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Effect of different N fertilization of vine on the tryptophan, free and total indole-3-acetic acid concentrations

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Summary

The compound responsible for UTA (untypical ageing off-flavour) in wines is o-AAP (o-amino acetophenone). It is formed from IAA (indole-3-acetic acid), triggered by sulfurylation. The aim of this study was to evaluate the effect of different N fertilizer supply on this precursor in must and wine, making use of a long-term nitrogen fertilization experiment. Trp (Tryptophan) as well as free and conjugated IAA were determined in musts and wines produced from grapevines supplied with 0, 30, 60, 90 or 150 kg N ha^{-1} year^{-1}. IAA concentrations in musts and wines varied highly over years (1994-1999), wines containing about half of the total IAA of musts. The IAA concentration in must was positively correlated with the concentration of amino acids in must, however, nitrogen supply did not have any effect, neither on Trp nor on IAA concentrations.

Key words: Nitrogen fertilization, tryptophan, indole-3-acetic acid, o-amino acetophenone.

Abbrevations: IAA: indole-3-acetic acid, o-AAP: o-amino acetophenone, Trp: tryptophan, UTA: untypical ageing off-flavour.

Introduction

The untypical ageing off-flavour (UTA) is a complex phenomenon, which is still not fully understood. UTA was first observed in the 1988 vintage and has often caused rejection of wine certifications. In 1994, rejection of more than 20 % of the wines was associated with UTA (Christoph *et al.* 1995). Ortho-aminoacetophenone (o-AAP) was identified as the characteristic compound, with an odour threshold of 0.5 - 1.5 µg l^{-1} (Rapp *et al.* 1993, Christoph *et al.* 1995). The bouquet has been described as odour taint such as acacia blossom or floor polish, but other types of UTA can also be found, e.g. a flavour caused by anthranilic acid, indole or skatol. As larger quantities of o-AAP are formed only after fermentation, o-AAP is often not found in young wines. Kynurenine, IAA and Trp have been discussed as possible precursors of o-AAP. If Trp were the only nitrogen source, o-AAP would be formed during fermentation (Rapp *et al.* 1995). However, further fermentation studies showed that the conversion of Trp into o-AAP is not relevant. Finally, IAA was found to be an obvious precursor of o-AAP, although very small quantities of indole lactic acid are converted into o-AAP as well (Dollmann *et al.* 1996, Christoph *et al.* 1998). Influenced by sulfurylation, a coupled oxidative degradation of IAA causes the formation of o-AAP (Christoph *et al.* 1998, Hoenicke *et al.* 2001 a). As a consequence, the IAA concentration in must has been considered as stress indicator, increasing stress leading to higher IAA concentrations (Müller 2000). It is assumed that UTA is the result of a stress reaction of grape. In addition to high yield and early harvest, especially drought and nitrogen deficiency and possibly high UV radiation are possible stress factors (Schwab *et al.* 1996, Köhler *et al.* 1995). A low concentration of amino acids in grapes and musts or high concentrations of proline are known to be stress indicators (Prior 1997). The response of Trp in grapes to stress conditions is yet unknown. Being the only amino acid absorbing UV light at wavelengths below 300 nm, Trp levels may be reduced by enhanced UV-B radiation (Grossweiner 1994). Under stress conditions, vines form a thinner leaf canopy which may increase UV-B absorption of clusters. While this assumption was partially confirmed for UV-B absorbing foils, defoliation led to contrasting results in the same experiment (Jähnisch 1998). Our study examines whether different N ferilizer supply affects the IAA concentration in must and wine and whether Trp can be used as an indicator of N deficiency stress.

Material and Methods

Field experiment: The experiment was carried out in the Rheingau, Germany, in a vineyard fertilized with different amounts of N (0, 30, 60, 90, 150 kg N ha^{-1} year^{-1}) since 1986. In 1977 Riesling vines grafted on 5C were planted (1 plant 2.6 m^{-2}) with permanent green cover in every second row. The soil was loamy sand, originating from tertiary sea sand containing 1.4 % humus, the pH-value was approximately 7.6. Vineyard details have been described by Prior (1997). Each treatment was repeated 4 times and arranged in a completely randomised design. Each replication was used for micro-vinification. Must and wine samples were stored at -20 °C.

