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Ambivalence of the influence of nitrogen supply on o-aminoacetophenone in 'Riesling' wine

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Summary

AAP (o-aminoacetophenone) is the aroma substance responsible for the untypical ageing off-flavour (UTA). The impact of nitrogen supply on the formation of AAP was investigated between 1994 and 1999. The experiment was carried out in the Rheingau (Germany) with six fertigation treatments of annual quantities of N (0, 30, 60, 90, 150 kg·N·ha^{-1}). Results indicated that the long-term varied N fertilization affected AAP concentration in wine as much as the year. Whereas a better N supply of the grapes due to effects of the year coincided with lower AAP values, the fertilization effect was reversal: higher N fertilization increased the concentrations of AAP. AAP did not correlate with its precursor IAA and only slightly with antioxidative capacity. Neither varying yield nor soluble solids could explain the high variance of AAP. An indicator for potential AAP formation could not be found, neither in must nor in wine.

K e y w o r d s : N fertilization, untypical ageing, o-aminoacetophenone, indole-3-acetic-acid, antioxidative capacity.

A b b r e v i a t i o n s : untypical ageing (UTA), o-aminoacetophenone (AAP), indole-3-acetic-acid (IAA), water soluble antioxidative capacity (ACW), total soluble solid (TSS), titritable acid (TA).

Introduction

In the early nineties, the untypical ageing off-flavour (UTA) was first observed in German wines. Within a short time, young wines developed a bouquet characterized by odour taints like acacia blossom or naphthalene. UTA has also occurred in other European countries (Rapp and Versini 1995, Gessner *et al*. 1998). More than ten years ago, o-aminoacetophenone (AAP) was identified as the characteristic compound with an odour threshold of about 0.5 to 1 µg l^{-1} in wine (Rapp *et al*. 1993). More than five years ago, investigations on the biochemical mechanisms of AAP formation in wine showed that the oxidative degradation of indole-3-acetic acid (IAA) triggered by sulfurylation leads to the formation of AAP. An addition of antioxidants can inhibit this reaction (Christoph *et al*. 1998, Gessner *et al*. 1998). Oenological treatments can neither cause nor inhibit UTA (Jakob 1993, Rapp and Versini 1995, Köhler 2000). The only oenological method known so far, the addition of ascorbic acid, can only delay or reduce but not safely prevent the formation of UTA (Gessner *et al*. 1999). According to empirical studies, UTA is caused by various stress reactions of the vine (Jakob 1993, Löhnertz 1996). The first investigations on the importance of different stress factors focussed on drought, high yield and reduced fertilization (Pohl 1992, Jakob 1993). Early harvest has been considered as the most important negative influence (Wohlfahrt 1993, 1995, Miltenberger *et al*. 1993, Köhler *et al*. 1995, Schwab *et al*. 1996, 1999). High yield was reported to be one stress factor (Löhnertz 1996, Schwab *et al*. 2001, Müller 2002); or the main factor (Walg 2003) causing UTA, but this was not clearly confirmed by experiments. The inconsistency was attributed to low yield (Schwab and Peternel 2001) or optimum water and nutrient supply (Rapp and Versini 1995). In wines made from grapes grown with permanent green cover UTA occured more often (Wohlfahrt 1995, Schwab *et al*. 1996, Schwab 1998, Seiter 2000, Löhnertz *et al*. 2002). Dry weather has a negative influence (Schwab *et al*. 1996, Sponholz *et al*. 1997), but water deficiency always leads to nutrient deficiency, because there is no medium to transport nutrients. Since the eighties, the number of vineyards with green cover has been increasing; concurrently, recommended N fertilization has been reduced. Both factors have resulted in an extremely reduced nitrogen supply in German vineyards. Therefore, nitrogen deficiency has been considered as a factor causing UTA, as evidenced by negative correlations between N in must and UTA in wine (Gessner *et al*. 1995). N fertilization generally reduces UTA (Schwab *et al*. 1996), although, in a 15-year N fertilization experiment, Müller (1999) did not find differences in the occurence of UTA in wines. Seiter (2000), however, observed that N supply inhibited the formation of UTA, but the study did not include field replicates. The report study mentions, that the effect of N supply on the formation of AAP was not obvious. After a period of intense research, few published studies have dealt with viticultural aetiology, although the problem is still of great economic importance for German wine production. In 2004, UTA was the most important cause of rejection of wine certifications in Baden (Krebs and Bärmann 2005). Our study evaluated the extent that nitrogen supply to vines during various years is responsible for the formation of AAP, and whether N fertilization prevent formation by affecting antioxidant levels, or the precursor, IAA. It also examined whether the precursor or other substances can be used as indicators of UTA risk in wine.

