Research Article

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Atypical ageing defect in Pinot Blanc wines: a comparison between organic and conventional production management systems

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Abstract

BACKGROUND: Atypical ageing (ATA) is an aroma defect that occurs in white wines and entails a loss of varietal aromas as well as scents of wet mop, shoe polish and dish rag. 2-Aminoacetophenone (2AAP) – a degradation product of indole-3-acetic acid (IAA) – has been described as the main odour-active compound and chemical marker responsible for this off-flavour. A stress reaction in the vineyard triggered by climatic, pedological and viticultural factors can ultimately cause ATA development in wines and remarkably affect wine quality. The aim of this research was to investigate the influence of three grapevine management systems on the occurrence of ATA. The experiments were carried out on Pinot Blanc grape samples from vines cultivated using one conventional and two organic approaches. The management systems mainly differed for the fertilisation regime and the weed control.

RESULTS: The amino acid profiles as well as 2AAP and its precursors were quantified in musts and wines using ultra-high-performance liquid chromatography coupled to a high-resolution mass spectrometer. The results showed the existence of a strong vintage effect, while no influence of the use of different agronomic systems was observed.

CONCLUSION: The study revealed that an efficient implementation of different grapevine production systems did not affect ATA development in Pinot Blanc wines. This finding is of great relevance for winegrowers and winemakers as it demonstrates that a well-planned organic management system correctly adjusted to the climatic conditions does not pose a threat towards the development of ATA-related compounds in wine.

Supporting information may be found in the online version of this article.

Keywords: atypical ageing; Pinot Blanc; UHPLC-HRMS; aroma fault; 2-aminoacetophenone; organic wine

INTRODUCTION

Atypical ageing (ATA) is a wine aroma fault occurring in white wines characterised by an early loss of varietal aromas as well as nuances of mothball, acacia blossom, soap and dirty rag, among others.[1] 2-Aminoacetophenone (2AAP) – a degradation product of indole-3-acetic acid (IAA) – has been described as the main odour-active compound and chemical marker responsible for this off-flavour. Depending on the aroma intensity of the wine, it can be perceived at concentrations ranging from 0.5 to 10.5 μg L^{-1}.[2,3] It seems that a stress reaction in the vineyard triggered by climatic, pedological and viticultural factors can ultimately cause ATA development in wines, affecting the quality and shortening the shelf-life.[4] Besides climatic limitations and soil composition, the use of different viticultural practices represents a valuable tool to improve grape quality and reduce or prevent ATA development. So far, research has focused on the impact of harvest time,[5-7] fertilisation,[8,9] water status[10,11] and UV radiation.[12] While all those factors are linked to ATA, to the best of our knowledge the use of different grape production approaches on the development of this aroma fault has not yet been evaluated.

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In recent years, organic farming has been widely discussed as an eco-friendly and sustainable alternative to conventional agriculture and, following the approval of the European Green Deal, it is now being promoted and implemented in the production of several crops, including grapevines.[13] Consumer perception of organic wines is generally positive,[14] but lower accumulation of polyphenols,[15] higher levels of oxidation compounds[16] as well as reduced must nitrogen (N) concentrations[17] might affect the quality and cause premature ageing.

As the antioxidant capacity and N supplementation are strictly related to ATA development,[4,18] the aim of this study was to investigate the influence of three grapevine management systems – one conventional and two organic approaches – on the occurrence of this aroma fault. White wines are characterised by a higher risk of ATA development[19] and therefore Pinot Blanc grapes were chosen for the study. ATA precursors were quantified in wines and musts, and the amino acid (AA) profiles together with ammonium (NH_4^+) and glutathione (GSH) accumulation – indicators of N status[8] and antioxidant activity[20] – were measured in the grape juices. The potential development of 2AAP was assessed in the wines after an accelerated ageing process (T6).[21]

MATERIALS AND METHODS

Study site and field determinants

The experiment was conducted at the Edmund Mach Foundation in San Michele all'Adige, Trento, Italy (46° 11′ 46.2″ N, 11° 08′ 12.8″ E; 236 m above sea level) in a vineyard planted with Pinot Blanc vines on SO4 rootstock in 2009 during the 2016–2018 growing seasons. The soil consisted of a glacial till with loam texture (45.6% silt, 44% sand and 10.4% clay). Soil organic matter was 31.5 (g kg^{-1} dry weight) and pH 7.9. Vines were spaced 0.5 m (within rows) by 2.8 m (between rows) and cultivated with a 'pergola semplice trentina' (single curtain) system. Supplemental irrigation was operated, when necessary, to all the managements together through drip irrigation (2 L h^{-1}).

Grapevine treatments

Since 2012, the vineyard has been cultivated following three different agronomic management systems: a conventional (C) and two organic approaches (O1 and O2) (Table 1). Mechanical in-row weed control was implemented in each treatment and, only for C, glyphosate (Roundup, Bayer, Germany) was administered in autumn and throughout the year as needed. The fertilisation regime entailed the use of a granular commercial fertiliser (NPK 12:12:17) applied in April every year to the vines cultivated with the C approach. For O1 it involved the use of cattle manure applied biyearly (20 tons ha^{-1}) while inter-row mulching was performed annually in the O2 system. This last fertilisation practice was performed by sowing, cutting and leaving on the land surface a mix of grasses and leguminous plants. To obtain consistency with regard to the N intake, it was ensured that roughly 36 units of N per year were provided with the C and O1 treatments. Considering the vegeto-productive balance, an estimation of the N provision to the O2 plants was carried out and found to be equivalent to the other systems. Pneumatic leaf removal was operated for the C and O1 treatments, while secondary shoots were removed only for O2. Grapes cultivated using the C approach were the only ones to be mechanically trimmed; shoot winding was carried out on O1 and O2 plants. Finally, grape cluster thinning was performed manually in O2, mechanically (pneumatically) in O1 and by application of gibberellic acid (GA) in the C system. Besides O2, where the inter-row space was alternately used to produce mulch during October–May, there was permanent green cover in every inter-row for the C and O1 treatments.

