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ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · AUGUST 2006

Impact Factor: 2.91 · DOI: 10.1021/jf0605446 · Source: PubMed

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Influence of Vineyard Location and Vine Water Status on Fruit Maturation of Nonirrigated Cv. Agiorgitiko (*Vitis vinifera* L.). Effects on Wine Phenolic and Aroma Components

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The influence of site on grape and wine composition was investigated for *Vitis vinifera* L. cv. Agiorgitiko in the Nemea appellation area in southern Greece. Three nonirrigated plots were studied during the 1997 and 1998 vintages, which were typically very hot and without summer rainfall. Vines were subjected to different water regimens as a result of the variation of soil water-holding capacity and evaporative demand. Vine water status was determined by means of predawn leaf water potential. Differences in vine water status between sites were highly correlated with the earliness of shoot growth cessation and veraison. Grape composition was monitored during fruit ripening. Water deficit accelerated sugar accumulation and malic acid breakdown in the juice. Early water deficit during the growth period was demonstrated to have beneficial effects on the concentration of anthocyanins and total phenolics in berry skins. A similar pattern was observed for the phenolic content of wines elaborated after vinification of grapes harvested on each plot, in both seasons. Limited water availability seemed to increase glycoconjugates of the main aromatic components of grapes as a quantitative increase in levels of bound volatile compounds of the experimental wines was observed under water deficit in both years. Wines produced from grapes of stressed vineyards were also preferred in tasting trials.

KEYWORDS: Grapevine; *Vitis vinifera*; Agiorgitiko; soil; climate; terroir; water deficit; leaf water potential; vegetative growth; grape ripening; wine composition; phenolic compounds; glycosylated aroma precursors; sensory evaluation

INTRODUCTION

Environmental factors (topographical, agro-pedological, climatic), usually described by the French term "terroir", have been acknowledged to influence grape and wine quality. Although the precise dependence of the major grape berry attributes on environmental conditions remains uncertain, a good viticultural site is considered to enhance complete ripening of appropriately chosen grape cultivars (*1*) either by creating favorable mesoclimates (*2*) or by achieving adequate but not excessive vine vigor through its ability to control soil fertility (*3*) and, above all, water supply (*4*).

Water conditions have long been recognized as an important factor determining winegrape quality, thereby affecting wine sensory attributes. Many papers have reported extensively on the effects of water deficit on the accumulation of various grape metabolites. Most of these studies concern irrigation trials (*5*−*8*), whereas very few investigate the impact of water conditions in nonirrigated situations, in relation to environmental factors such as soil and climate (*9*, *10*). Vine water status effects on berry components are often contrasting, mainly because of different irrigation dosages leading to various levels of water stress. Furthermore, grape response to moderate irrigation might also be cultivar-dependent as *Vitis vinifera* varieties have been shown to respond differently to water stress (*11*).

Grape-derived secondary metabolites are the principal sources of wine color, aroma, and flavor. Considerable research has been conducted regarding the phenolic compounds of the skin as they play an important role in the quality of red grapes, conferring much of the color and structural properties of wines. Vine water status is reported to affect the rate of accumulation of phenolic compounds in maturing grapes (*12*, *13*). Most of theses studies show a clear positive effect of water deficit on berry phenolic composition.

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10.1021/jf0605446 CCC: $33.50
Published on Web 06/15/2006

Table 1. Soil Characteristics of the Studied Plots

characteristic	P	H	A
geological origin	quaternary alluvium	tertiary limestone (Pliocene)	quaternary alluvium
sand (%) (0–50 cm)	16	16	23
silt (%) (0–50 cm)	68	51	37
clay (%) (0–50 cm)	14	32	38
soil type	silty loam	loam on soft limestone bedrock	sandy clay loam with hardpan
organic matter content (%)	1.75	1.14	1.05
organic matter C/N ratio	9.3	12.2	5.3
soil pH (0–50 cm)	7.8	7.8	6.7
lime (%) (0–50 cm)	51.8	68.3	
gravel content	low	low	low
soil depth	deep	shallow	deep
water logging	never	never	all year round

Table 2. Climatic Parameters of the Studied Plots for the Growth Period (April–September)

	1997			1998		
parameter	P	H	A	P	H	A
mean lowest temperature (°C)	11.4	14.4	8.0	12.7	15.9	10.1
mean highest temperature (°C)	26.1	24.6	23.1	27.6	26.4	23.5
mean daily temperature (°C)	19.1	19.1	16.1	20.6	20.8	17.6
no, of days with T_{max} [a] $\geq$ 30 °C	64	52	23	74	61	28
no, of days with $T_{max} \geq$ 35 °C	8	5	3	18	10	2
heat summation (base 10 °C)	1741	1758	1226	1957	1998	1410
rainfall (mm) Oct–March	371	417	372	338	359	360
rainfall (mm)	89 (27[b])	71 (11)	87 (26)	63 (0)	67 (0)	65 (8)
evapotranspiration (mm)[c]	503	512	458	549	559	503
water balance[d]	–162	–185	–142	–211	–212	–186

[a] Highest daily temperature. [b] Summer rainfall (June–Aug). [c] (*62*). [d] Rainfall − 0.5 × ETo.

Whereas soluble phenolic compounds determine some aspects of berry quality, volatile metabolites are responsible for wine varietal aroma (*14*, *15*). These compounds occur in grapes in free and glycosylated forms, with the latter predominant (*16*). Although their proportion is not directly related to wine organoleptic properties, the total concentration of glycosylated aroma compounds in grapes could provide an indication for their enological potential (*17*). Previous studies on this subject were mainly focused on the effects of vine and cluster microclimate on the glycosylated fraction of grape and wine aroma (*18*−*20*). Few data exist regarding the effect of either environmental parameters (*21*−*23*) or vine water status (*24*−*26*) on the volatile components of grapes and wines.

Water shortage is the dominant environmental constraint for grape production in the Nemea vine-growing region in southern Greece, where grapevines are exposed to high water stress during summer. Especially in the case of nonirrigated vineyards, grapevine vegetative and reproductive growth depends mainly upon site variables affecting the extent and seasonal timing of water deficits (*27*). Although drip irrigation has been steadily increasing in Nemea, most of the vineyards in this area remain dry-farmed, mainly because of legal restrictions. *V. vinifera* cv. Agiorgitiko is a Greek black variety cultivated almost exclusively in Nemea, giving Denomination of Origin, deeply colored, red wines. Its aromatic profile is often characterized as "spicy", "vanilla-like", or "red currant". These aromatic notes could be attributed to volatiles formed during fermentation from enzymatic hydrolysis of glycoconjugated precursors of the grapes.