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Material and Methods

Field experiment: The experiment was carried out since 1985 in the Rheingau, Germany, in a vineyard fertilized with annual quantities of N at 0, 30, 60, 90 and 150 kg·N·ha^{-1}. In 1977 *Vitis vinifera* 'Riesling' vines grafted on 5C were planted (one plant per 2.6 m^2) with permanent green cover in every second row. The soil was loamy sand containing 1.4 % humus and with a pH of 7.6. Vineyard details were described by LINSENMEIER *et al*. (2006). Each treatment was replicated four times and arranged in a completely randomised design. Weather conditions, harvest date, yield, amount of *Botrytis* and must composition see Tabs 1 and 2. In 1999 at veraison a thinning was carried out, so that no yield data are available. Grapes from each replication were fermented in 10-l glass flasks. The yeast strain used was "Champagne Epernay Geisenheim". Must and wine samples were stored at -20 °C. Bottled wines were stored at 14 °C.

Determination of free and total IAA: The method to determine free and total IAA using HPLC-FLD (HOENICKE *et al*. 2001) was slightly modified as described in LINSENMEIER *et al*. (2004). For solid-phase extraction of the sample, an anion exchange material (SAX) was used. Total (free and bound) IAA was analysed after alkaline hydrolysis and solid-phase extraction using prior RP18 and then a SAX. A HP 1090 chromatograph with a RP-18 column (Merck LiChrospher 5 µm, 250 x 3 mm) was used for the subsequent HPLC-FLD analysis. Solvents used were: 0.1 % TFA (solvent A), acetonitrile (solvent B). The fluorescence detector was a HP 1046A with an excitation of 255 nm and an emission of 360 nm.

Determination of arginine: Arginine (Arg) and other amino acids were measured using a HPLC system (Spectraphysics) according to PRIOR (1997). Samples were extracted with sulfosalicylic acid and derivatised with dansyl chloride. Chromatographic separation was carried out on an RP-18 column (Merck LiChrospher 5 µm, 250 x 3 mm) using a ternary gradient and the following solvents, A: 2 l bidestilled water, 7 ml acetic acid, 160 µl triethylamine; B: methanol; C: acetonitrile. For detection, a fluorescence detector (Jasco 820 FP) was used with an excitation of 298 nm and an emission of 546 nm.

Antioxidative potential: The antioxidative potential of the frozen wines from the years 1998 and 1999 was determined in 2004 by means of the Photochem® Analyser (AnalytikJena). In the sample, superoxide radicals are produced, which react with the antioxidants. Free radicals react with a chemoluminescent substance; by means of the luminescence created, antioxidants are quantified as sum parameters. Water soluble antioxidative capacity (ACW) was determined as ascorbic acid equivalent. Lipid soluble antioxidative capacity (ACL) was determined as trolox equivalent.

Determination of o-aminoacetophenone: The aged bottled wines were extracted and analysed with pentane/dichlormethan (2:1). After concentrating the extract with a vigreux column, AAP was analysed by GC-MS using a Siemens Sichromat 2 with a DB-wax column (RAUHUT and KÜRBEL 2002, unpublished method). The vintages 1994 to 1998 were analysed in autumn-winter 2001/02, the vintage 1999 was analysed in winter 2002/03. Wines of the vintage 1995 of 0 and 150 kg·N·ha^{-1}-treatment were only available in one of the four field replicates. Apart from 1995, all replicates were examined.