Sampling and winemaking

To ensure homogeneity across the investigation, plants were chosen after building a vigour map. For every year of the trial, ten grape replicates from each agronomical system were sampled on the same day. Each replicate – consisting of 25 kg grapes hand-picked at technological ripeness from adjacent vines – was brought to the experimental vinery at the Edmund Mach Foundation. There, grapes were crushed–destemmed (Ares 15; Omac, Chiuduno, Italy) and pressed (20 L Hydropress, Speidel, Ofterdingen, Germany) until 60% w/v yield was reached. Grape juices were sampled in quadruplicate and, following the addition of NaN_3 (100 mg L^{-1}), were frozen at −20 °C until analysis. The remaining musts were vinified. 50 mg L^{-1} $K_2S_2O_5$ (Dal Cin, Concorezzo, Italy) together with 1 mL hL^{-1} of pectolytic enzymes (Zimopec P110L; Perdomini-IOC, San Martino Buon Albergo, Italy) were added to the juices which were settled at 10 °C for 24 h and then racked with a turbidity of ∼100 NTU. Inoculation was carried out with 200 mg L^{-1} of a commercial active dry yeast (EC-1118; Lallemand, Blagnac Cedex, France) previously rehydrated at 37 °C for 30 min. Fermentation was conducted at 18–20 °C in a temperature-controlled room. On the third day of fermentation, diammonium phosphate (250 mg L^{-1}) was supplemented to the musts. Upon alcoholic fermentation, wines were racked and $K_2S_2O_5$ (130 mg L^{-1}) was added. Sterile bottling was performed in quadruplicate 6 months after the end of alcoholic fermentation.

Sample preparation and analysis by high-resolution mass spectrometry (HRMS)

This study entailed the use of ultra-high-performance liquid chromatography (UHPLC; Ultimate R3000, Thermo Fisher Scientific,

Table 1. Agronomic parameters of the grapevine management systems under evaluation

	Conventional (C)	Organic 1 (O1)	Organic 2 (O2)
Weed control in row	Chemical and mechanical	Mechanical	Mechanical
Permanent grass cover/inter-row mowing	Yes	Yes	Yes
Fertilisation	Mineral	Organic (every 2 years)	Green manure
Pneumatic leaf removal	Yes	Yes	No
Secondary shoot removal	No	No	Yes
Mechanical trim	Yes	No	No
Shoots winding up on the higher wire	No	Yes	Yes
Thinning of bunches	Gibberellic acid	Mechanical	Manual

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Waltham, MA, USA) coupled to a high-resolution mass spectrometer (Q-Exactive hybrid Q-Orbitrap, Thermo Fisher Scientific) equipped with a heated electrospray ionisation (HESI-II) interface working in positive ionisation mode. Specifics of the standards and solvents used to perform the analyses are reported in the Supporting Information (Tables S1–S3).

Amino acids, amines, ammonium and glutathione quantification

For the detection and quantification of AAs, amines, NH_4^+ and GSH, a stock solution was prepared by dissolving the standards in water. To enhance the solubility and extend the lifetime of the standard mix, a few drops of 37% hydrochloric acid and methanol (MeOH) were added to the solution. On average, concentrations of 300 mg L^{-1} for the AAs (except for Asn, Gln and Trp, which was 750 mg L^{-1}), 600 mg L^{-1} for the amines, 2000 mg L^{-1} for NH_4^+ and 50 mg L^{-1} for GSH were obtained. The calibration solutions were prepared directly in HPLC vials at concentrations ranging from 0.001 to 50 mg L^{-1} of each native analyte, with the exception of Trp, for which concentrations ten times higher were reached.

Must samples were diluted 50 times in water and injected together with the calibration curve in the analytical column (Raptor Biphenyl 3 × 150 mm; Restek, Bellefonte, PA, USA) with a mobile phase of 0.1% formic acid (FA; solvent A) and MeOH (solvent B) at a flow rate of 0.5 mL min^{-1}. The chromatographic separation was obtained with a linear ramp: 95% A, 5% B in 1 min; 5% A, 95% B in 15 min and held for 3 min. In 0.5 min, eluents returned to the initial condition and were held for 4 min for column re-equilibration. Injection volume was 10 µL, of which 5 µL were of sample/standard and 5 µL were *O*-phthalaldehyde (OPA), a derivatisation reagent.[22]

ATA precursors

To determine ATA precursors in musts and wines, the method developed by Roman *et al*.[23] was used. Wine samples were filtered with 0.45 µm polytetrafluoroethylene filters directly into HPLC vials, while musts were first diluted five times with water and then filtered. Except for Trp, the method entailed the use of a preconcentration and purification solid-phase extraction online system to reduce the matrix effects and enhance the sensitivity for the target compounds.

2AAP quantification

2AAP analysis was performed on the wines after an accelerated ageing process (heat treatment at 40 °C, 6 days)[21] using the method described by Nardin *et al*.[18]

Statistical evaluation

Data analysis was performed on the concentrations of targeted compounds using XLSTAT 2021.5 (Addinsoft, New York, USA) while Statistica 13.1 Software (Tibco, Palo Alto, USA) was employed to generate the box plots. The Kruskal–Wallis test (multiple pairwise comparison, Steel–Dwass–Critchlow–Fligner; $P \leq 0.05$) was used for comparing the effects of different treatments and influence of the harvest year on the AA profile, NH_4^+, amines, GSH, ATA precursors and 2AAP amounts. An analysis of covariance (ANCOVA modelling) was carried out to predict the possible 2AAP production considering the AA profiles in musts and 2AAP precursors in wines as known variables. Principal component analysis (PCA) was performed using R Statistical Software (version 2.14.0; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Amino acids, biogenic amines, ammonium and glutathione content in grape musts

Table 2 describes the accumulation of the AAs, while Table 3 reports the contents of amines, NH_4^+ and GSH detected in the musts. Considering all data collected for the 3 years of the trial, different treatments did not affect the AA profiles of the grape juices (Kruskal–Wallis, $P \leq 0.05$). However, considering the samples by single vintage and not by treatment, all AAs significantly differed in their concentrations, with the exception of Asp, Glu, Hyp and Met. Overall, 2018 was characterised by relatively low AA contents compared to 2016 and 2017: besides γ-aminobutyric acid (GABA), Phe, Lys, Pro and Ser, all AA concentrations were higher in the first 2 years of the trial. GABA accumulation was lower in 2016 compared to the other years. Phe concentration was the most affected by the vintage year as, for this specific AA, the amounts detected were substantially different for each year of the trial. Interestingly, its accumulation decreased over time, with higher values detected in 2016 and lower amounts measured in 2018. Pro and Ser concentrations were significantly higher in 2016 compared to 2018.

