest reduction was quantified in Tempranillo; the leaves of the main grapevine shoot decreased by 17 % in size, and those of the lateral shoots by 29 %, which, furthermore, coincided with being the cultivar with significantly larger leaves.

The leaf size of the main and lateral shoots is a varietal property (FANIZZA and RICIARDI, 1990 ; PALLIOTI *et al.*, 2000). The cultivar had a significant effect on leaf size in both irrigation conditions. The leaves of the main and lateral shoots of Tempranillo were significantly larger than the other cultivars studied, while the leaves of Chardonnay belong to the group of significantly smaller leaves.

V - SPECIFIC WEIGHT

The specific weight of the leaves of the main shoot was greater than that of the leaves on the lateral shoots in all the cultivars both in L and NL, except in Airén under L conditions (table III).

On the whole of the grapevine, significant differences in the specific weight of the leaves were not

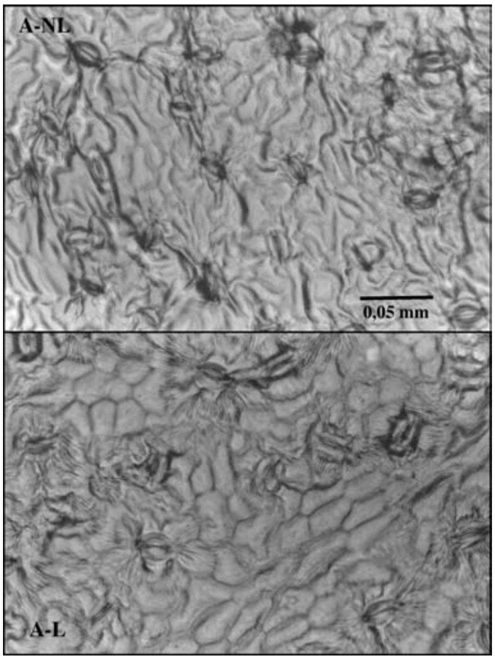

Fig. 2 - Abaxial leaf surface of Airén grapevine under non-water limitation (top) and water limitation (bottom) conditions.

Surface abaxiale des feuilles de cépage Airén en régime d'irrigation non contrainte hydrique (haut) et contrainte hydrique (bas).

observed among irrigation treatments in any of the cultivars. Nevertheless, in Garnacha tinta and Tempranillo, water limitation caused a significant reduction in the specific weight of the leaves of the main shoot. However, in Airén under L there was a significant increase in the specific weight of the lateral shoot leaves. WILLIAMS and GRIMES (1987) observed that the specific weight of the leaves under L conditions was greater than that of NL conditions; they contributed this fact to the higher temperatures that the leaves maintain under these circumstances as the dry weight per unit of the leaf area depends on temperature. The presence of one sole vine shoot on the grapevines and its vertical position caused little shading of the leaves and it is probably because of this that differences were not observed in the specific weight of the leaves amongst irrigation treatments.

The specific weight of the leaves of the main shoot and on the whole of the vine was significantly altered by genotype. Nevertheless, the specific weight of the leaves developed on the lateral shoots was not specifically determined by the cultivar (table III). Tempranillo presented significantly higher specific weight values on the whole of the vine under both conditions of water availability, whilst Airén presented lower values. Whilst SLAVCHEVA (1990) obtained differences in the specific weight of five cultivars, PALLIOTTI *et al.* (2000)

Table III

Main, lateral and whole vine leaf area properties at the end of the season in two-year-old Garnacha tinta (G), Tempranillo (T), Chardonnay (C) and Airén (A) grapevines grown in 35-L lysimeters under water limitation (L) and non-water limitation conditions (NL), in three phases of the cycle.

Phase S = slow growth phase (days 126-186), phase E = exponential growth (days 186-228) and phase L = Lag phase growth (228-263) and in the whole of the cycle. Factorial analysis of variance (CUL = cultivar, IT = irrigation treatment, CUL·IT = interaction).

Caractéristiques de la surface foliaire (feuilles primaires, latérales et entières) à la fin de la saison pour des cépages Grenache (G), Tempranillo (T), Chardonnay (C) et Airén (A) âgés de deux ans, ayant poussé dans des lysimètres de 35-L en conditions de contrainte hydrique (L) et en régime de non-contrainte hydrique (NL), sur trois phases du cycle.