Statistical analysis: An ANOVA was carried out using Fischer´s test to detect treatment and vintage effects on vine performance, must components and wine AAP. Significant differences (α = 5 %) between

Table 1

Development stage, meteorological data (German Meteorological Service, Geisenheim) and soil moisture measured as mm available field capacity (AFC) between veraison and 60 °Oe

	1994	1995	1996	1997	1998	1999
Bloom	22. Jun	28. Jun	25. Jun	16. Jun	20. Jun	17. Jun
Veraison	16. Aug	20. Aug	31. Aug	26. Aug	19. Aug	18. Aug
Harvest	11. Oct	20. Oct	31. Oct	22. Oct	26. Oct	11. Oct
Mean temperature (°C)	11.6	10.7	9.2	10.5	10.6	11.1
Sum of precipitation (mm·year^{-1})	428.5	681.8	472.5	438.4	587.3	558.7
mm AFC (0-90 cm)	29	47	50	86	47	44

Table 2

Botrytis infection, yield and must composition during 1994-1999 (*: data missing, yield was estimated according average riesling yield in Rheingau 1999). Significant differences between years are indicated by different letters (α < 5 %)

	1994	1995	1996	1997	1998	1999
Botrytis	low	high	low	low	high	very high
Yield (kg·ar^{-1})	84 b	49 a	91 c	148 d	80 b	160*
TSS (°Oe)	78 a	83 c	87 d	87 d	88 d	82 b
TA (g·l^{-1})	11.7 c	13.7 d	11.9 c	10.5 b	10.3 b	8.8 a
Arg (mg·N·l^{-1})	25 a	78 b	229 d	38 a	110 c	28 a

means are indicated by different letters. With total data a two-way anova was carried out, significant differences between the N-treatments in the vintages were tested with one-way anova. The portion of variance is calculated by dividing the sum of squares of the effect from the total sum of squares. Linear regression were performed and assured with $\alpha = 5$ %.

Results

Quality parameters in must: Must components including minerals, Arg, total soluble solids (TSS), titritable acid (TA) were affected by N fertilization (Tab. 3). With increasing fertilization there were increases in yield, leaf area and pruning yield. Vintage year was partly confounded with the effect of fertilization. This is especially true for must Arg. Whereas fertilization accounted for 7 % of the variance of Arg concentration, vintage accounted for 84 %. The highest Arg storage in the grapes was found in 1996, but it was also elevated in 1995 and 1998. Within an individual year, fertilization accounted for 45 to 75 % of the Arg variance. On average, N fertilization increased Arg concentration continuously up to the double of the content found in the zero treatment. The response of must mineral concentration to N fertilization varied. Whereas must P considerably decreased with increasing applied N, must K slightly increased. TSS and TA decreased with applied N, but this effect was not observed in all years.

AAP concentration: N fertilization increased AAP concentration in wine in all years but 1999 (Fig. 1). The effects of vintage year and fertilization were highly significant but they only explained 20 % and 17 % respectively of the AAP variance. The residual variance was very high. Over all, the highest AAP concentrations were observed in 1999. In other years, N fertilization explained 16 to 35 % of the AAP variance. Compared to when no N was applied, N fertilization of 60 kg·ha^{-1} increased AAP concentration by 45 %, whereas fertilization with 150 kg·ha^{-1} increased AAP by 125 %.