The aim of the present work was to examine the association between environmental parameters and fruit and wine quality (with emphasis on the phenolic and aroma components) from a study of Agiorgitiko vines growing under field conditions and subjected to different water deficit conditions imposed by soil and climate factors.

MATERIALS AND METHODS

Site Variables and Experimental Plots. *Soils.* The study was carried out in three nonirrigated vineyard plots located in the Nemea vine-growing region in southern Greece (37° 81′ N, 22° 66′ E) during two consecutive years (1997 and 1998). The three plots were primarily selected to represent the major soil and climate types of the region and to include what appeared to be distinctive differences in fruit ripening capacity, and wine quality potential. The first plot was situated on a uniform, calcareous silty loam, with nearly 70% of silt in the surface layers, situated on a flood plain at the main altitude zone of 300 m. Soil water-holding capacity was ≈190 mm (*28*) due to the high silt percentage. The second plot was set on a shallow calcareous silty loamy soil developed on a soft limestone bedrock and situated on west-facing hill slopes of the main plain of Nemea, at an altitude of 500 m. The water-holding capacity was lower because of the shallow rooting (70 mm). The third plot was set at a plateau of 700 m, on a clayey loamy soil, with a hardpan of nearly 70% clay at 140 cm of depth forming a barrier for water drainage, thus leading to permanent water logging during the growing season. The three vineyard sites were identified as P (plain), H (hill slope), and A (altitude). The main soil characteristics of the studied plots are presented in **Table 1**.

Climatic Conditions. The Nemea area has a Mediterranean-type climate with a mean temperature for the growth period of 19.9 °C (1973−1989) and a mean annual rainfall of 750 mm, of which <20% occurs during the growth cycle. Climatic data were recorded on the three altitude zones with a Lambrecht thermohygrograph (temperature−humidity recorder, Lambrecht GmbH, Göttingen, Germany) housed in a proper weather instrument shelter, at a height of ≈1.5 m from the ground (**Table 2**). Weekly rainfall was monitored using a cylindrical rain gauge placed at a distance of ≈4 m from the weather shelter.

Among the two years of the study, 1997 was warm with an average temperature of the growth period (from April to September) of 19.1 °C, close to the long-term mean (data from the lowest zone, P). The year 1998 was even warmer, with an average temperature from April to September of 20.6 °C at the main altitude zone. No considerable climatic variations were recorded among the two lower altitude zones during these two vintages concerning temperature and rainfall averages.

As expected, the mountainous plot always presented lower temperatures throughout the growing season, resulting in a heat deficit and a lower evaporative demand as compared to the two lower altitude plots (**Table 2**).

Rain conditions were characterized by a wet winter preceding each growth period, indicating that soils were close to field capacity at budbreak, and a low growth season rainfall, particularly during the summer months (only 27 mm in 1997, entirely due to a brief storm in late August, and no summer rainfall in 1998). Thus, the water balance for the growth period became highly negative, leading to exceptionally dry summer conditions in both years. Variation between plots was low.

Plant Material. The vineyards were all planted with *V. vinifera* L. cv. Agiorgitiko vines, grafted onto 41B rootstock. To minimize the effect of nonenvironmental factors, special care was taken to achieve maximum uniformity in viticultural conditions. Vine age was between 15 and 22 years and planting density between 4000 and 5000 vines per hectare with a vine spacing of 2−2.2 m between rows and 1−1.2 m within a row. The trellis system was composed of one fixed wire located at 0.5 m and two paired wires at 1.0 and 1.5 m. A vertical shoot-positioned training system was used. Vines were spur pruned. Hedging was fixed at 1.5 m of height. Yield was limited by pruning to 12 buds per vine. Each experimental site comprised eight adjacent rows with five border vines on either side of a single row. Measurements and samplings were carried out in the four inner rows according to a completely randomized design.

Viticultural Variables. *Vine Water Status.* Measurements of vine water status were made at predawn (between 3 h prior to and at dawn) on five sample dates between the end of June and early September, during the 1997 and 1998 growing seasons. At each sampling time, leaf water potential (Ψ_d) was measured using the pressure bomb technique (*29*) on six randomly selected mature leaves of the outer part of the canopy.

Vine Development and Vigor. On 25 vines per plot, the length of one shoot per vine was measured every 10 days until growth cessation. To prevent hedging, these shoots were each tightened vertically on a stick firmly attached to the posts of the trellis system. On each plot, yield was determined at harvest as a mean of the total number of vines per row (four rows per plot). Date of veraison was noted when 50% of berries reached this phenological stage (onset of berry ripening manifested by a change in color in red varieties).

Assessment of Berry Ripening. Berry composition was measured on a weekly basis, from veraison through harvest, on a sample of ≈1000 berries collected randomly from the middle four rows of each experimental block.

Each sample was counted and weighed to determine mean berry mass. Berries were then pressed, and the must, after a gentle centrifugation, was analyzed for soluble solids (°Brix) by refractometry and total acidity by titrimetry with 0.1 NaOH. Malic acid and tartaric acid in juice were analyzed simultaneously by high-performance liquid chromatography after filtration and passage through Sep-Pak C_{18} and detection by means of measurement of UV absorption at 210 nm (*30*). Pulp ripening speed was calculated by assessing the sugar/acid ratio as a function of a climatic index as described by Duteau (*31*). According to this author, during the first 4 weeks after veraison, the S/TA ratio is a linear function of $\sum \{[(T_{av-10}) + (T_{max-10})]/2\}$. The slope of the linear regression represents the pulp ripening speed.

Two hundred berries were separated to determine the total phenolic potential of grapes (*32*). Grapes were hand peeled, and the skins were suspended in 250 mL of a hydroalcoholic solution (12% v/v ethanol, 5 g/L tartaric acid, pH adjusted to 3.2 with 1 M NaOH) and then ground using an Ultraturax ball grinder for 1 min. After 6 h of stirring, the mixture was centrifuged at 3000 rpm for 10 min, and the supernatant was filtered through glass wool. Anthocyanins were determined at 520 nm optical density in HCl media (*33*). Tannins were measured indirectly by the total phenolics index on the same extract, at 280 nm optical density (*34*). All measurements were carried out with a Shimadzu UV-1240 Mini spectrophotometer, using quartz and glass cells with 1 cm path lengths.