Phase L = phase de croissance lente (jours 126 à 186), phase E = croissance exponentielle (jours 186 à 228) et phase R = croissance ralentie (jours 228 à 263) et sur l'ensemble du cycle. Analyse factorielle de la variation (CUL = cépage, IT = régime d'irrigation, CUL·IT = interaction).

	Number of leaves			Size of leaves (cm²)			Specific weight (mg/cm²)		
	Main	Laterals	Vine	Main	Laterals	Vine	Main	Laterals	Vine
CUL	** x	**	**	**	**	**	**	ns	**
IT	**	**	**	**	**	**	**	*	ns
CUL·IT	**	**	**	ns	*	ns	ns	ns	*
G-NL vs.G-L	**	**	**	**	**	ns	**	ns	ns
T-NL vs. T-L	ns	**	**	*	**	*	*	ns	ns
C-NL vs. C-L	ns	**	**	*	*	ns	ns	ns	ns
A-NL vs. A-L	**	**	**	**	**	*	ns	**	ns
G-NL	51	277 c	328 c	62.1 b	32.8 b	37.3 b	8.60 a	6.95	7.37 b
T-NL	43	229 d	272 d	80.5 a	37.4 a	44.0 a	9.98 a	7.19	7.98 a
C-NL	49	391 a	440 a	61.0 b	23.5 c	27.7 c	8.96 a	6.71	7.21 b
A-NL	48	343 b	391 b	68.5 b	31.5 b	36.0 b	7.10 b	6.57	6.70 c
G-L	37 b y	104 c	143 c	54.8 c	27.1 a	34.5 b	7.30 bc	6.86	7.06 c
T-L	39 b	89 c	130 c	67.1 a	26.6 a	39.2 a	8.23 a	7.34	7.82 a
C-L	53 a	196 a	249 a	53.6 c	18.6 b	26.1 c	7.72 ab	7.24	7.45 b
A-L	37 b	154 b	192 b	61.7 b	25.8 a	32.8 b	6.69 c	7.14	6.97 c

x : ns,*,**, non significant or significant at P = 0.05 or 0.01, respectively.

y : Mean separation by Duncan's multiple range test at P = 0.05.

did not observe differences between the Cabernet franc and Trebbiano toscano cultivars.

VI - STOMATAL DENSITY

The values of stomatal density obtained in this trial (table IV) are found within the range of 129-254 stomata/mm² recorded in other works (CARBONNEAU, 1980 ; DÜRING, 1980 ; DÜRING and SCIENZA, 1980 ; ERIS and SOYLU, 1990 ; ALBUQUERQUE-REGINA, 1993 ; PALLIOTI *et al.*, 2000). Nevertheless, KLIEWER *et al.* (1985) reported values of up to 350 stomas per mm² in the Napa Gamay cultivar. Stomatal density does not seem to differ between the leaves of lateral and main shoots or between the different lobes of the leaf (PALLIOTI *et al*., 2000).

The cultivar and the interaction of the cultivar with water availability had a significant effect on stomatal density; water availability only had a significant effect on the second measurement day (table IV). However, only Airén presented a significantly lower stomatal density under L than under NL on both measurement days (figure 2); this suggests that the leaves of this cultivar present an alteration in their anatomical properties when they are under water shortage conditions. Likewise, DÜRING and SCIENZA (1980) observed that Riesling was able to modifiy stomatal density when the crop was passed from a glasshouse to the field.

When all the grapevines in the trial are considered, the stomatal density of the grapevines under NL and L separately is not directly related to stomatal conductance (figure 3) nor to the assimilation rate (figure 4) coinciding with the observations of FANIZZA *et al.*, (1989) and ALBUQUERQUE-REGINA (1993). Nevertheless, alterations were significant for the Airén grapevines (statistical analysis not presented); KLIEWER *et al.* (1985) observed that a cultivar with greater stomatal density coincided with being that which presented higher conductance rates.

Genotype had a significant effect on stomatal density when cultivars are grown under the same water availability conditions. This observation is consistant with other authors (DÜRING and SCIENZA, 1980; SCIENZA and BOSELLI, 1981; KLIEWER *et al.*, 1985; FANIZZA *et al*, 1989; ERIS and SOYLU, 1990 ; ALBUQUERQUE-REGINA, 1993). Under NL, Garnacha tinta and Airén presented the highest values; under L, Garnacha tinta stood out for presenting signi-

Table IV

Stomatal density (number·mm^{-1}) measured on two days of the cycle on main leaves of two-year-old Garnacha tinta (G), Tempranillo (T), Chardonnay (C) and Airén (A) grapevines grown in 35-L lysimeters under water limitation (L) and non-water limitation conditions (NL).