Regression analyses: Yield and TSS were unrelated to AAP formation. Over all vintages, yield did not correlate with AAP concentration in wine (Fig. 2, Tab. 4). In 1996, low AAP levels correlated positively with yield. In 1995, however, the lowest yield and the highest AAP concentration were found in the wine produced from the highly fertilized vines, resulting in a negative correlation between yield and AAP content. Considering all years, a higher TSS was linked with a slight tendency towards lower AAP concentrations. Within the vintages 1995 and 1999, however, a positive regression coefficient was found. IAA concentration in wine did not correlate with AAP formation (Fig. 2, Tab. 4), regardless of whether single vintages or all years were examined. We found tendency towards negative correlations between water soluble antioxidative capacity (ACW) and AAP. In the years 1998 and 1999, when ACW was measured, it accounted for less than 10 % of the AAP variance. Even in a multiple regression of IAA and the antioxidants on AAP, no better correlation was found. Over all data, Arg correlated negatively with AAP, whereas positive correlations were found within individual vintages. A similar contradiction was observed for total IAA in must and AAP in wine. Considering all years, a highly significant negative correlation was found. Within the vintages, the coefficient of determination was lower and not significant; moreover, a positive correlation was

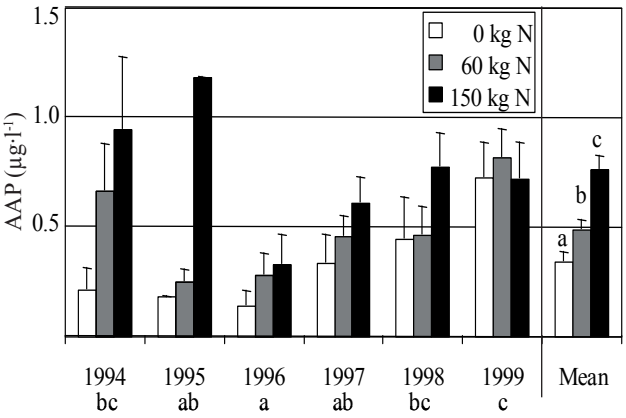

Fig. 1: AAP concentrations in wine (µg·l^{-1}) as related to long-term nitrogen fertilization with 0, 60 and 150 kg·N·ha^{-1} (1994-1999). (1995 without replication in the 0 and 150 kg·N·ha^{-1}-treatment). Bars indicate standard error. Significant differences ($\alpha < 5$ %) are indicated by different letters.

Table 3

Influence of the N fertilization on must components and vine performance and portion of variance (PV) of the factors vintage and fertilization by two-way anova. Mean of the four replicates in the years 1994-1999 (must parameters), 1994-1998 (yield), 1997-1999 (pruning yield), only 1998 (leaf area). Significant differences between treatments are indicated by different letters. (*, **, ***: significant factor effect at $\alpha < 5$ %, 1 %, 0.1 %)

		Must parameters					Generative and vegetative performance		
		TSS (°Oe)	TA (g·l^{-1})	P (mg·l^{-1})	K (mg·l^{-1})	Arginine (mg·N·l^{-1})	Yield (kg·ar^{-1})	Leaf area/ leaf (cm^2)	Pruning yield (kg·ar^{-1})
	0	86.2 c	11.5 b	150 d	862 a	53 a	75.8 a	147.4 a	17.9 a
kg	30	84.1 ab	11.3 b	109 c	890 ab	78 b	91.8 b	177.5 ab	22.9 b
N·ha^{-1}	60	85.4 bc	11.2 ab	92 b	889 ab	84 b	91.5 b	182.0 b	24.3 b
	90	82.3 a	11.2 ab	79 a	919 b	101 c	90.3 b	176.5 ab	24.6 b
	150	84.6 bc	10.9 a	71 a	912 b	110 c	89.4 b	181.7 b	23.9 b
	Year (Y)	46.3***	86.6***	16.8***	82.9***	84.4***	83.2***	-	22.8***
PV	Fert. (F)	5.7*	1.4*	60.1***	1.1	6.5***	2.5***	31.1	26.1***
	Y x F	7.8	1.9	9.3***	2.4	2.3	2.4	-	2.6