Vinifications and Wine Analysis. Harvest date was determined for each site when the increase in sugar accumulation slowed and anthocyanin concentration began to decrease. Small-scale vinifications were carried out using 300 kg of grapes from each experimental vineyard. Destemmed and crushed grapes were vinified in stainless steel 350 L containers, adding 5 g of sulfur dioxide/100 kg of grapes and 15 g/hL of the same active dry yeast. Maceration was conducted by pumping the juice over, twice a day. Fermentation and maceration lasted 14 days for all vinifications. In all cases, malolactic fermentation occurred without inoculation and was fully completed. Wines received no wood treatment and were bottled after a 2 month conservation period during which they were stored at 15 °C in glass 25 L recipients.

Wine analysis was performed 3 months after harvest. Alcoholic degree and titrable acidity of the wines were determined according to OIV methods (*35*). Total anthocyanins and total phenolics index were determined according to the spectrophotometric methods described previously. Tanins were measured by transformation of procyanidins to anthocyanidins in acid media at 100 °C and measurement at 550 nm optical density (*36*). Wine color intensity was determined as the sum of optical densities at 420, 520, and 620 nm (*33*). All measurements were done in triplicate.

Extraction and Determination of Wine Glycosylated Aroma Compounds. *Isolation of Wine Glycosylated Aroma Compounds.* The protocol used was that of Williams et al. (*37*) slightly modified. One hundred and fifty milliliters of wine was centrifuged and neutralized to pH 7 with 1 N NaOH. Samples were continuously liquid/liquid extracted for 72 h using 100 mL of freshly distilled dichloromethane every 24 h to retrieve any volatile compounds and then dried over Na_2SO_4. The solvent-stripped juice was concentrated under vacuum and then passed through a glass column packed with preactivated C_{18} reversed-phase adsorbent (470 × 15 mm) at a rate of 3 mL/min. After loading, the column was flushed with a volume of water equal to 3 times the volume of the applied sample to eliminate any sugars, acids, and other low molecular weight polar compounds. The glycosidically bound fraction was eluted with 100 mL of methanol and concentrated to dryness under vacuum at 30 °C.

The dried glycosidic extract was redissolved in 10 mL of a citrate−phosphate buffer (equal volumes of 0.1 M citrate and 0.2 M Na_2HPO_3 solutions at pH 5). Ten milliliters of an enzymatic solution possessing β-glucosidase activity (20 mg of Novoferm 12G dissolved in 10 mL of a citrate−phosphate buffer at pH 5) was added to 5 mL of the above solvent-stripped glycosidic extract. After the addition of 1 mL of 1-octanol−glucoside as an internal standard (250 μg/mL in deionized water), the mixture was stirred, sealed, and placed in a water bath at 37 °C for 24 h under nitrogen. The mixture was continuously liquid/liquid extracted with 100 mL of dichloromethane at 40 °C for 24 h. The extracts were collected, dried over Na_2SO_4, filtered, and then concentrated using a Vigreux column at 40 °C to a final volume of 500 μL for GC-MS analysis.

Gas Chromatography−Mass Spectrometry (GC-MS) Analysis. Extracts were analyzed using a Carlo Erba Fisons 8000 series gas chromatograph equipped with a CP-Wax 52CB fused silica capillary column (50 m × 0.32 mm i.d.; 0.25 μm film thickness). Operating conditions were as follows. Injections were made in splitless mode (60 s) with an injection volume of 1 μL. The injector temperature was set to 220 °C, and the oven temperature was programmed to rise from 50 °C (3 min isothermal) to 230 °C at 3 °C/min and then was held isothermal for 20 min. The carrier gas was helium N60 at a flow rate of 1.3 mL/min. The gas chromatograph was coupled to a mass detector MD800-EI (electron impact). Mass spectra were recorded at 70 eV scanning upward from *m*/*z* 35 to 350 each second, with a 0.1 s delay between scans. Spectral recording was automatic throughout separation. Identification of compounds was carried out by comparison of the retention time and mass spectra with those from NIST Library or published data. Compound concentrations were determined by assuming a 1:1 response factor between extracted components and internal standard for the total ion chromatograms. Thus, the results are considered to be semiquantitative and are expressed as micrograms of volatile compounds per liter of wine.

Sensory Evaluation of Wines. Sensory analysis of the experimental wines was conducted in both seasons by a panel of 20 experienced tasters drawn from the Faculty of Enology of the University of Bordeaux II staff members and students. Each of the wines was descriptively assessed for its appearance, nose, and palate. A global appreciation

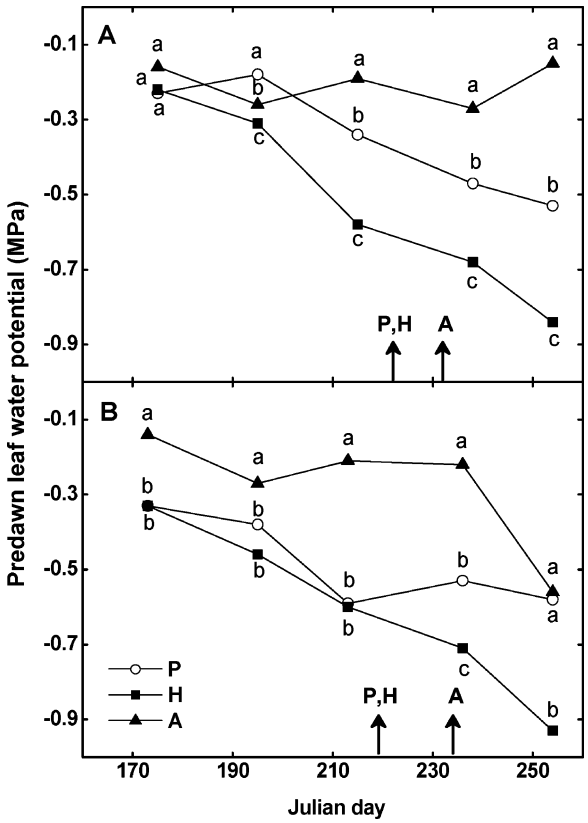

Figure 1. Influence of site on the seasonal variation of predawn leaf water potential in 1997 (**A**) and 1998 (**B**). Each point represents the mean of six replicates. Statistically significant differences by Newman–Keuls test (0.05) are indicated by different letters. Arrows at abscissa indicate veraison.

note was also given on a scale of 1−20. The scores of all judges for each wine were summed. Wine scores were statistically analyzed as a complete randomized design with one factor (site).