Considering the impact of the different treatments on the AA profiles within the individual years, some meaningful effects were noticed (Kruskal–Wallis, $P \leq 0.05$). In 2016, higher concentrations of Cit, Gln and His were observed in samples from the O2 system compared to O1. In the same year, C samples were characterised by higher amounts of Phe, Pro, Tyr and Trp as opposed to O1. With regard to 2018, O1 samples displayed higher accumulations of Ile, Leu and Val compared to O2, while the concentrations of Asp and Glu were observed to be lower for O2 in comparison with the other treatments in the same year. The effects of the different agronomic systems on the accumulation of Ser and Thr were found to be inconsistent. More specifically, compared to O2, O1 was associated with lower amounts of those compounds in 2016 and higher concentrations in 2018. Due to limitations of the analytical method, Cys and CysCys as well as Leu and Ile were quantified together. Asn, Gly and Orn were not detected.

By looking at the data obtained for the 3 years of the trial, the use of different agronomic systems did not affect the accumulation of biogenic amines in the grape musts. Nevertheless, their concentrations varied across the years (Kruskal–Wallis, $P \leq 0.05$). Contrary to 2017 and 2018, in 2016 the accumulation of ethanolamine and ethylamine was lower. The amount of methylamine was found to be higher in 2018 compared to the other years. Putrescine, cadaverine, histamine, spermine, spermidine, tyramine and tryptamine were not detected in the must samples. Considering the impact of the different treatments on the accumulation of biogenic amines within the individual years, a meaningful effect was recorded only for methylamine in 2018 (Kruskal–Wallis, $P \leq 0.05$). For this vintage, higher amounts of this compound were detected in the musts obtained from grapes cultivated using the C system as opposed to the organic treatments. Finally, by looking at the concentrations across all years, 2016 was characterised by a lower accumulation of biogenic amines.

The different agronomic systems had a significant impact on the concentrations of NH_4^+ in the musts: considering all data from the 3 years of the trial, the C treatment was associated with higher amounts compared to O2 (Kruskal–Wallis, $P \leq 0.05$; Fig. 1).

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Table 2. Statistical distribution (Kruskal–Wallis test; multiple pairwise comparison, Steel–Dwass–Critchlow–Fligner) of the minimum, maximum and median concentrations of the amino acids (mg L^{-1}) detected in musts from grapes cultivated using different agricultural management systems

	2016									2017									2018									Year significance		
	Conventional			Organic 1			Organic 2			Conventional			Organic 1			Organic 2			Conventional			Organic 1			Organic 2					
	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	2016	2017	2018
Amino acids																														
GABA	2.08	4.37	7.84	1.68	2.39	5.01	0.94	3.47	5.80	1.39	5.29	7.52	3.19	5.86	7.71	2.27	4.94	5.67	4.05	5.16	5.97	1.47	5.97	8.76	1.17	4.64	6.05	B	A	A
Asp	1.05	1.84	2.45	1.18	1.60	2.13	1.10	1.94	2.98	0.67	1.54	3.51	0.93	1.78	2.40	1.25	1.44	2.09	1.49	1.86a	2.15	0.94	2.09a	2.68	0.76	1.46b	1.81			
Glu	5.59	11.3	17.3	5.82	8.44	11.2	6.77	9.50	12.8	4.73	8.96	15.8	6.96	11.5	13.6	5.43	10.8	18.4	6.10	9.06a	14.2	5.33	9.89a	13.6	3.94	6.22b	6.80			
Ala	4.69	9.83	14.7	6.16	7.70	10.6	0.87	9.28	13.6	2.39	7.14	14.3	7.59	9.15	13.6	5.85	9.11	13.5	3.21	4.85	11.0	4.54	5.00	7.38	3.63	4.90	6.69	A	A	B
Arg	12.4	46.8	57.7	18.6	29.7	46.7	24.2	42.2	68.0	17.9	46.8	99.4	31.7	45.0	73.0	19.3	37.9	82.8	9.49	23.2	81.7	7.71	26.5	53.3	5.86	19.0	35.0	A	A	B
Cys + CysCys	<LOD	0.007	0.022		<LOD	0.008	<LOD	0.005	0.007	<LOD	0.005	0.014	<LOD	0.008	0.010	<LOD	0.006	0.008		<LOD	0.002		<LOD	0.005		<LOD	0.003	A	A	B
Cit	<LOD	0.31ab	0.83	<LOD	0.25b	0.38	<LOD	0.42a	0.66		<LOD	0.80	<LOD	0.26	0.49		<LOD	0.69		<LOD	0.62		<LOD	0.25		<LOD	0.22	A	A	B
Phe	0.45	1.28a	5.88	0.19	0.39b	2.47	0.39	1.27ab	3.97	0.17	0.32	4.84	0.25	0.37	0.68	0.24	0.44	1.01	0.11	0.20	0.82	0.10	0.25	0.36	0.12	0.24	0.29	A	B	C
Gln	4.90	13.8ab	50.5	5.50	6.90b	12.5	9.06	13.6a	23.6	3.21	8.50	31.9	6.10	8.99	15.5	4.53	8.63	19.1	3.34	6.80	16.7	2.29	6.19	10.3	1.69	4.37	7.42	A	A	B
Hyp	<LOD	0.44	1.08	<LOD	0.52	0.89		<LOD	1.55	<LOD	0.39	0.59	<LOD	0.41	0.79	<LOD	0.47	0.91		<LOD	0.71		<LOD	0.63	<LOD	0.33	0.90			
Ile + Leu	0.71	1.52	5.34	0.64	0.83	2.54	0.30	1.51	3.62	0.45	0.94	4.15	0.76	1.03	1.26	0.68	0.97	1.42	0.39	0.68ab	1.46	0.31	0.76a	1.33	<LOD	0.54b	0.69	A	A	B
His	0.56	1.66ab	3.37	0.59	0.77b	1.51	1.09	1.29a	1.78	0.52	1.49	3.05	0.92	1.32	1.79	0.57	1.09	1.87	<LOD	0.64	2.02	<LOD	0.76	1.45		<LOD	0.77	A	A	B
Lys	<LOD	0.19	0.47		<LOD	0.34	<LOD	0.20	0.37	<LOD	0.25	0.79	<LOD	0.33	0.74	<LOD	0.27	0.84	<LOD	0.22	0.54		<LOD	0.47		<LOD	0.29	B	A	B
Met	<LOD	0.32	2.35	<LOD	<LOD	1.89		<LOD	1.85		<LOD	0.70	<LOD	0.29	0.71	<LOD	0.30	2.28		<LOD	1.24		<LOD	3.73	<LOD	0.62	1.39			
Pro	6.49	10.4a	16.7	4.93	6.72b	11.8	0.24	8.51ab	13.5	3.47	6.64	20.1	<LOD	7.57	10.6	5.46	7.86	10.4	4.11	5.73	15.0	6.31	7.17	11.2	5.41	5.77	9.29	A	AB	B
Ser	1.98	4.24ab	6.18	2.12	3.38b	5.38	3.31	4.47a	6.39	1.74	2.90	5.76	2.33	4.09	5.74	2.25	3.69	5.88	1.87	3.11ab	5.73	1.43	3.23a	6.09	1.17	2.26b	2.99	A	AB	B
Tyr	0.18	0.60a	0.94	0.20	0.33b	0.54	0.20	0.50ab	0.83	0.21	0.48	0.98	0.31	0.42	0.75	0.20	0.43	0.66	0.10	0.25	0.61	<LOD	0.27	0.44	<LOD	0.15	0.31	A	A	B
Thr	4.46	12.1ab	15.5	6.14	7.81b	11.7	6.64	10.0a	16.5	3.71	9.43	18.7	6.99	11.4	13.4	5.98	8.81	15.6	4.37	6.57ab	13.3	2.70	7.60a	11.8	2.06	5.16b	7.00	A	A	B
Trp	0.20	0.78a	2.53	<LOD	0.21b	1.00	0.22	0.65ab	2.12	<LOD	0.32	2.47	<LOD	0.28	0.49	<LOD	0.31	0.64		<LOD	0.86		<LOD	0.33		<LOD	0.24	A	A	B
Val	1.24	2.73	7.37	1.07	1.47	3.83	0.51	2.43	5.19	0.75	1.67	6.10	<LOD	1.83	2.37	1.20	1.70	2.45	0.71	1.25ab	2.74	0.62	1.42a	2.43	0.43	0.96b	1.21	A	A	B
Asn		<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD				
Gly		<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD				
Orn		<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD				
FAN	8.46	25.3[ab]	39.3	11.2	16.4[b]	24.0	15.2	22.3[a]	31.8	8.79	21.7	47.6	16.0	22.1	35.0	10.9	19.9	39.7	6.97	13.1	38.3	5.80	14.7	26.6	4.81	10.6	16.9	A	A	B