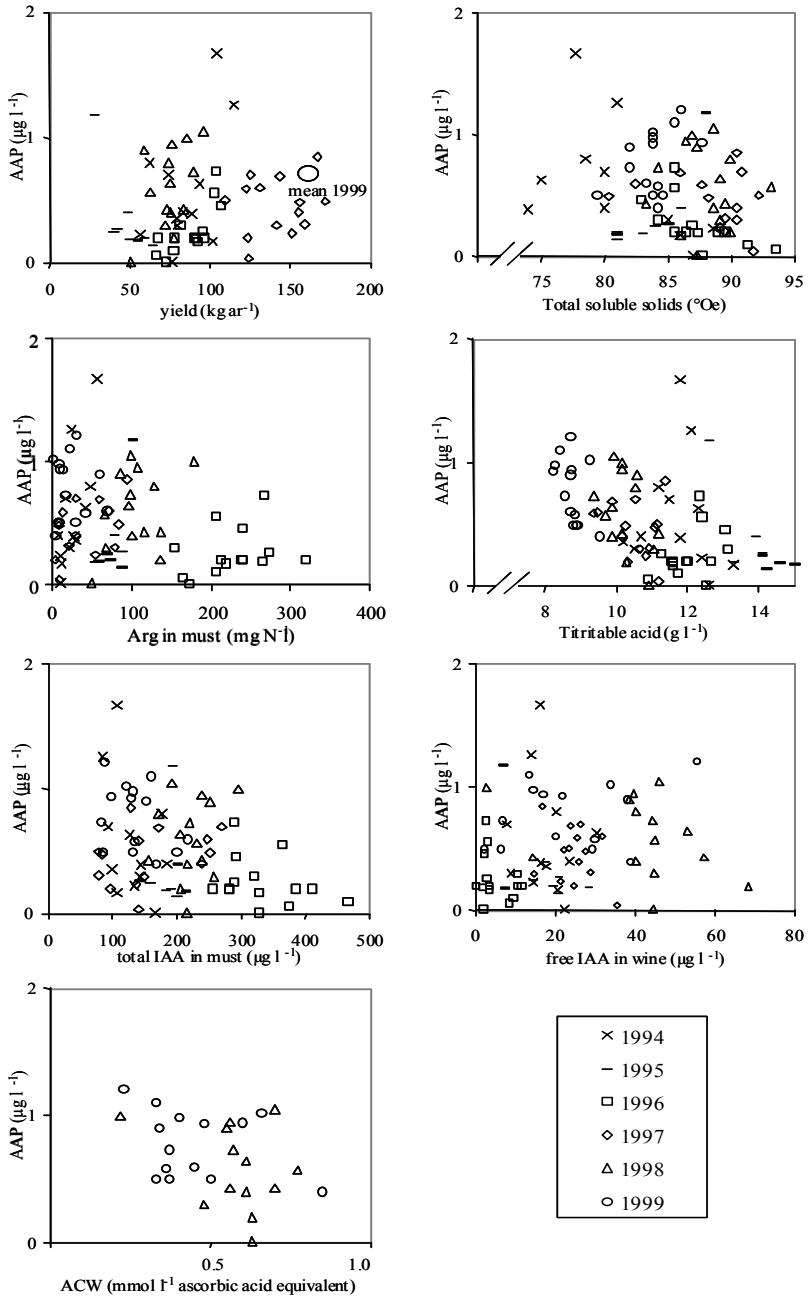

Fig. 2: Relation between yield, wine- and must-composition and resulting AAP concentration in wine (1994: n = 11, 1995: n = 8, 1996-1998: n = 14 each).