Statistics. Data were analyzed by means of linear regression (determination coefficient) and analysis of variance (ANOVA), followed by the Newman−Keuls test for the comparison of means using the statistical software SPSS (SPSS Inc., Chicago, IL). All quoted coefficients of correlation are significant for $P < 0.05$ unless indicated otherwise.

RESULTS AND DISCUSSION

Vine Water Uptake Conditions in 1997 and 1998. On June 24, 1997 (Julian day 175), predawn leaf water potentials (Ψ_d) were close to zero on the three plots, showing no limitation in water availability (**Figure 1**). From the beginning of July (Julian day 195), Ψ_d became significantly different between plots. Water deficit continued to increase during the growing season on plots H and P. Water deficit was always more intense on H than on P, mainly because of the shallow vine rooting on plot H. In early September (Julian day 254) water stress was severe in H ($\Psi_d = -0.84$ MPa) but not in P ($\Psi_d = -0.53$ MPa). Vines on plot A did not face water deficit stress because of soil characteristics (water logging linked to the subsoil clay depression) and lower evaporative demand (**Table 2**). In 1998, which was an overall warmer vintage, water deficit appeared earlier on H and P ($\Psi_d = -0.33$ MPa at the end of June) and developed through veraison without significant difference (**Figure 1**). Ψ_d became significantly more negative on H during the maturation period (Julian days 236 and 254). Climatic conditions were similar for these two plots on both seasons (**Table 2**); thus, water uptake differences reflect mostly soil ability to supply water to vines. No water deficit was recorded on plot A until the end of August. On this plot, moderate water deficit appeared ($\Psi_d = -0.56$ MPa) only on the final measurement day (Julian day 254). This may be attributed to the disappearance of the water table as a result of the extremely hot climatic conditions in 1998. Thus, under nonirrigated conditions, site strongly influenced vine water status, mainly by altering soil water retention capacity and depth, although differences in evaporative demand could play an important role as well.

Table 3. Influence of Site on Vine Development and Vigor Parameters

	1997			1998		
parameter	P	H	A	P	H	A
final shoot length (cm)	287 ab[a]	226 a	323 b	282 ns	277 ns	315 ns
shoot growth cessation (Julian days)	207 ab	196 a	213 b	202 a	195 a	217 b
yield (t ha^{-1})	14.9 b	9.5 a	15.3 b	22.1 b	12.6 a	21.2 b

[a] Statistically significant differences by Newman–Keuls test (0.05) are indicated by different letters; ns, not significant.

Vegetative Growth and Yield. Shoot growth cessation typically occurs in the Nemea area in late July when soil-available water becomes limiting and evaporative demand highest. In both seasons, shoot growth occurred earlier on plot H, although significant differences were observed only between plots A an H (**Table 3**). On plot A, shoot growth was the highest among plots, accompanied by a significantly later growth cessation in both years. This resulted in longer shoots (>300 cm), although significant differences were observed only between plots A an H in 1997. The maximum rates of shoot elongation were 6.5, 6.6, and 6.9 cm/day for P, H, and A, respectively, in 1997 and 5.4, 4.7, and 5.8 cm/day in 1998. Yield was significantly lower in both seasons on plot H; thus, this plot where vine water uptake was severely limited was the less vigorous among the three. Plot A, characterized by the absence of water limitations during the growth period, can be termed as the most vigorous among the studied plots.

Vegetative growth is known to be highly dependent upon vine water status (*38*). Decline in vegetative growth is strongly associated with reduced soil water availability (*39*). Under the conditions of our experiment, the earliness of shoot growth cessation and veraison was positively correlated with both the earliness of water stress, expressed by the average Ψ_d from set to veraison, and the intensity of water stress, expressed by the average Ψ_d from veraison to harvest (**Table 4**). Water deficit accelerated growth cessation ($r = 0.896$, $p = 0.05$; and $r = 0.944$, $p = 0.01$, respectively, for preveraison and postveraison mean Ψ_d) and the onset of ripening ($r = 0.915$, $p = 0.01$; and $r = 0.944$, $p = 0.01$, respectively, for preveraison and postveraison mean Ψ_d), the three sites and two vintages considered together ($n = 6$). Previous work (*40*) also reported that increased vine vigor was associated with lateness in phenology. Final shoot length correlated only with postveraison mean Ψ_d ($r = 0.833$, $p = 0.05$). Sustained shoot growth under favorable water conditions is undesirable because it impairs berry ripening due either to competition for assimilates or to excessive bunch shading by leaves (*41*).

Yield and berry weight were less affected by vine water status (**Table 4**). In agreement with this result, Goodwin and Jerie (*42*) found no effect of irrigation on berry weight, whereas other authors (*39*, *43*) found a strong correlation between berry growth and water supply. According to McCarthy (*44*), water deficit, when applied after veraison, has only minor effects on berry

Table 4. Correlations between Vine Water Status and Vine Development and Berry Composition at Harvest

parameter	mean Ψd set–veraison	mean Ψd veraison–harvest	mean Ψd set–harvest
final shoot length (cm)	0.630 ns [a]	0.833 *	0.771 ns
shoot growth cessation (Julian days)	0.896 *	0.944 **	0.947 **
veraison (Julian days)	0.915 **	0.944 **	0.943 **
yield (t ha^{-1})	0.193 ns	0.447 ns	0.397 ns
berry weight (g)	0.520 ns	0.544 ns	0.550 ns
sugar (°Brix)	–0.759 ns	–0.925 **	–0.905 *
titrable acidity (g of tartaric acid L^{-1})	0.779 ns	0.743 ns	0.747 ns
malate (g L^{-1})	0.779 ns	0.871 *	0.860 *
tartrate (g L^{-1})	–0.845 *	–0.937 **	–0.910 *
ripening speed	–0.973 ***	–0.977 ***	–0.992 ***
anthocyanins (mg kg^{-1})	–0.811 *	–0.666 ns	–0.750 ns
total phenolics index	–0.821 *	–0.636 ns	–0.729 ns

[a] *, **, and *** represent significance at $p < 0.05$, 0.01, and 0.001, respectively; ns, not significant.

size at harvest, which seems to be the case in our experiment as water stress did not become severe until after veraison and only in plot H (Ψ_d values > -0.6 MPa prior to veraison).