Determination of Trp and free and total IAA: The method reported by Hoenicke *et al.* (2001 a, 2002) to determine free and total IAA using HPLC-FLD was slightly modified. For the determination of free IAA and Trp, 2 ml of

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the sample were mixed with 2 ml indole-3-propionic acid (0.1 mg l^{-1}) as an internal standard and neutralized with NaOH. For solid-phase extraction (SPE), an anion exchange material (SAX) was used (Merck LiChrolut® 500 mg). Total (free and bound) IAA was analysed after alkaline hydrolysis (4 M NaOH, 4 h 110 °C) and SPE using prior RP18 (Merck LiChrolut® 500 mg) and then a SAX (Merck LiChrolut® 500 mg). An HP 1090 chromatograph with a RP-18 column (Merck LiChrospher 5 µm, 250 mm x 3 mm) was used for the subsequent HPLC-FLD analysis. The temperature of the column was kept at 35 °C (± 0.8). The following solvents were used: 0.1 % TFA (solvent A), acetonitrile (solvent B). The gradient was 0 min: 95 % A, 15 min: 60 % A, 25 min: 60 % A, 27 min: 0 % A, 29 min: 0 % A, 31 min: 95 % A, 40 min: 95 % A. The flow rate was 0.7 ml min^{-1}. The fluorescence detector was a HP 1046A with an excitation of 255 nm and an emission of 360 nm.

Determination of free amino acids: Amino acids were quantified by means of HPLC (Spectraphysics) according to PRIOR (1997) and BLESER (1999). Samples were extracted with sulfosalicylic acid and derived with dansyl chloride. Chromatographic separation was carried out on an RP-18 column (Merck LiChrospher 5 µm, 250 mm x 3 mm) using a ternary gradient and the following solvents, A: 2 l bidest. 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.

Statistical analysis: To calculate the significance of means an ANOVA was carried out using Fischer´s test at a significance level of 5 %. Bars in the figures indicate standard error. Significant differences between years are indicated by different letters above the columns; significant differences caused by nitrogen fertilization in a year are shown by different letters in the table.

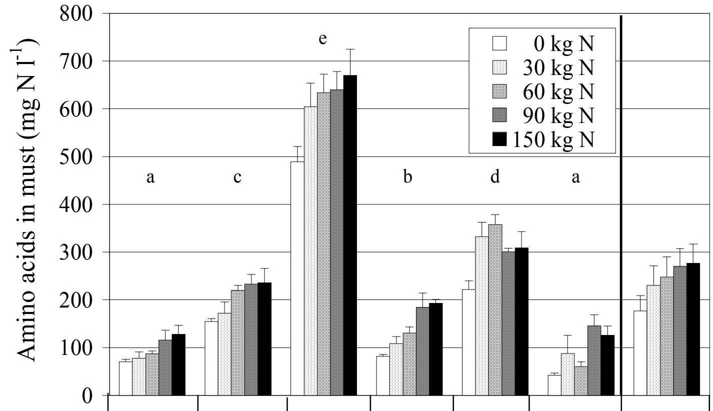
Table

Mean temperature and sum of precipitation from April to September 1994 - 1999 and means of 30 years (1971-2000) (data from the German Meteorological Service, Geisenheim)

Year	Mean temperature °C	Sum of precipitation mm
1994	16.57	239
1995	16.20	340
1996	14.92	229
1997	15.99	223
1998	15.89	318
1999	16.78	307
Mean of 30 years	15.55	281

Results

Amino acids in must: The amino acid concentration in must strongly responded to weather conditions (Table, Fig. 1). In 1996, at low temperatures, we found extremely high concentrations of total amino acids in must: approximately 600 mg N l^{-1}. This was 6 times the concentration found in 1994 and 1999, the two warmest years. In 1998, a year with high rainfall, the amount of amino acids was still about 4 times higher than in 1994, and even in 1995, a warm year without drought, the amino acids were twice those in 1994. Regardless of the weather, the effect of nitrogen deficiency was significant. In each year, the levels of amino acids decreased with decreasing nitrogen fertilization. The Trp concentration of the must essentially depended on weather, *e.g.* in 1996 it was 6 times higher than in 1999. The



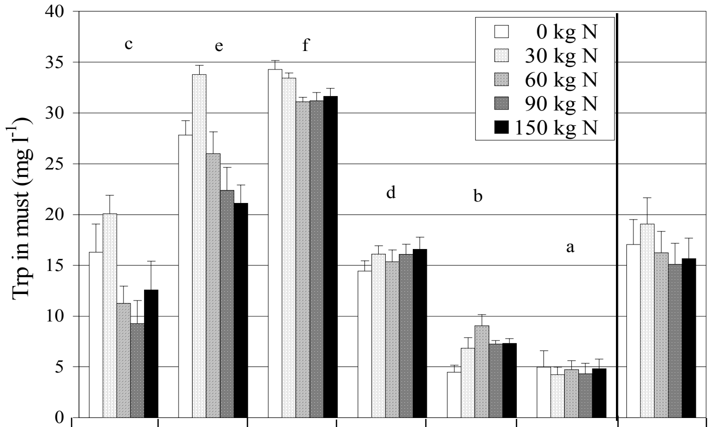
	1994	1995	1996	1997	1998	1999	Mean
0 kg N	69 a	152 a	496 a	85 a	272 a	49 a	187 a
30 kg N	76 a	169 ab	613 ab	111 a	383	95 abc	241 b
60 kg N	86 ab	217 bc	643 b	132 ab	405	68 ab	259 bc
90 kg N	97 b	218 c	648 b	188 b	348	153 bc	275 c
150 kg N	128 b	234 c	682 b	196	352	133	287 c