For each year, different lower-case letters in the same row indicate differences between treatments ($P \leq 0.05$), while different upper-case letters indicate significant differences between vintages ($P \leq 0.05$).
Abbreviations: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cit, citrulline; Cys, cysteine; CysCys, cystine; GABA, γ-aminobutyric acid; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Hyp, hydroxyproline; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine.
Free available nitrogen (FAN) was calculated as the sum of the N atoms contained in Asp, Glu, Ala, Arg, Cys, CysCys, Phe, Gly, Gln, Ile, Leu, Hys, Lys, Met, Pro, Ser, Tyr, Thr, Trp, and Val. LOD, limit of detection; Max, maximum; Med, median; Min, minimum.

Table 3. Statistical distribution (Kruskal–Wallis test; multiple pairwise comparison, Steel–Dwass–Critchlow–Fligner) of the minimum, maximum and median concentrations of biogenic amines, glutathione and ammonium (mg L^{-1}) detected in musts from grapes cultivated using different agricultural management systems

	2016									2017									2018									Years significance		
	Conventional			Organic 1			Organic 2			Conventional			Organic 1			Organic 2			Conventional			Organic 1			Organic 2					
	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	2016	2017	2018
Biogenic amines																														
Ethanolamine	<LOD	0.52	0.68		<LOD	0.54		<LOD	1.23	<LOD	0.49	0.76	<LOD	0.64	0.81	<LOD	0.52	0.72	<LOD	0.61	0.74	<LOD	0.69	0.85	<LOD	0.54	0.76	B	A	A
Ethylamine	<LOD	0.11	0.46		<LOD	0.25		<LOD	0.46	<LOD	0.47	2.16	<LOD	0.45	2.16	<LOD	0.13	1.62	0.25	0.80	1.15	<LOD	0.41	0.97	0.19	0.68	1.03	B	A	A
Methylamine	<LOD	0.31	0.87	<LOD	0.38	0.58	<LOD	0.42	0.60	<LOD	0.48	1.13	<LOD	0.47	1.11	<LOD	0.42	0.99	0.66	1.10[a]	1.54	<LOD	0.57[b]	1.12	0.25	0.66[b]	0.98	B	B	A
Phenylethylamine		<LOD	0.004		<LOD	0.005		<LOD	0.006		<LOD	0.012		<LOD	0.014		<LOD	0.014		<LOD	0.007		<LOD	0.019		<LOD	0.005			
Putrescine		<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD				
Cadaverine		<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD				
Histamine		<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD				
Spermine		<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD				
Spermidine		<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD				
Tyramine		<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD				
Tryptamine		<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD			<LOD				
∑ Amines	0.46	1.05	1.94	0.37	0.90	1.09	0.38	1.00	1.47	0.73	1.25	3.72	0.71	1.53	4.10	0.35	1.02	3.08	1.56	2.64	3.18	0.61	1.66	2.93	0.96	1.79	2.78	B	A	A
Others																														
GSH		<LOD	0.62		<LOD	0.35	<LOD	<LOD	0.26		<LOD	0.59		<LOD	0.50		<LOD	0.28	<LOD	0.05	0.14	<LOD	0.11	0.23	<LOD	0.08	0.13	A	A	B
NH_4^+	2.84	20.3	81.0	1.39	10.0	54.7	2.96	8.17	82.1	4.13	23.7	37.9	7.27	16.9	77.1	3.07	9.40	22.3	5.37	43.0[a]	78.1	2.46	20.0[b]	36.8	<LOD	9.41[ab]	69.2			

For each year, different lower-case letters in the same row indicate differences between treatments ($P \leq 0.05$), while different upper-case letters indicate significant differences between vintages ($P \leq 0.05$). Abbreviations: GSH, glutathione; LOD, limit of detection; Max, maximum; Med, median; Min, minimum; NH_4^+, ammonium.