Berry Composition through Ripening. *Must Characteristics.* Must sugar content was highest on plot H (**Figure 2**) even though titrable acidity levels on plots H and P were similar. Must from plot A exhibited the lowest sugar content and the highest total acidity. These results are in general agreement with previous reports (*41*) of grapes from water-stressed vines having higher concentrations of soluble solids and lower titrable acidity at harvest. However, because malic acid is the main acid contributing to the decrease in acidity and its breakdown is primarily temperature dependent, the climate factors might explain in part the lower acidities on plot A. For the three sites and the two vintages ($n = 6$), a strong linear correlation was observed ($r = -0.925$, $p = 0.01$) between sugar content at harvest and the intensity of water deficit (average Ψ_d from veraison through harvest), whereas no correlation was found with the earliness of water deficit (average Ψ_d preveraison) (**Table 4**). Titrable acidity in musts at harvest was poorly correlated to water conditions, which agrees with previous results (*39*). Titrable acidity was closely related to malic acid concentration ($r = 0.945$, $p = 0.001$; $n = 30$) but not to tartaric acid concentration ($r = 0.013$, ns; $n = 30$), a fact that has also been reported for other cultivars (*4*). Similarly to the results reported by previous workers (*8*, *9*), malic acid decrease was enhanced by water deficits during the maturation period ($r = 0.871$, $p = 0.05$). Higher malic acid degradation in water-stressed vines has been attributed to the reduction of canopy density and, therefore, cluster shading by leaves, resulting in higher temperatures in the cluster zone (*45*). On the contrary, tartaric acid showed higher values at harvest in water-stressed vines and was affected by both the earliness ($r = -0.845$, $p = 0.05$) and extent ($r = -0.937$, $p = 0.01$) of water deficit. Similar results have been reported for Tempranillo in an irrigation experiment (*5*). The most highly significant correlation was established between water deficit and ripening speed of the pulp ($r = -0.988$, $p = 0.001$), suggesting that water deficit clearly accelerated the ripening process. Contrary to our findings, Sipiora and Gutierrez Granda (*46*) presented evidence that severe water stress, imposed by irrigation cutoff prior to veraison, delays fruit maturity. These authors reported midday values of leaf water potential close to -1.6 MPa immediately after veraison for the cutoff treatments. It is well documented that the period of rapid sugar accumulation at the onset of veraison is particularly sensitive to water deficit (*47*). Our results indicate that such values for midday Ψ were not reached until just before harvest, and only in 1998 (data not shown). Furthermore, previous research has reported a tendency for accelerated ripening following irrigation cutback toward harvest (*41*). As photosynthesis and translocation are impaired by water deficits, the acceleration of the ripening process under mild water deficit is probably related to the indirect effects of water stress such as reduced vegetative growth and favorable canopy microclimate. In other field experiments (*48*), the advanced maturation speed was associated with a reduction in berry size. However, as already exposed, water stress did not coincide with a reduction in berry size under the conditions of our experiment.

Phenolic Compounds in Berry Skins. Over the ripening period, the increase in the concentration of anthocyanins and total phenolics in skins was not continuous in most cases as it was followed by a stabilization or decrease just before harvest. Similarly, Sommers (*49*) found a decrease in the anthocyanin concentration in ripening grapes starting 30 days after veraison. Values were generally higher in all plots in 1998 than in 1997, which might be associated with the overall better ripening conditions in 1998.

Berries on plot H with the highest water deficit had the highest anthocyanin content and total phenolics index through ripening in both years, except for a decline near harvest in 1997 (**Figure 3**). Many studies indicate the positive impact of moderate water deficit stress on phenolic compound concentration in grape berries. Yet, the increase in anthocyanin concentration under water deficit is seldom caused by higher levels of synthesis but appears more often to be a consequence of smaller berry size of the stressed vines (*12*, *50*). However, according to the findings of other workers (*51*), increased anthocyanin concentration under water deficit stress could not be attributed only to berry size. Our results support that view, as berry weight was poorly correlated to water supply in the conditions of our experiment. The higher concentration of phenolic compounds in the grapes of water-stressed vines (plot H) might be the result of either better cluster microclimate (*52*) or favorable accumulation conditions due to reduced intraplant competition for assimilates (*15*). It can also be hypothesized that, in the case of late-maturing varieties, such as Agiorgitiko in Nemea, earliness in phenology due to water stress may improve overall winegrape composition (*53*).

When regressed on Ψ_d averages (**Table 4**), anthocyanin content and total phenolics index in skins were strongly correlated to early water deficits ($r = -0.811$, $p = 0.05$; and $r = -0.821$, $p = 0.05$, respectively) but not to the intensity of water stress ($r = -0.666$, ns; and $r = -0.636$, ns, respectively), contrary to total soluble solids, which were mostly affected by the intensity of water stress. Ojeda et al. (*13*) reported an inhibition of phenolic biosynthesis when intense water deficits were applied during the first phase of berry growth (preveraison), defining a threshold value of a predawn leaf water potential of -0.6 MPa. In our experiment, Ψ_d did not exceed this value until after veraison and only in plot H; therefore, it can be assumed that water deficit stress always remained moderate prior to veraison.