Table 4

Determination coefficient R^2 according to the linear regression with the data of all years (1994-1999, n = 76), only the single fertilization treatments (0, 60, 150 kg·N·ha^{-1}, 0; 150: n = 17 each, 60: n = 20) and only the single years (1994: n = 12, 1995: n = 8, 1996-1999: n = 14 each). AAP in wine as dependent parameter and the independent parameters yield (data of 1999 missing), Arg, TSS, TA, total must IAA, free wine IAA and ACW (only 1998-1999). Negative correlations were indicated with "-" ($^+$, *, **, ***: significant at α < 10 %, 5 %, 1 %, 0.1 %)

	All years	0 kg·N	60 kg·N	150 kg·N	1994	1995	1996	1997	1998	1999
Yield	0.02	< 0.01	0.06	< -0.01	0.26$^+$	-0.61*	0.56**	0.02	0.24$^+$	-
TSS	-0.04$^+$	-0.06	-0.02	-0.02	-0.10	0.65*	-0.36*	-0.07	-0.02	0.18
Arg	-0.06*	-0.10	-0.21*	-0.15$^+$	0.51**	0.43$^+$	0.08	0.19	0.14	< -0.01
TA	-0.19***	-0.40**	-0.21*	< 0.01	< -0.01	-0.60*	0.18	-0.06	-0.16	-0.23$^+$
Total must IAA	-0.11**	-0.11	-0.19*	-0.18$^+$	-0.16	0.01	-0.08	0.11	0.04	-0.11
Free wine IAA	0.02	0.08	-0.01	0.06	-0.02	-0.32	-0.07	-0.08	-0.15	0.09
ACW	-0.15$^+$	-0.03	-0.42	-0.11	-	-	-	-	-0.09	-0.09

found in half of the vintages. Furthermore, Fig. 2 shows that even very low concentrations of the precursor IAA - partly below 3 $\mu g \cdot l^{-1}$ - can lead to sensorially important AAP concentrations of up to one $\mu g\ l^{-1}$.

Discussion

This study dealing with the formation of the untypical ageing off-flavour was carried out in the six years between 1994 and 1999, a period in which the weather conditions varied extremely (Tab. 1). The use of a range of N fertilization treatments allowed us to compare the effect of a continuous increase of N fertilization to that of a better N supply due to seasonal influences. Increasing fertilization led to a continuously increasing storage of amino acids in the grapes (Linsenmeier *et al*. 2004). The Arg concentrations in must clearly illustrate the highly significant impact of N fertilization on N supply, which was exceeded by the impact of the year (Tab. 2, 3). Vines were well supplied with N in the years 1995, 1996 and 1998; whereas years 1994, 1997 and 1999 appear to be N-stress years. Extreme weather conditions were observed in 1994 and 1996. 1994 was the hottest and dryest year, with early harvest and low TSS (Tab. 1). 1996 was the only year to have temperatures below the mean of 30 years; harvest was late at high TSS.

The 1999 vintage differed from the other vintages. The vines were subjected to different cultivation measures (defoliation, thinning) and had the strongest botrytis infection with simultaneous acetic acid rot which triggered an early harvest. Thus, the impact of the vintage year resulted from several factors such as weather conditions, date of harvest, grape health and cultivation measures, which could not be seperated from each other.

On average, N fertilization increased AAP concentration in the wine. This observation contradicts former non replicated studies finding that N fertilization appeared to reduce the formation of UTA or had no impact on it (Schwab *et al*. 1998, Seiter 2000, Müller 1999). In the present trial N fertilization increased the formation of fruity esters, which could reduce the UTA off-flavour (Linsenmeier *et al*. 2005). Nevertheless AAP-concentration correlates with UTA intensity in aged wines (Linsenmeier *et al*. 2007).