Fig. 1: Amino acid concentrations in must (mg N l^{-1}) as related to long-term nitrogen fertilization with 0, 30, 60, 90 and 150 kg N ha^{-1} $year^{-1}$ (1994-99). Bars indicate standard error. Significant differences between years are indicated by different letters (graph), significant differences between fertilizer treatments within a year are shown by different letters (Table). The significance level is 5 %.

annual differences of the Trp concentration were similar to those of the total amino acid concentration (Fig. 2). The only exception was 1998, when low Trp values coincided with high amino acid concentrations in must. The considerable differences between the annual average values of amino acid and Trp levels can be attributed to the respective weather conditions (Table). Hot years such as 1994 and 1999 resulted in low values, while in the cool year 1996 the Trp and amino acid values were very high. However, in contrast to other amino acids, *e.g.* arginine and glutamine, N supply did not seem to have consistent effects on the concentration of Trp (Fig. 2) and proline (not shown) in must.

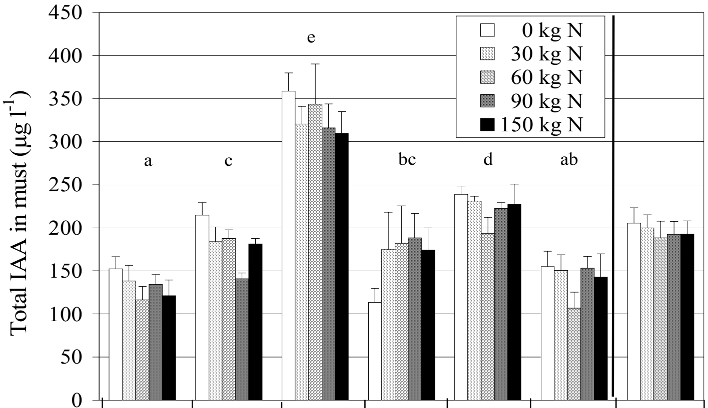
IAA in must: In contrast to the accumulation of amino acids, the accumulation of IAA in grapes was not affected by nitrogen supply, but was strongly different in various years (Fig. 3). In 1996, the total concentration of IAA (free and conjugated IAA) in must was very high at all N levels (>300 µg total IAA l^{-1}) whereas the average total IAA concentration ranged between 130 and 200 µg l^{-1} in other years with significant differences between years. Thus the levels of IAA in grapes were affected by weather conditions in the same way as the amino acids: under stress conditions (high temperature, drought) less IAA was found (Fig. 4).

The concentrations of free IAA were small (1994-1997: 2-7 µg IAA l^{-1}) (Fig. 5). In 1998, there was no free IAA in must and in 1999 no free IAA could be found in several musts of field replicates, *i.e.* the averages were below the



	1994	1995	1996	1997	1998	1999	Mean
0 kg N	16.3 bc	27.8 b	34.3 c	14.4	4.5 a	5.0	17.1 b
30 kg N	20.1 c	33.8 bc	33.4 bc	16.1	6.9 ab	4.2	19.1 c
60 kg N	11.3 ab	26.0 b	31.1 a	15.3	9.1 b	4.7	16.2 ab
90 kg N	9.3 a	22.4 ab	31.2 a	16.1	7.2 b	4.3	15.1 a
150 kg N	12.6 ab	21.1 a	31.6 ab	16.6	7.3 b	4.8	15.7 ab

Fig. 2: Tryptophan (Trp mg l^{-1}) concentrations in must as related to long-term nitrogen fertilization (For details: Fig. 1).



	1994	1995	1996	1997	1998	1999	Mean
0 kg N	152	215 b	359	114	239 a	155	206
30 kg N	138	184 b	320	175	231 ab	151	200
60 kg N	116	188 b	343	182	194 ab	107	188
90 kg N	134	141 a	316	188	223 b	153	192
150 kg N	121	181 b	310	174	227 ab	143	193

Fig. 3: Total IAA (µg l^{-1}) concentrations in must as related to long-term nitrogen fertilization (For details: Fig. 1).