A close positive relationship was observed between total soluble solids in the grape juice and phenolic compounds in the skins. The correlations relating total soluble solids and both skin anthocyanins and skin total phenolics, all sampling dates, plots, and years considered together ($n = 30$), were highly significant ($r = 0.662$, $p = 0.001$, for anthocyanins; and $r =$

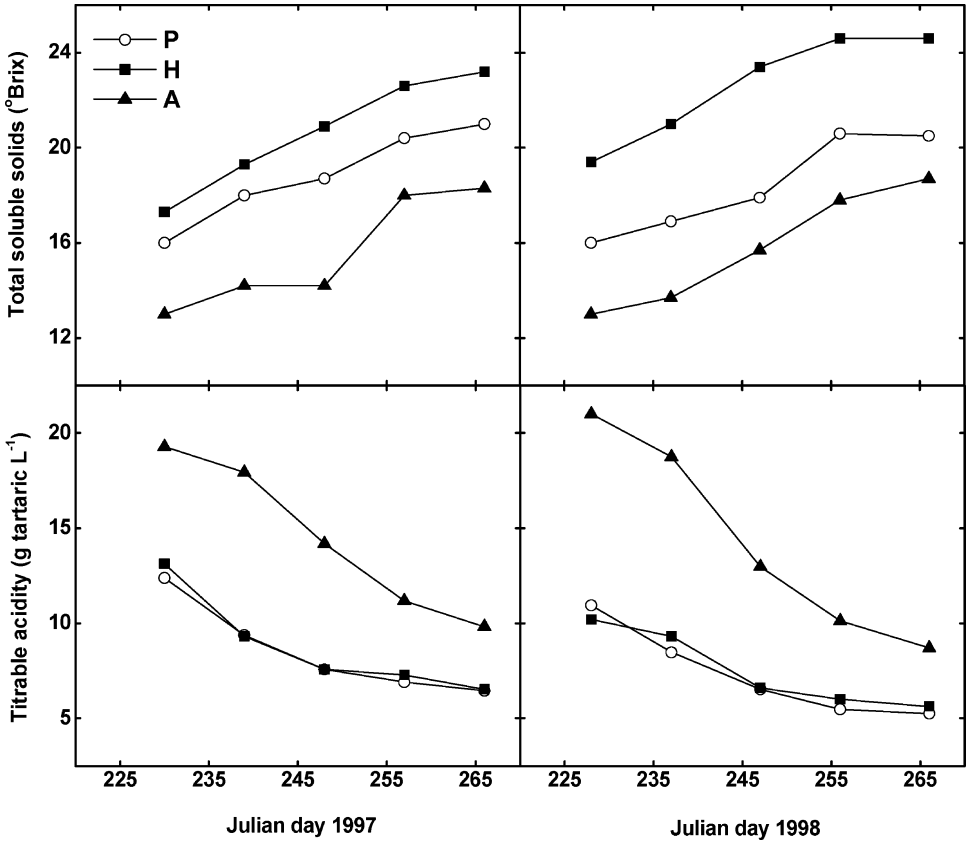

Figure 2. Influence of site on must soluble solids and total acidity evolution during ripening in 1997 and 1998.

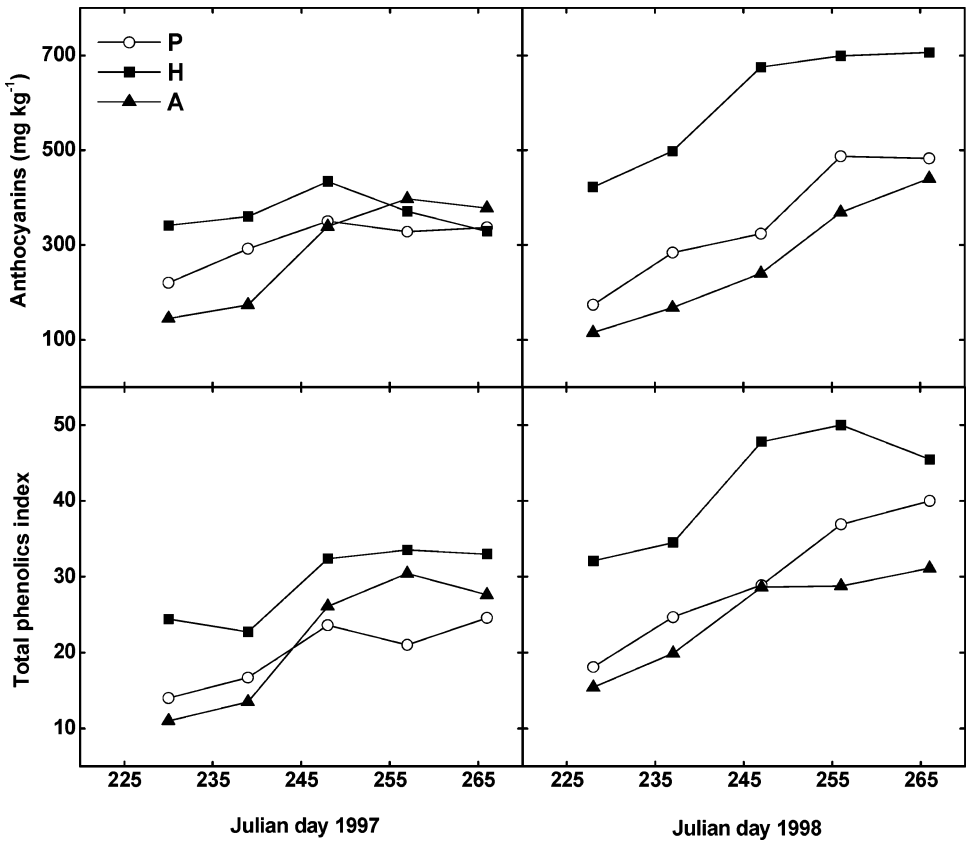

Figure 3. Influence of site on skin anthocyanin and total phenolics evolution during ripening in 1997 and 1998.

0.662, $p = 0.001$, for total phenolics index). Previous research found that higher levels of soluble carbohydrates in berries during ripening contribute to better polyphenol synthesis (*54*).

Wine Composition and Quality. *Phenolic Compounds.* Wines from plot H exhibited in both vintages the highest alcohol and phenolic contents (**Table 5**). The intensity values for plot

Table 5. Influence of Site on Wine Composition

	1997			1998		
parameter	P	H	A	P	H	A
alcohol (% vol)	11.7 a[a]	13.8 b	11.3 a	12.6 b	15.1 c	12.0 a
titrable acidity (g of tartaric acid L^{-1})	4.7 a	5.5 b	7.5 c	5.8 a	5.8 a	7.6 b
anthocyanins (mg L^{-1})	335 b	445 c	296 a	542 a	633 b	526 a
tannins (g L^{-1})	2.15 a	3.00 b	2.01 a	2.46 b	4.88 c	2.03 a
total phenolics index	44.2 a	56.8 b	39.4 a	49.0 a	86.7 b	44.1 a
color intensity	3.6 a	6.7 c	5.1 b	8.8 a	15.0 b	8.3 a

[a] Statistically significant differences by Newman–Keuls test (0.05) are indicated by different letters.