Vines receiving no N were obviously stressed (weak growth, pale and small leaves, sluggish fermentations due to low amino acid contents in must). UTA is not necessarily caused by stress of the vines; on the contrary, in this study, the vines which were obviously more stressed had lower AAP concentrations. This result contradicts former studies, in which vine stress conditions triggered UTA formation (Jakob 1993, Müller 2000). Fertilization leads to changes in yield and ripeness which are considered to be the main factors causing UTA formation. These parameters, however, had no impact on the accumulation of AAP in our study. Many authors recommend harvest at the moment of "physiological ripeness" in order to avoid UTA (Schwab and Peternel 2001, Müller 2002, Rapp and Versini 2002). In the present study, the zero-N treatment indeed showed the highest TSS. Yet, TSS can not be used as unique quality parameter for ripeness; other parameters like amino acids and minerals have to be included (Schwab and Peternel 2001), and the low Arg concentrations in response to zero N indicate that physiological ripeness was not greater than that from the other treatments. Thus, in this study, physiological ripeness can not explain the N fertilization effect. Higher N supply caused by annual differences coincided with lower AAP formation. In 1996, the lowest AAP concentrations were found in the wine, in 1999 the highest. Taking into consideration the fertilization effect, we can conclude that this was not caused by higher N supply but by other variables that varied by the year (water relations, late harvest). Early harvest and low physiological ripeness in 1994 and 1999 can explain the effect of the vintage. Many authors state that UTA formation is increased by green cover (Wohlfahrt 1994, Schwab *et al*. 1996, Seiter 2000, Löhnertz *et al*. 2002). The present results indicate that this effect, as far as it concerns AAP accumulation, can be attributed to competition for water rather than nitrogen.

Drought is said to have an important impact on UTA formation (Pohl 1993, Rapp and Versini 1995, Löhnertz 1996, Müller 2002). According to Sponholz *et al*. (1997), water deficiency in the period between veraison and 60 ° Oe leads to UTA-vintages. Schwab *et al*. (1996) found a high UTA risk at a soil moisture of less than 30-40 mm available water capacity in sandy soil. In the present study, soil moisture was obviously above permanent wilting point. As water and nutrient deficiency are prerequisites of an effect of yield on UTA, this partly explains the absence of a yield effect. But the zero treatment did not show a yield effect either. The water relations in response to various N fertilization treatments may differ due to the stronger growth of vines and green cover at higher nitrogen fertilization. Vines under the highest fertilizer treatment, however, did not exhibit any water deficiency visually, which was confirmed by soil moisture. The effects low water relations can have without leading to veritable water stress conditions are of interest, but there has been very little research on this subject focussed on UTA. Further trials will have to evaluate the effect of water relations on UTA formation.

Hoenicke (2002) found an accumulation of 0.7 $\mu g \cdot l^{-1}$ AAP in spite of IAA values below the detection limit. On the other hand, Pour Nikfardjam *et al*. (2005) mention a IAA value of 50 $\mu g \cdot l^{-1}$ as critical for UTA risk. According to our results, however, it is impossible to determine IAA minimum values neither for AAP formation itself nor for the formation of AAP concentrations which are sensorily perceived. When wines were spiked with unnaturally high amounts of IAA, the AAP concentration increased in proportion (Christoph *et al*. 1998). In the present study, however, the IAA concentration in wine did not correlate with AAP. Furthermore IAA does not respond to N supply (Linsenmeier *et al*. 2004). Hoenicke (2002), however, found a consistent correlation of $R^2 = 0.5$. Yet, this result was possibly not only caused by substrate effects of IAA, but also by other hidden impacts, since the samples were obtained from different treatments (date of harvest, fermentation temperature, yeast strain). Early harvested grapes resulted in the highest AAP concentrations (at high free IAA contencentrations). In the sample harvested two

weeks earlier, however, lower free IAA contencentrations in wine coincided with lower AAP values. Early harvest probably had an effect on amino acids, minerals and phenoles, too. Due to the portions of variance not explained by regression, Hoenicke (2002) concluded that there had to be other factors influencing AAP formation.