Factorial analysis of variance (CUL = cultivar, IT = irrigation treatment, CUL·IT = interaction).

Densité stomatique (nombre·mm^{-1}) mesurée pendant deux jours au cours du cycle sur des feuilles primaires de cépages de Grenache (G), Tempranillo (T), Chardonnay (C) et Airén (A) âgés de deux ans ayant poussé dans des lysimètres de 35-L en conditions de contrainte hydrique (L) et en régime de non-contrainte hydrique (NL).

Analyse factorielle de la variance (CUL = cépage, IT = régime d'irrigation, CUL·IT = interaction).

	DOY 219	DOY 248
CUL	**x	**
IT	ns	**
CUL·IT	*	**
G-NL vs.G-L	ns	ns
T-NL vs. T-L	ns	ns
C-NL vs. C-L	ns	ns
A-NL vs. A-L	*	**
G-NL	192.6 a y	182.2 b
T-NL	134.4 b	157.2 c
C-NL	131.3 b	153.9 c
A-NL	171.2 a	195.6 a
G-L	210.5 a	174.1 a
T-L	122.0 b	149.9 b
C-L	122.8 b	148.8 b
A-L	140.5 b	150.7 b

x ns,*,**, non significant or significant at P = 0.05 or 0.01, respectively.
y Mean separation by Duncan's multiple range test at P = 0.05

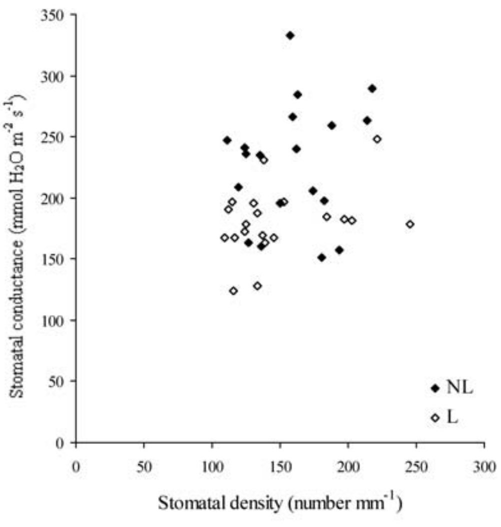

Fig. 3 - Relationship between stomatal conductance and stomatal density for two-year-old grapevines under non-water limitation (NL) and water limitation (L) conditions during day 219.

Rapport entre la conductance stomatique et la densité stomatique pour des cépages âgés de deux ans en régime de non-contrainte hydrique (NL) et de contrainte hydrique (L) au 219e jour.

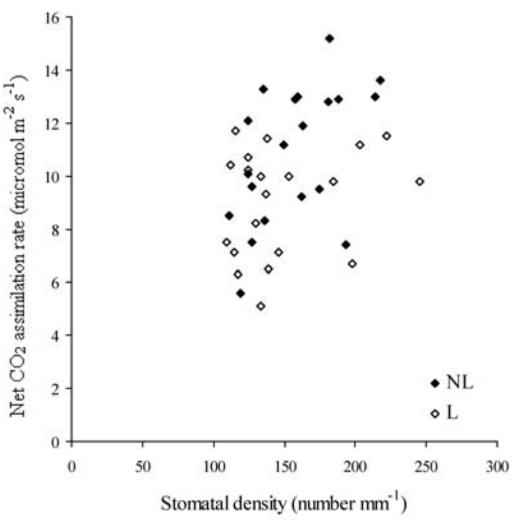

Fig. 4 - Relationship between net CO2 assimilation rate and stomatal density for two-year-old grapevines under non-water limitation (NL) and water limitation (L) conditions during day 219.

Rapport entre le taux d'assimilation net du CO_2 et la densité stomatique pour des cépages âgés de deux ans en régime de non-contrainte hydrique (NL) et de contrainte hydrique (L) au 219e jour.

Table V

Production of dry matter per annual vine and per unit of mean foliar surface area and water use efficiency of two-year-old Garnacha tinta (G), Tempranillo (T), Chardonnay (C) and Airén (A) grapevines grown in 35-L lysimeters under water limitation (L) and non-water limitation (NL).

Factorial analysis of variance (CUL = cultivar, IT = irrigation treatment, CUL·IT = interaction).