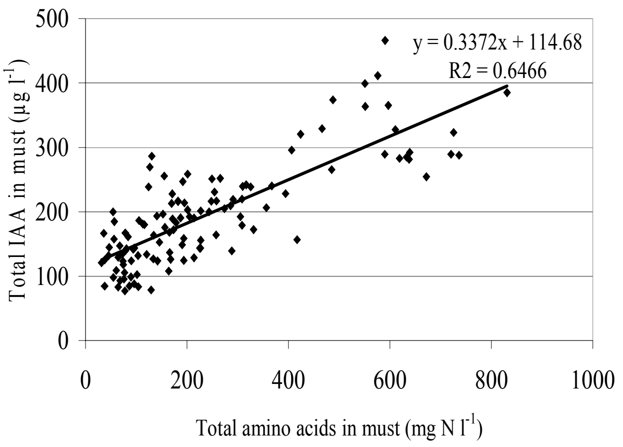


Fig. 4: Relationship between amino acid concentrations (mg N l^{-1}) and total IAA concentrations (µg l^{-1}) in must in 6 years including all nitrogen levels (For details: Fig. 1).

detection limit of 1 µg l^{-1}. No relationship was found between the concentrations of free IAA in must and N supply. On the other hand, free IAA levels differed significantly between years; they were, however, not correlated to water supply or temperature in various years (not shon).

IAA in wine: The concentration of total IAA in wines was considerably lower compared to must (Fig. 6), *i.e.* only between 10 % (1995) and 70 % (1997) of the total IAA of the must were found in wine. The highest value was determined in 1997, it was 6 times higher than in 1995, when the total IAA in wine was lowest. In 1996 and 1999, there was a tendency towards higher total IAA values in wine in the 0 kg N ha^{-1} treatment, whereas in 1997, the total IAA concentration was lowest in this treatment, as was shown for must.

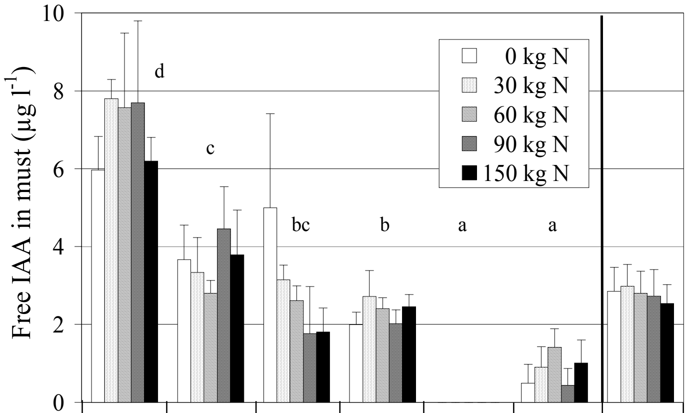
The concentrations of free IAA were considerably higher in wine than in must (Fig. 7). In 1996, the lowest average values were measured (5 µg l^{-1}), whereas in other years the IAA concentrations ranged between 15 and 25 µg l^{-1}. Weather and N supply did not seem to have a consistent effect on the IAA concentrations.

Discussion

Different long-term N supplies had no effect on the Trp concentrations of must (Fig. 2). Nevertheless, the annual differences indicate that Trp also responded to seasonal influences, *e.g.* weather. Experiments using UV absorbing sheets have shown that high UV radiation may cause a reduction of the amino acid accumulation in grapes (Schultz *et al.* 1998). The hypothesis, however, that at low N supply high UV radiation lowers Trp concentrations in grapes due to a reduced canopy density was not confirmed.