H wines were also significantly higher than those of the other studied plots, in both vintages, which is normal as anthocyanins are the components that make the highest contribution to color intensity (520 nm optical density). Especially in 1998, total phenolics index, tannins, and color intensity were almost 2-fold higher in plot H wines than for plots P and A wines. Plot A wines had the lowest phenolic contents in both years, although values did not always differ significantly from those of plot P wines. Greater color intensity in plot A wines was observed in 1997, probably because of a higher degree of ionization of anthocyanins due to its elevated acidity. All values were higher in 1998 than in 1997, probably as a result of higher grape maturity, due to a more pronounced water deficit in all sites.

Wine phenolic content correlated closely with grape skin phenolic content ($r = 0.759$, $p = 0.05$, for anthocyanins; and $r = 0.710$, $p = 0.05$, for total phenolics index). Because skin contact time was the same for all vinifications and the skin-to-must ratio (a consequence of berry size) had little influence on the extraction of anthocyanins and other phenolic compounds in wines, wine phenolic content and color were a direct consequence of higher levels and possibly higher extractability of grape skin phenolics. Alcoholic degree of wines was also a direct consequence of must sugar concentration ($r = 0.783$, $p = 0.05$).

Glycoconjugated Aroma Compounds. The volatiles isolated from the enzyme hydrolysates are listed in **Table 6**, classified as major groups according to their chemical similarity and biosynthetic pathway. Wine-making parameters (such as ethanol or skin cell wall thickness) can also affect the extraction of glycocnojugates, thus altering wine composition. The present study should therefore be regarded as an initial approach to improve our knowledge about the Agiorgitiko varietal aroma and to understand the influence of site-related factors on the aromatic constitution of its wines. Furthermore, although analysis of grapes can minimize the influence of extraction parameters, wine samples are produced from a larger amount of grapes and better reflect vineyard and winery conditions.

The aromatic compound constitution of wines differed only slightly between plots or vintages. The concentration of free volatiles was very low in comparison to the hydrolysates (data not shown). In red varieties, examination of the glycosidically bound fraction is expected to give a more complete indication of berry aroma potential (*55*).

Among the chemicals studied, the hexenols and hexanol are considered to be responsible for the herbaceous aroma of wines with a threshold of 2 mg/L (*21*). Plot H wines had the highest concentrations of these compounds, although without direct influence on flavor. The sum of bound 2-phenylethanol and benzyl alcohol followed different trends between the two years of study. Glycosides of aromatic alcohols were greater in plot P in 1997 and in plot H in 1998. In all cases, bound benzyl alcohol was more abundant than bound phenyl ethanol, which coincides with the results for the variety Syrah (*18*).

Volatile phenols originate from the shikimic acid pathway passing through a phenylalanine step, like the soluble phenolic compounds examined previously. Terpenols and C13-norisoprenoids are both products of carotenoid degradation. In a previous work, >100 volatile compounds were identified in Agiorgitiko grapes submitted to carbon dioxide atmosphere, with a higher contribution of volatile phenols and norisoprenoids (*56*). These groups of grape-derived volatile compounds were also found to be predominant in Syrah (*17*) and Cabernet Sauvignon (*57*) and thus could be responsible for the typical aroma of many red winegrape varieties. In our experiment, the total amount of bound volatile phenols was higher in both years in wines of plot H, more clearly in 1998. With regard to terpenols, Agiorgitiko is a neutral type of variety with contents much lower than the sensory threshold values reported for Muscat grape varieties (*58*). However, the general trend was that the plot H wines presented the highest levels of terpenyl glucoconjugates in both vintages. Likewise, the total amount of bound norisoprenoids was clearly higher in plot H wines in 1998, whereas no differentiation was observed in 1997.

It has been reported that higher maturity levels correspond usually to higher levels of bound terpenols and C13-norisoprenoids (*59*). With regard to vine water status effects on berry and wine aroma potential, previous works have reported contrasting results. McCarthy and Coombe (*60*) found a significantly lower rate of accumulation of bound terpenes in ripening Riesling grapes under irrigation, whereas Escalona et al. (*25*) reported a positive relationship between irrigation and aroma potential at harvest in Tempranillo. Under the conditions of our experiment, limited water availability was found to improve the aroma and flavor of Agiorgitiko wines, especially during the driest vintage. It is possible that the higher levels observed under limited water supply are related to higher cluster exposure due to reduced vine vigor. Modification of canopy microclimate has been shown to affect the levels of grape glycoconjugates in several cultivars (*15*, *18*, *19*). Recent studies have indicated that soil characteristics and water supply have a large influence on the degradation of carotenoid contents of grapes, thereby possibly affecting the presence of substances with a high aroma impact at harvest, such as norisoprenoids (*61*). Previous research has also suggested that glycoside accumulation is independent of sugar translocation (*15*). Our results show that an increase in secondary metabolites accompanies an increase in must soluble solids.

The total contents of glycosides in wines were also shown to depend on year, a fact that is in agreement with previous workers (*18*, *23*) who found a strong year-to-year variation in the aroma potential of other winegrape varieties. Comparing the sum of glycosides of the main groups of aroma compounds between years, glycoconjugates were always higher in 1998 than in 1997. The vintage effect was particularly intense on glycosides of volatile phenols, monoterpenes, and norisoprenoids, which in most cases increased by >50% in 1998 over 1997, a fact that could explain the better sensorial properties of the 1998 wines (**Figure 4**).