Production annuelle de matière sèche par cep et par unité de surface foliaire et usage efficace de l'eau de cépages Grenache (G), Tempranillo (T), Chardonnay (C) et Airén (A) âgés de deux ans ayant poussé dans des lysimètres de 35-L en conditions de contrainte hydrique (L) et en régime de non-contrainte hydrique (NL).

Analyse factorielle de la variance (CUL = cépage, IT = régime d'irrigation, CUL·IT = interaction).

	Production of dry matter		WUE
	(g)	($g.m^2$)	($g.L^{-1}$)
CUL	* [x]	**	ns
IT	**	ns	**
CUL·IT	**	ns	**
G-NL vs.G-L	**	ns	*
T-NL vs. T-L	**	ns	ns
C-NL vs. C-L	**	ns	**
A-NL vs. A-L	**	ns	*
G-NL	351.3 ab [y]	682.0 ab	2.633
T-NL	334.3 bc	654.8 bc	2.677
C-NL	312.9 c	710.0 a	2.417
A-NL	366.1 a	605.4 c	2.621
G-L	141.2 b	692.8 a	3.056
T-L	151.1 ab	639.6 a	2.843
C-L	166.1 a	709.0 a	2.788
A-L	159.8 a	563.0 b	2.839

[x] ns,*,**, non significant or significant at P = 0.05 or 0.01, respectively.

[y] Mean separation by Duncan's multiple range test at P = 0.05.

ficantly higher stomatal densities (figure 5). DÜRING and SCIENZA (1980) consider that greater stomatal density in leaves when they are under dry conditions is an adaptation strategie to drought and is inversely related to ABA content of the leaves. Other authors do not observe any direct relationship between stomatal density and the resistance to drought of the cultivars (ERIS and SOYLU, 1990), nor to the physiological activity of the leaf (FANIZZA *et al.*, 1989; ALBUQUERQUE-REGINA, 1993), but that the genetic differences in the assimilation of CO_2 are due to the physiological control of their opening rather than to variation in size or number (CHAVES, 1991).

VII - DRY MATTER PRODUCTION

The production of dry matter of the experimental vine (table V) was lower than that referred to field conditions (ARAUJO and WILLIAMS, 1988; MULLINS *et al.*, 1992; ARAUJO *et al.*, 1995), as the pot limits growth, and, therefore, productivity (BRAVDO *et al.*, 1972; CONRADIE 1990 and 1991; MILLER *et al.*, 1996 a and b).

The cultivar and the irrigation treatment as well as the interaction between both factors was significant (table V). Water limitation caused a mean reduction of 55 % in the production of dry matter throughout the season compared to NL. In all the cultivars, this reduction was significantly higher. The cultivar which most reduced the dry matter produced when it was subjected to conditions of water limitation was Garnacha tinta (60 %), while Chardonnay decreased production by 47 %.

When the cultivars were under the same conditions, the production of dry matter differed significantly among the cultivars, as BRAVDO *et al.* (1972) and RIES (1986) observed under conditions of high water availability. Under conditions of NL, the Airén cultivar stood out for being the most productive, whilst Chardonnay was the least productive; therefore, under conditions of high water availability it was the cultivar from the dry area which was the most productive, these results are in contradiction with KRAMER and BOYER (1995). Under L conditions, both Chardonnay and Airén produced significantly more dry matter than Garnacha tinta.

Relationship between morphological and anatomical properties of leaves and dry matter production

The mean leaf area of the vine significantly determined the production of dry matter in a linear manner (figure 6) either when taking into consideration all the grapevines together, each irrigation treatment or each cultivar separately. The relationship between the production of dry matter and total leaf area was proven previously (BRAVDO *et al.*, 1972; MILLER *et al.*, 1996 a and b). The equation of the graph line of the grapevines under NL differed significantly to that of L; however, the slope did not differ significantly (statistical analysis not presented), which indicates that the increase in leaf area in the grapevines caused the same increase in production of dry matter in NL as in L. The graph lines of the cultivars differed significantly from each other (statistical analysis not presented). For the same leaf area, Garnacha tinta and Chardonnay produced more dry matter than Airén, leaving Tempranillo in an intermediary position (figure 6).

The production of dry matter per unit of mean leaf area turned out to be a property which depended exclusively on the cultivar (table V). It was not sigificantly influenced by water availability and the interaction between both factors was not significant. In both water availability treatments, the Airén cultivar stands out for presenting significantly lower values.