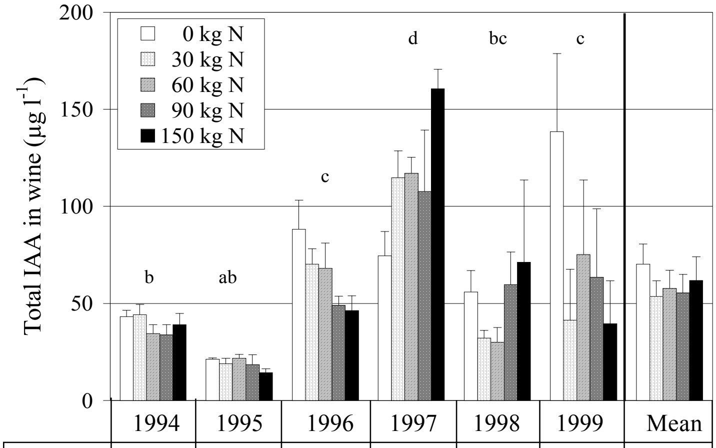
Must contained very low amounts of free IAA. The high concentrations of total IAA in grapes mainly consisted of conjugated IAA (98 %) (Figs 3 and 5). A correlation between the levels of amino acids and IAA in must was confirmed (Fig. 4). Thus, amino acid and IAA levels may be linked. The latter is considered to be a stress indicator (Müller 2000). While a lack of N supply caused a lowering of amino acid levels in grapes (Prior 1997, Bleser 1999), even long-term differences of N supply did not affect the IAA level in grapes. Hoenicke *et al.* (2001 b) report that different soil management did not affect IAA concentrations. Within one year, no correlation between amino acids and total IAA concentration in the must can be found. Therefore, the hypothesis that IAA concentrations may be used as an indicator for stress conditions of grape is not supported.

After fermentation, large quantities of free IAA are released or formed (Fig. 7). However, only about half of the total IAA concentration in must was found in wine (Fig. 6). Thus, the results of Hoenicke *et al.* (2002), who measured similar total IAA values in must and wine, are not confirmed. There was no relationship between the concentration of IAA in must and the level of free IAA in wine. Since the conver-



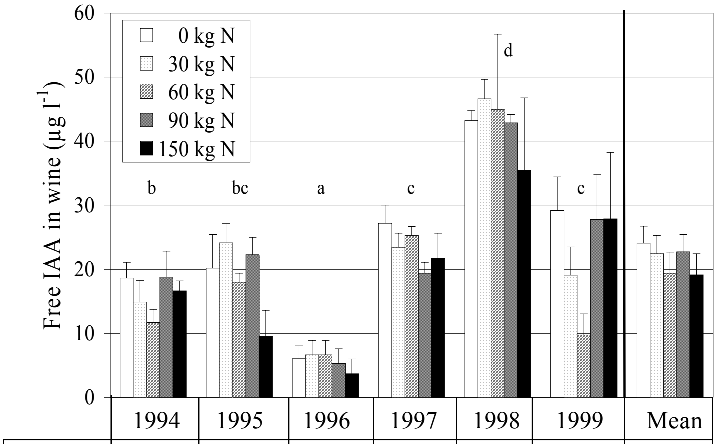
	1994	1995	1996	1997	1998	1999	Mean
0 kg N	6.0	3.7	5.0	2.0	n.d.	0.5	2.8
30 kg N	7.8	3.3	3.2	2.7	n.d.	0.9	3.0
60 kg N	7.6	2.8	2.6	2.4	n.d.	1.4	2.8
90 kg N	7.7	4.5	1.8	2.0	n.d.	0.4	2.7
150 kg N	6.2	3.8	1.8	2.5	n.d.	1.0	2.5

Fig. 5: Free IAA (µg l^{-1}) concentrations in must as related to long-term nitrogen fertilization. 1998: IAA concentration not detectable (n.d.). For details: Fig. 1.



	1994	1995	1996	1997	1998	1999	Mean
0 kg N	43	21	88 b	75 a	56	138	70
30 kg N	44	19	70 ab	115 ab	32	41	54
60 kg N	34	22	68 ab	117 ab	30	75	58
90 kg N	34	19	49 a	108 a	60	63	55
150 kg N	39	14	46 a	161 b	71	40	62

Fig. 6: Total IAA (µg l^{-1}) concentrations in wine as related to long-term nitrogen fertilization (For details: Fig. 1).



	1994	1995	1996	1997	1998	1999	Mean
0 kg N	18.6	20.2 b	6.0	27.2 b	43.2	29.2	24.1
30 kg N	14.9	24.1 c	6.6	23.4 ab	46.6	19.1	22.5
60 kg N	11.7	18.0 b	6.7	25.3 ab	45.0	9.7	19.4
90 kg N	18.8	22.3 bc	5.3	19.4 a	42.9	27.8	22.7
150 kg N	16.6	9.5 a	3.7	21.7 ab	35.5	27.9	19.2

Fig. 7: Free IAA (µg l^{-1}) concentrations in wine as related to long-term nitrogen fertilization (For details: Fig. 1).

sion rates of IAA to o-AAP are in the order of 20 % (Christoph *et al.* 1998), even the relatively low concentrations of free IAA in the 1996 wine were sufficient to produce wines tained by UTA. Regardless whether the conjugated IAA represents a substrate for conversion, all examined wines had abundant precursors for the formation of o-AAP, irrespective of year and N supply.

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