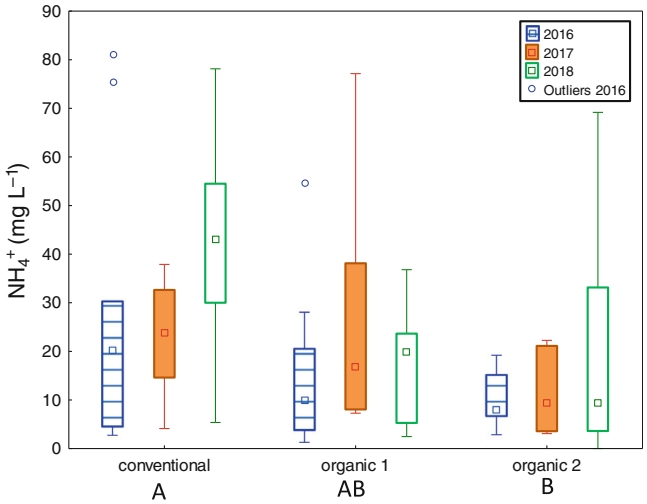

Figure 1. Box plots of the distribution of ammonium (NH^{4+}; mg L^{-1}) in must samples obtained from grapes cultivated using different agronomic systems. Different upper-case letters indicate significant differences between treatments considering data from all years (Kruskal–Wallis test; $P \leq 0.05$).

Looking at the effect of the treatments within the individual years, the only difference was found between the C and O1 systems in 2018: the first displayed a higher accumulation of NH_4^+ compared to the second (Table 3).

The effect of the agronomic management on the accumulation of GSH was not assessed as the median values for this compound were found to be below the limits of detection (LOD). However, by comparing the data from the 3 years, 2018 was characterised by a lower accumulation of GSH as opposed to 2016 and 2017 (Kruskal–Wallis, $P \leq 0.05$; Table 3).

2AAP precursors in musts

No effects of the agronomic management on the accumulation of 2AAP precursors were observed when the data from the 3 years of the trial were considered (Fig. 2). Nevertheless, different treatments affected the concentrations within the singular vintages (Kruskal–Wallis, $P \leq 0.05$; Supporting Information, Table S5). Tryptophan (TRP) accumulation in grapevines was influenced by the management system only in 2016: the C approach was associated with higher amounts compared to O1 (778 *vs.* 210 µg L^{-1}). For this compound, 2018 was characterised by a vintage effect as TRP concentrations were found to be significantly lower when

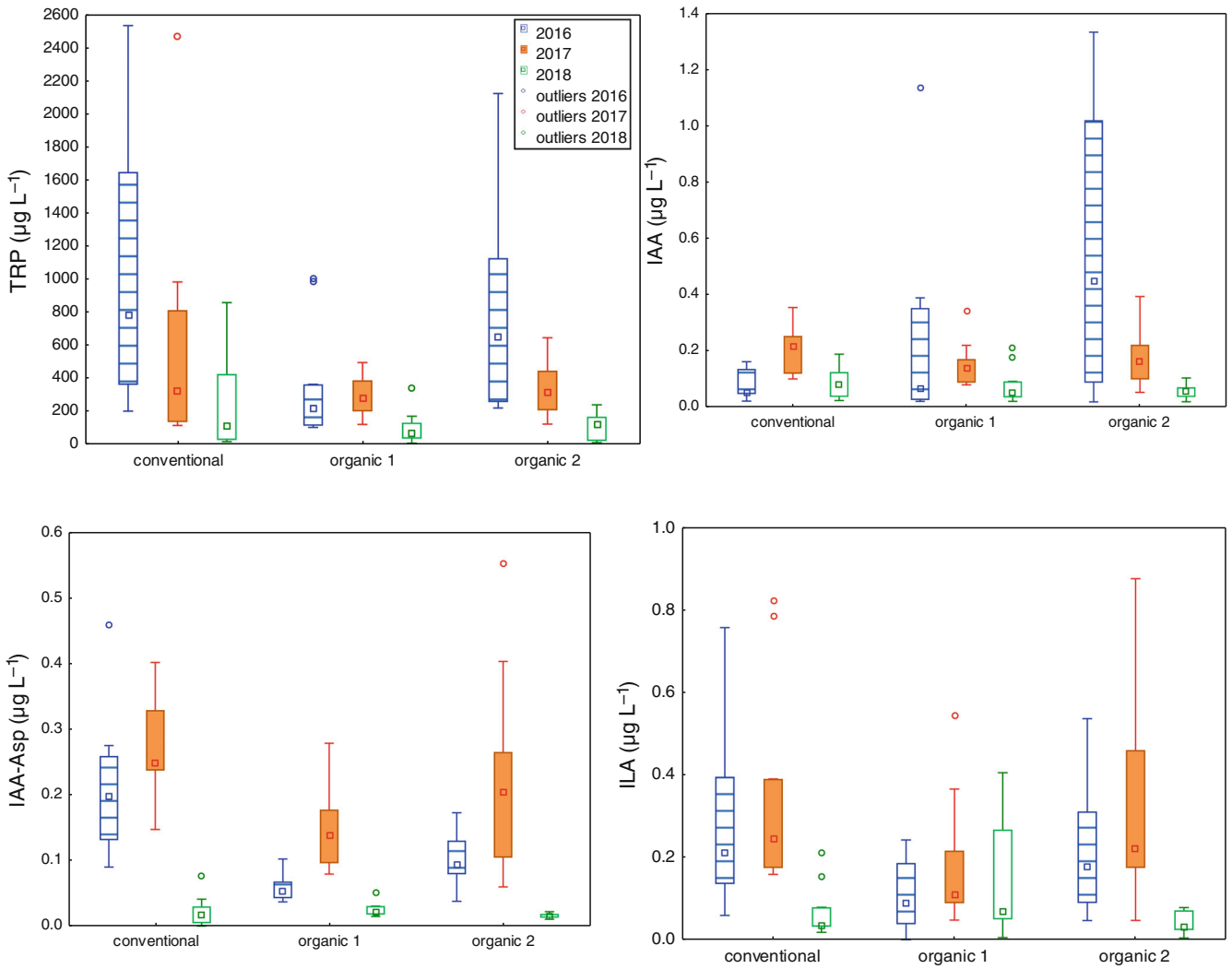

Figure 2. Box plots of the distribution of tryptophan (TRP), indole-3-acetic acid (IAA), *N*-(3-indolacetyl)-DL-aspartic acid (IAA-Asp) and indole-3-lactic-acid (ILA) in must samples obtained from grapes cultivated using different agronomic systems; concentrations are reported in µg L^{-1}.

compared to 2016 and 2017. With regard to free IAA, the amounts of this precursor fluctuated according to the vintage year, with a significant difference between 2017 (higher) and 2018 (lower). *N*-(3-Indolyalcetyl)-DL-aspartic acid (IAA-Asp) concentrations varied greatly between years. For this compound systems-related differences were observed in 2016 and 2017, where grapes