The linear correlation between productivity and leaf size was significant for the cultivars Tempranillo and Airén (table VI), however when the data was pooled by grouping all the cultivars together, the adjustment was not seen to be significant; therefore, in this trial, leaf size has not turned out to be a productivity indicator as was stated by BRAVDO *et al.* (1972).

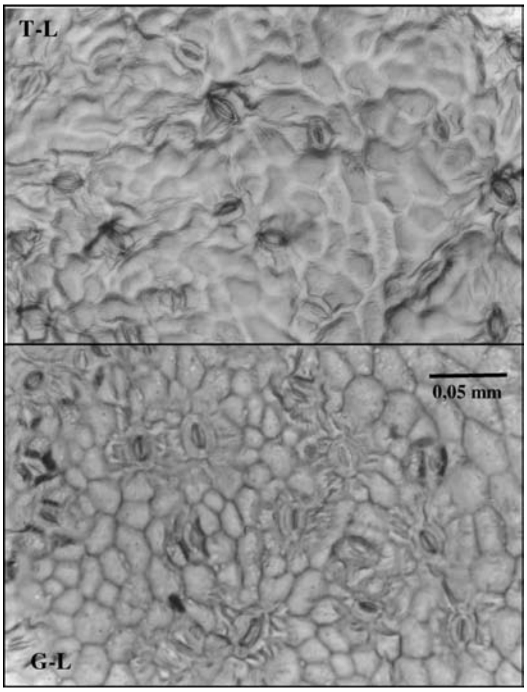

Fig. 5 - Abaxial leaf surface of Tempranillo (top) and Garnacha tinta (bottom) grapevines under water limitation conditions.

Surface abaxiale des feuilles de cépages Tempranillo (haut) et Garnacha tinta (bas) en conditions de contrainte hydrique.

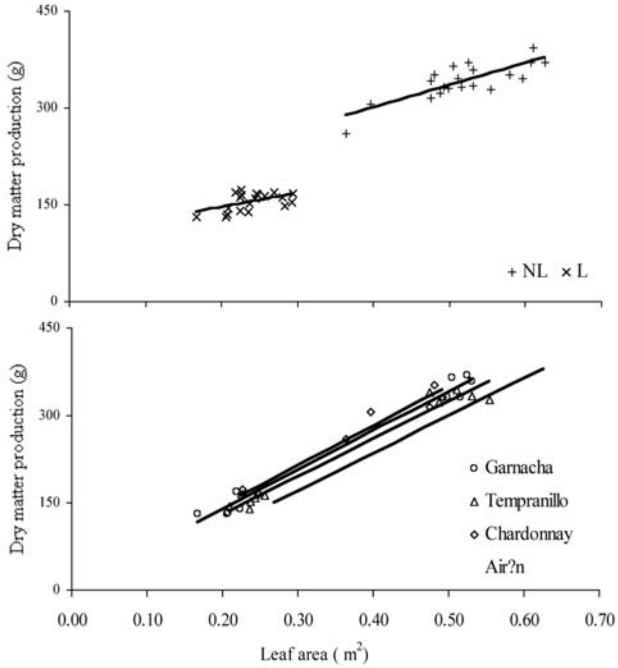

Fig. 6 - Relationship between dry matter production and mean leaf area over the season in two-year-old Garnacha tinta (G), Tempranillo (T), Chardonnay (C) and Airén (A) grapevines grown in 35-L lysimeters under non-water limitation (NL) and water limitation (L) conditions.

Regression for pooled data: y = 628.x + 10 **, regression for non-water limitation condition: y = 345.x + 162**, regression for water limitation condition y = 214.x + 103*, regression for Garnacha tinta grapevines: y = 674.x + 3**, regression for Tempranillo grapevines: y = 648.x**, regression for Chardonnay grapevines: y = 684.x + 8**, regression for Airén grapevines: y = 643.x – 23**.

Rapport entre la production de matière sèche et la surface foliaire moyenne pendant la saison pour des cépages Garnacha tinta (G), Tempranillo (T), Chardonnay (C) et Airén (A) âgés de deux ans ayant poussé dans des lysimètres de 35-L en régime de non-contrainte hydrique (NL) et de contrainte hydrique (L).

Régression pour la combinaison de données : y = 628.x + 10 **, régression en régime de non-contrainte hydrique: y = 345.x + 162**, régression en conditions de contrainte hydrique y = 214.x + 103*, régression pour le cépage Garnacha Tinta : y = 674.x + 3**, régression pour le cépage Tempranillo : y = 648.x**, régression pour le cépage Chardonnay : y = 684.x + 8**, régression pour le cépage Airén : y = 643.x – 23**.