Sensory Evaluation of Wines. A strong relationship was found between mean Ψ_d for the period from flowering to harvest and the global appreciation note given to the experimental wines (**Figure 4**). The negative regression denotes that the decrease in Ψ_d (thus the increase in vine water deficit) is translated in a higher global quality of wines. The site that produced the best wines in both years was plot H followed by plots P and A, the

Table 6. Influence of Site on the Glycoconjugated Aroma Contents of Agiorgitiko Wines in 1997 and 1998 (Amounts in Micrograms per Liter of Wine)

	1997			1998		
compound	P	H	A	P	H	A
C6 compounds						
1-hexanol	138	164	99	116	238	132
(*Z*)-3-hexen-1-ol	56	64	57	102	113	143
(*E*)-2-hexen-1-ol	64	114	39	28	96	101
total	**258**	**342**	**195**	**246**	**447**	**376**
alcohols						
benzyl alcohol	545	207	272	1058	1524	744
2-phenyl ethanol	185	173	154	269	453	248
total	**747**	**426**	**474**	**1395**	**2034**	**1087**
volatile phenols						
methyl salicylate	6	2	5	12	27	16
benzoic acid	5	9	4	5	5	7
4-methylphenol	2	2	7	1	2	
dimethoxyphenol isomer				11	5	1
4-vinylguaiacol		17	1		49	
3-methoxybenzenemethanol	2		3	7	6	3
vanillin	6	8	3	7	16	2
acetovanillone	10	2	9	10	5	16
3,4-dimethoxyphenol	2	1		8	7	6
zingerone	7	6	4	8	14	9
butyrovanillone	1	1		2	6	
homovanillyl alcohol isomer	3	7	2	24	10	25
cis-dihydroconiferyl alcohol	4	4	3	3	10	3
4-hydroxybenzaldehyde	14	17	6	27	10	7
3,4,5-trimethoxyphenol	13	5	5	77	65	98
syringaldehyde	4	14	2	20	24	22
3,5-dimethoxybenzenemethanol	4	4	2	2	30	5
total	**85**	**102**	**57**	**224**	**299**	**220**
terpenols						
trans-furan-linalool oxide	4	5	2	2	5	3
geraniol	12	21	4	33	55	25
(*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol	16	21	16	16	52	35
(*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol	13	21	10	17	35	16
7-hydroxyterpineol	24	60	15	54	136	72
2,6-dimethyl-2,6-octadiene-1,8-diol isomer		5			8	
2,2,6-trimethyl-6-vinyltetrahydropyran	6	7	6	4	8	3
3,7-dimethyl-1,5-octadiene-3,7-diol (terpenediol 1)	12	23	13	25	73	30
3,7-dimethyl-1,5-octadiene-3,7-diol (terpenediol 2)	4	7			8	
total	**91**	**170**	**66**	**151**	**380**	**184**
C13-norisoprenoids						
3,4-dihydro-3-oxo-actinidol (isomer 1)	1	1	1	2	4	2
3,4-dihydro-3-oxo-actinidol (isomer 2)	3	5	2	6	12	5
3,4-dihydro-3-oxo-actinidol (isomer 3)	2	2	2	3	5	3
3-hydroxy-β-damascone	2	6	5	12	37	2
3,4-dehydro-7,8-dihydro-β-ionone	2	3	2	6	13	3
3-oxo-α-ionol (isomer 1)	5	5	4	8	13	9
3-oxo-α-ionol (isomer 2)	4	6	2	3	9	3
3-hydroxy-7,8-dihydro-β-ionol (isomer 1)	12	23	9	25	66	36
3-hydroxy-7,8-dihydro-β-ionol (isomer 2)	2	3	2	5	14	6
3-hydroxy-5,6-epoxymegastigm-7-ene-9-one	4	3	2	6	13	6
3-hydroxy-7,8-didehydro-β-ionol	13	16	7	22	35	23
grasshopper ketone	66	22	40	103	301	142
vomifoliol	28	17	22	34	105	15
total	**144**	**112**	**100**	**240**	**633**	**259**

latter being the least typical for a Nemea Appellation red wine. Wine sensory scores were also higher in 1998 than in 1997 for all three sites, presumably due to the drier climatic conditions of 1998. Statistical analysis showed a highly significant year and site effect on wine tasting note (data not shown). When the three sites and two years were considered together, there was a marked reduction in global wine score, being higher in plot H in 1998 and lower in plot A in 1997 (H98 > H97 = P98 > P97 > A98 = A97). These observations coincide with the higher levels of phenolic compounds and aroma glycoconjugates in wines produced from plot H, especially in 1998.

The results of the above two-year terroir experiment clearly demonstrate that deficit water status imposed by soil and climate parameters was linked to high enological potential for red grape variety Agiorgitiko. Furthermore, the influence of water conditions on grape quality seems to be mostly linked to the limiting effect of low water uptake on vine vigor rather than to a reduction in berry weight. Low vine water status induced early shoot growth cessation and accelerated the ripening process, resulting in higher sugar in the must and higher berry and wine phenolics. Analysis of bound volatile components in wines suggested that low water uptake had a positive effect on the aromatic potential of grapes. This positive effect on wine quality was confirmed by sensory evaluation. The higher global quality of grapes on the hill slope in both years may reflect advanced physiological maturity due to limited water supply. Further investigations will be necessary to define more accurately the dependence of grape volatile secondary metabolites on individual environmental and viticultural parameters, under controlled conditions. Finally, it remains to be elucidated in the

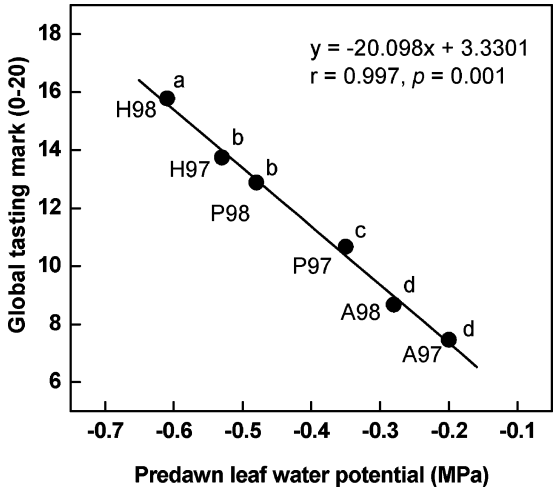

Figure 4. Relationship between the average predawn leaf water potential from set through harvest and wine sensory evaluation. Data labels refer to three sites (P, H, A) and two seasons (1997 and 1998). Statistically significant differences by Newman–Keuls test (0.05) are indicated by different letters, all years and sites considered together.

future whether the effects of soil and climate on fruit composition and wine quality are mostly mediated through their influence on vine water status or if certain site parameters such as temperature daily and seasonal variation, heat summation, air saturation deficit, or nitrogen nutrition exert also an independent role on berry ripening.

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Received for review February 24, 2006. Revised manuscript received May 12, 2006. Accepted May 15, 2006.

JF0605446