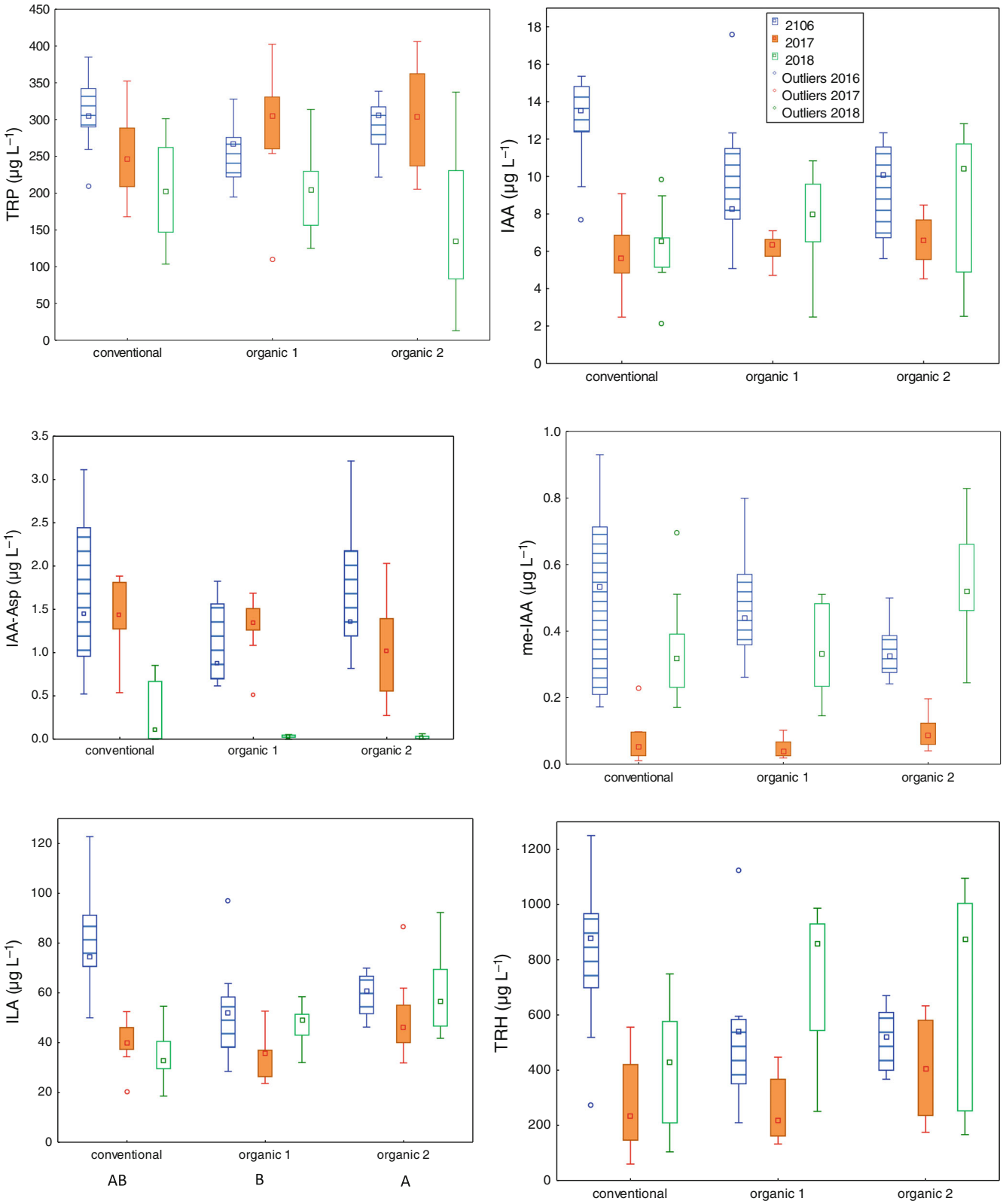

Figure 3. Box plots of distribution of tryptophan (TRP), indole-3-acetic acid (IAA), *N*-(3-indolacetyl)-DL-aspartic acid (IAA-Asp), methyl-indole-3-acetate (me-IAA), indole-lactic-acid (ILA) and tryptophol (TOH) in wines obtained from grapes cultivated using different agronomic systems. Upper-case letters indicate differences between treatments considering data from all years (Kruskal–Wallis test; $P \leq 0.05$); concentrations are reported in µg L^{-1}.

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cultivated with the C treatment displayed higher amounts (0.20 and 0.25 μg L^{-1}) of IAA-Asp compared to O1 (0.05 and 0.14 μg L^{-1}). DL-Indole-3-lactic acid (ILA) concentrations were not affected by the use of different agronomic systems but varied according to the vintage year: 2018 was characterised by lower amounts compared to the other vintages. *N*-(3-Indolylacetyl)-DL-alanine (IAA-Ala), methyl indole-3-acetate (me-IAA), indole-3-acetamide (IAM), indole-3-acetonitrile (IAN), DL-kynurenine (KYN), skatole (SKA), tryptamine (TAM) and tryptophol (TOH) were not detected in the must samples.

2AAP precursors in wines

Figure 3 reports the box plots of the distribution of the quantified 2AAP precursors in the wine samples. Except for ILA, considering all the data from the 3 years, the treatments did not affect the precursor concentrations in the wines. However, the use of different agricultural systems influenced their accumulation within the singular vintages (Kruskal–Wallis, $P \leq 0.05$; Supporting Information, Table S6). In 2016 TRP concentrations significantly changed according to the agronomic system: as opposed to O1, the use of C was associated with higher amounts of this precursor (304 *vs*. 267 μg L^{-1}). A vintage effect was recorded for 2018 where, compared to the other years, lower concentrations of TRP were measured. As opposed to 2017 and 2018, free IAA levels were higher in 2016. In this year, a treatment effect was noticed as well: wines obtained from grapes cultivated with the C system displayed higher amounts of unbound IAA compared to those cultivated with the O2 approach (13.5 *vs*. 10.0 μg L^{-1}). IAA-Asp concentrations were not affected by the treatments but differed across the years; as opposed to the other vintages, lower amounts were detected in 2018. In comparison with the other years of the trial, me-IAA amounts were lower in 2017. The accumulation of this precursor was affected by the treatments only in 2018, where the me-IAA amount measured in O2 samples was higher than that recorded for O1 (0.52 *vs*. 0.33 μg L^{-1}).