Table VI
Relationship between morphological and anatomical properties of leaves and productivity (g.vine-1) of two-year-old Garnacha tinta (G), Tempranillo, Chardonnay and Airén grapevines grown in 35-L lysimeters under water limitation (L) and non-water limitation conditions (NL).

Tableau VI - Rapport entre les caractéristiques morphologiques et anatomiques des feuilles et de la productivité (cépage-1) de cépages Garnacha tinta (G), Tempranillo (T), Chardonnay (C) et Airén (A) âgés de deux ans ayant poussé dans des lysimètres de 35-L en conditions de contrainte hydrique (L) et en régime de non-contrainte hydrique (NL).

	Leaf size (cm^2)		Specific weight ($mg.cm^{-2}$)		Stomatal density ($number.mm^{-2}$)	
G	y = 20.x – 479	ns	y = 168.x - 973	ns	y = -10.x + 768	ns
T	y = 17.x – 479	*	y = 146.x - 911	ns	y = 7.x + 26	ns
C	y = 18.x - 239	ns	y = -93.x + 921	ns	y = 13.x - 183	ns
A	y = 39.x - 1085	**	y = -251.x + 1980	ns	y = 12.x - 236	*
NL	y = 2.x + 286	ns	y = -9.x + 404	ns	y = 2.x + 277	*
L	y = -1.x + 186	ns	y = 6.x + 108	ns	y = -x + 180	*
Total	y = 5.x + 86	ns	y = -6.x + 294	ns	y = x + 192	ns

[z] ns,*,**, non significant or significant at P = 0.05 or 0.01, respectively.

The specific weight of the leaves was not related in a linear manner to the productivity of the vine in any of the cases studied (table VI).

Productivity was related in a linear manner to stomatal density when the grapevines in NL and L were taken into consideration separately; the graph lines differed significantly (statistical analysis not presented). Nevertheless, while under NL conditions the greater the number of stomas the higher the productivity, under L conditions the relationship was the reverse, as it was observed by CARBONNEAU (1980). Our results do not confirm that greater stomatal density is related to a better adaptation to drought (DÜRING and SCIENZA, 1980), if we consider that a cultivar is adapted to drought when it reaches high performance and quality even when conditions are not optimum (DÜRING and SCIENZA, 1980; ALBUQUERQUE-REGINA, 1993). The adjustment between the production of the dry matter and stomatal density of all the Airén grapevines was significant; as density increased so did productivity.

CONCLUSIONS

The drought adaptation strategies related to the morphological and anatomical properties of leaves which enable grapevines to avoid water limitation effects which were observed in this study were the following:

1.- Lesser development of leaf area in conditions of water limitation in all the cultivars; on average it was reduced by 57 % due to a significant reduction in the leaf size of main and lateral shoots and a lower number of leaves on lateral shoots; the latter was the parameter which was most affected by water limitation.

2.- The development of the leaf area in conditions of water limitation occurred on earlier dates than under conditions of total water availability. This enabled the grapevines to avoid drought by reducing their cycle and growing in periods in which ground humidity is favourable.

3.- In the Airén cultivar, a significant reduction in stomatal density was observed under conditions of water limitation. Stomatal density was positively related to photosynthesis and stomatal conductance.

All the strategies related to grapevine survival under drought conditions tend to reduce the productivity potential of dry matter (JONES, 1983). Nevertheless, there are significant differences amongst cultivars in the production of dry matter under drought conditions; Chardonnay and Airén produced significantly more dry matter than Garnacha tinta. The production of dry matter by the grapevine was directly related to the mean leaf area of the vine; the relationship depended on genotype. For the same leaf area, Airén turned out to be the cultivar which produced the least amount of dry matter, while Garnacha tinta and Chardonnay were the most efficient. The production of dry matter per unit of leaf area was not significantly altered by water limitation but it is an exclusively varietal property.

Of all the cultivars studied, Airén stood out for its different morphological and anatomical properties. It was one of the cultivars which produced significantly more dry matter throughout the season under both irrigation conditions. It coincided with being the one which developed a larger leaf area, standing out for the early development of the latter in spite of being a cultivar with late budbreak, for its greater development of lateral shoot leaves. The specific weight of the leaves was significantly lower than that of other cultivars. Under drought conditions, stomatal density fell significantly and presented a positive correlation between stomatal density and the physiological activity of the leaf.

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