Antioxidants are important for the prevention of UTA. White wines containing artificially high phenol concentrations and red wines are not bound to get UTA (Köhler *et al*. 1996, Sponholz *et al*. 1997, Schwab *et al*. 1999). The antioxidative effect of phenols or other scavengers reduces the accumulation of AAP from IAA after sulfurylation (Christoph *et al*. 1998, Gessner *et al*. 1998). Since AAP is formed by a pyrrole ring cleavage of IAA, superoxide radicals are required for this reaction (Hoenicke *et al*. 2002). The PCL method used in this experiment measures the activity of superoxide scavengers. It yields, at least theoretically, the antioxidative capacity relative to AAP formation. Yet, a relationship between water soluble antioxidative capacity (ACW) and AAP was only found in tendency. It has to be noted that ACW was determined after a storage of 4 to 5 years. Hoenicke *et al*. (2002) found a negative correlation of $R^2 = 0.38$ between the antioxidative capacity measured by means of an enzymatic method and the UTA intensity found in the wine. Measurements using the PCL resulted in a lower determination coefficient. A regression between antioxidative capacity and AAP concentration was not reported.

In this study, the antioxidative potential in wine was reduced by N fertilization (Linsenmeier *et al*. 2007). This finding is supported by the observation that N deficiency causes vines (and many other plants) to store increasing amounts of phenoles in the fruits due to stress reactions (Feucht and Treutter 1989, Löhnertz *et al*. 2002, Hilbert *et al*. 2003). The influence of viticultural management on the antioxidative potential in grapes and wines has not yet been sufficiently examined in spite of its importance for the UTA problem. We examined several parameters to determine if they could be used as indicators of UTA formation. These parameters do not directly impact AAP formation, but could work as early indicators for diagnosis. Generally, amino acids, individually or as sum, cannot be used for this purpose. As the most important amino acid in grapes, Arg correlates consistently with the total amino acid concentration. In comparison to free IAA, Arg was better correlated with AAP.

The overall negative correlation was caused by variation among years: in the years exhibiting high amino acid concentrations in the must, less AAP was found in the wine. The positive correlation within years reflects a reverse effect of N fertilization. This contradiction is due to the statistical problems of the regression of the total data. Data found in different years are not homogenous. Arg concentrations from the vintages 1998 and 1999 as well as from other years scarcely coincide. Conducting a regression of data from all years can miss hidden causal relationships and erroneously establish a correlation with Arg. This is generally true for non-homogeneous data, and thus also for the relationship between total IAA in must and AAP in wine. This highly significant negative correlation was caused by the effect of the year, too. The conditions of the storage of total IAA in the grapes are similar to those of AA, resulting in a strong correlation (Linsenmeier *et al*. 2004). Consequently, the concentration of total IAA in must cannot be used as an indicator.

Due to high negative correlations between N concentrations in must and AAP, N concentration has been considered as an indicator for UTA risk (Gessner *et al*. 1995, Sponholz *et al*. 2001). According to the present results, parameters concerning the N concentration in must (N concentration, amino acid concentration, ferm-N value) should not be used as indicators for AAP accumulation.

Conclusion

So far, the viticultural causes of UTA formation have not been explained sufficiently. Whereas differences in average annual AAP concentrations can be attributed to water relations and late harvest (physiological ripeness), neither these factors nor yield explain the fertilization effect, respectively the distribution of AAP within a year (and treatment). The causes of the effects of fertilization and year on AAP formation could not be revealed. The precursor IAA as well as the antioxidants which regulate the transformation of IAA to AAP do not correlate with AAP in the wine. Neither an IAA treshold value nor an antioxidant level critical for AAP formation could be found. Other parameters (yield, TSS, Arg) could not explain the high variance of AAP concentration in wines produced from equally treated variants either. Consequently, no indicator for AAP formation could be found. Further trials will have to reveal to what extent fermentation causes the high variance. The impact of water relations on the formation of UTA needs further investigation, too. In this experiment, the AAP formation occuring in wines produced from four field replicates differed considerably. Why did high quantities of AAP accumulate in one wine and not in another one produced from the same treatment in the same year? This question could not be answered. Thus, it will still be necessary to search for suitable methods for UTA prevention. Although it seemed to be sure that AAP is chemically accumulated in the course of the cooxidation of IAA, knowledge about the chemical conversions in the wine is still rather limited.

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