Considering the data from all years, the agronomic systems had a significant impact on the amounts of ILA, as O2 samples displayed significantly higher concentrations compared to O1 (Fig. 3). By looking at each individual year, this observation was confirmed only for 2017. In 2016, compared to O1 and O2, higher amounts were recorded for C (74.5 μg L^{-1}), and in 2017 O1 and O2 systems significantly differed, with higher concentrations detected for the second (36.1 *vs*. 45.0 μg L^{-1}). In 2018, a higher accumulation of ILA was observed in the wines obtained from grapes cultivated using the organic approaches (49.2 μg L^{-1} for O1 and 56.8 μg L^{-1} for O2) as opposed to C (32.8 μg L^{-1}). Finally, by evaluating the cumulative data for each individual year, it was noticed that in 2016 the concentration of ILA was higher compared to the other vintages. The accumulation of TOH was influenced by the treatments in 2016 and 2018. In the first year of the trial, a significant difference between its concentration for C (879 μg L^{-1}) and O2 (520 μg L^{-1}) systems was recorded. As for the last year, TOH amounts varied significantly between the C and O1 treatments with lower amounts detected for the first (431 *vs*. 858 μg L^{-1}). By comparing the data obtained for all vintages, 2017 was characterised by lower concentrations of TOH. IAA-ala, IAM, IAN, KYN, SKA and TAM were not detected in the wine samples.

2AAP in wines

The 2AAP content of the wines was evaluated after an accelerated ageing process;[21] the box plots of the distributions are reported in Fig. 4. The agronomical systems caused a significant difference in the concentration of 2AAP only in 2016, where the C treatment was associated with higher amounts compared to O1 (0.631 *vs*. 0.424 μg L^{-1}). A remarkable distinction between years was noticed, with much lower 2AAP concentrations in 2018 as opposed to the other vintages (Kruskal–Wallis, $P \leq 0.05$; Supporting Information, Table S6).

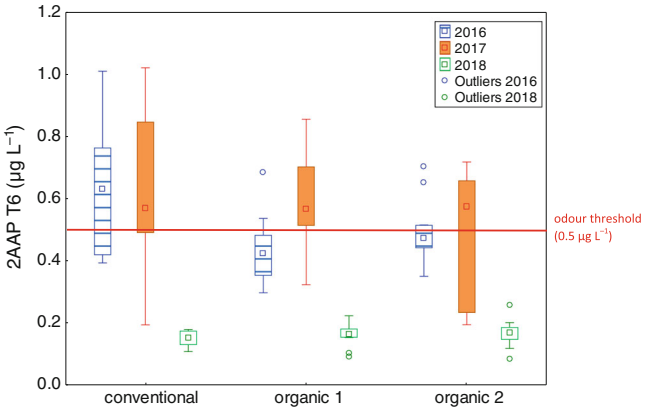

Figure 4. Box plots of distribution of 2-aminoacetophenone (2AAP) in wines obtained from grapes cultivated using different agronomic systems after accelerated ageing (40 °C, 6 days); concentrations are reported in μg L^{-1}.

Statistics

To describe the impact of the agronomic systems and the vintage effect on ATA development, a PCA with the concentrations of the main ATA precursors detected in wines and musts as well as 2AAP after ageing was performed (Fig. 5). Principal component 1 (PC1) explained 26.7% of the variance and principal component 2 (PC2) explained 17.1% of the variance, representing 43.8% of the total variance. F1 was strongly correlated with IAA-Asp and 2AAP, while F2 was strongly correlated with IAA and TRH.

In addition, to predict the formation of 2AAP in the wines after ageing (T6), the ANCOVA model of linearisation was used (Fig. 6). The factors considered in the creation of the model were vintages and treatments (qualitative variables) as well as AA concentrations in the musts and accumulation of 2AAP precursors in the wines (quantitative variables). Figure 6 shows the graph of the ANCOVA model representation with the predicted 2AAP (μg L^{-1}) values *versus* the observed 2AAP (μg L^{-1}) values for each year. The predicted model has an R^2 (coefficient of determination) of 0.86, indicating that about 86% of the variability of the dependent variable was described by the explanatory variables. To assess the goodness of the model, mean squared error (MSE) and root mean squared error (RMSE) were calculated and values of 0.014 and 0.12 were respectively obtained. Additionally, Fisher's *F*-test was executed and a value of <0.0001 was obtained. This means that a risk lower than 0.01% is taken when assuming the null hypothesis (no effect of the explanatory variable) to be wrong.

DISCUSSION

The aim of this study was to investigate the impact of three different agronomic systems on the development of ATA. Grapevine N status is a compelling factor affecting the formation of this sensorial defect[8,10,24] and it ultimately influences must AA composition and NH_4^+ accumulation.[25-27] Since the treatments under

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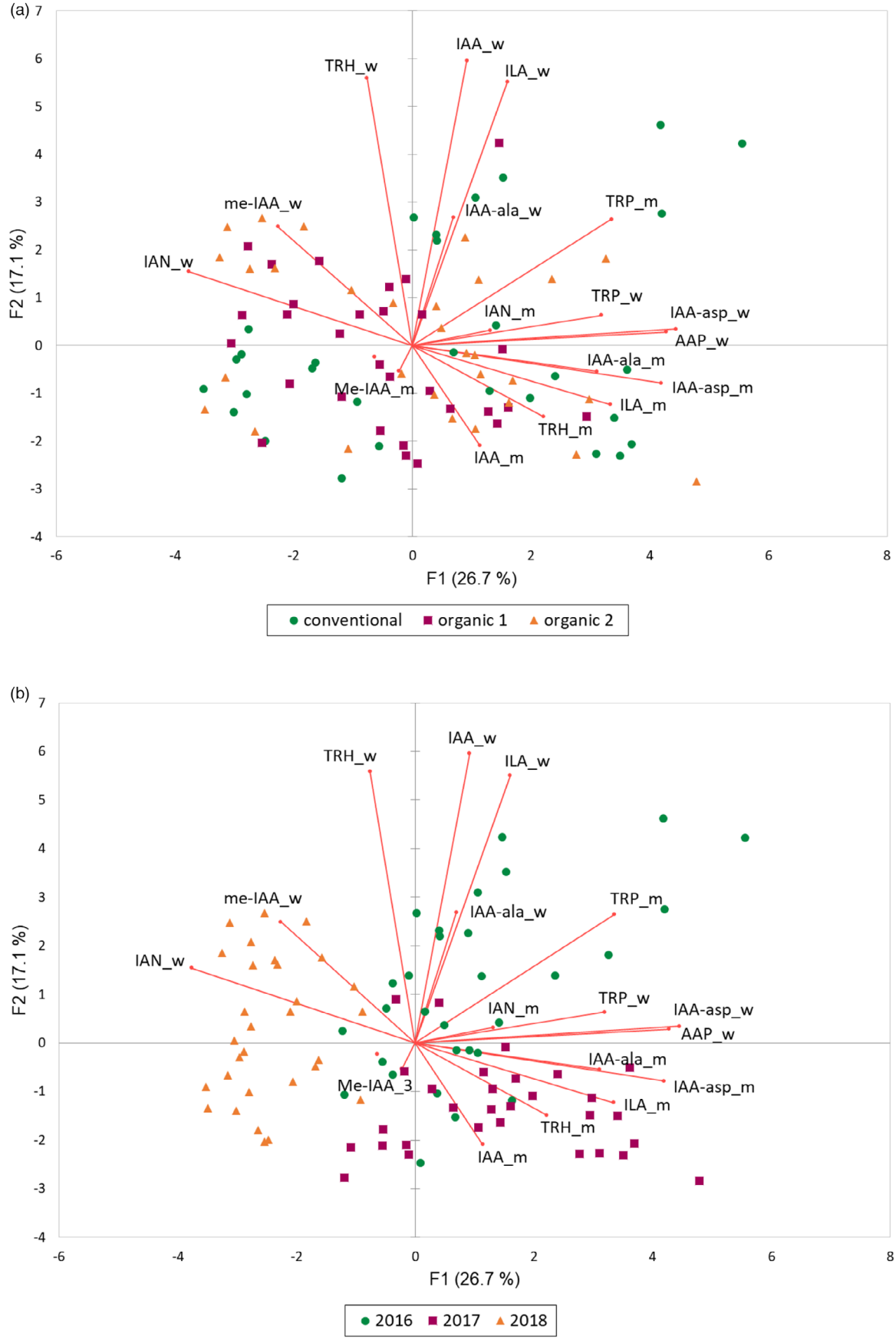

Figure 5. Principal component analysis (PCA) performed with atypical ageing (ATA) precursors and 2-aminoacetophenone (2AAP) detected in must (m) and wine (w) samples in relation to management system (a) and vintage (b). 2AAP values were measured upon accelerated ageing.

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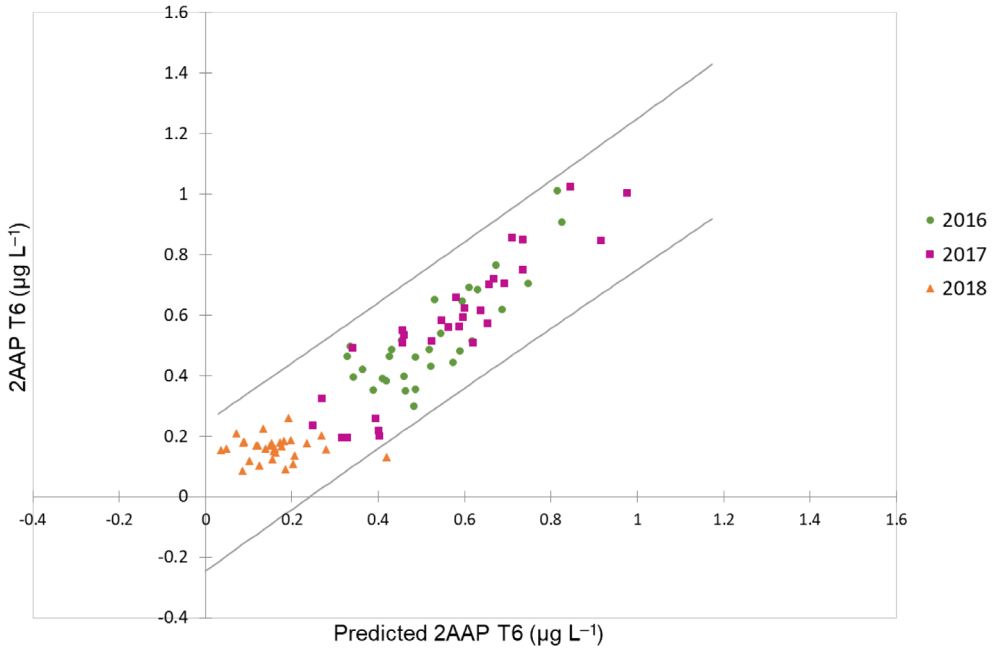

Figure 6. Chart of the ANCOVA model representation with the predicted 2-aminoacetophenone (2AAP) (µg L^{-1}) values after accelerated ageing (T6) *versus* the observed 2AAP (µg L^{-1}) values at T6. Confidence intervals identify potential outliers.

evaluation differed with regard to the fertilisation regime, the AA profiles along with NH_4^+ concentrations were measured and used to assess the impact of diverse plant nutrient supplementations on ATA formation. In disagreement with Garde-Cerdán *et al.*,[17] who found that organic grapes display lower concentrations of N compounds compared to non-organic ones, no treatment-associated differences were observed in relation to must AA composition in our trial. While the AA profiles mostly fell within the ranges reported in the literature,[28] their concentrations varied significantly across the vintages. Since seasonal conditions are known to affect their accumulation,[29,30] weather data were explored. The last year of the trial was characterised by mildly higher average temperatures (Supporting Information, Table S4) and a low accumulation of AAs. This is in agreement with the findings of Linsenmeier *et al.*,[8] who observed that the AA concentrations in must respond to seasonal influences. More specifically, as opposed to cooler years, they detected a higher amount of total AAs in a vintage characterised by low temperatures.

Among the quantified AAs, of special interest is Pro, which correlates with the osmotic stress levels: its concentration naturally rises with sugar accumulation during berry ripening and following external events such as high temperatures, rainfall and soil salinisation.[31-33] Compared to the other vintages, the concentration of Pro in the musts was significantly lower in 2018. This AA being a stress indicator in plants,[34] it was speculated that grapevines were less subjected to exogenous stress in that year.

GSH is a natural compound endemic to many plants[20] and is found in grapes as well.[35] Due to its ability to act as an antioxidant and inhibit the formation of free radicals[36,37] it has been evaluated as a tool in the reduction of ATA formation but found not to be very effective.[18] The impact of different agronomic systems on GSH accumulation could not be assessed but a variation of concentrations across the vintages was noticed.

To gain a better understanding of the vines' N status, NH_4^+ accumulation was measured: grapes cultivated with the O2 system displayed lower amounts compared to those produced with C. It was speculated that the use of green manure might have caused a reduction in the concentration of NH_4^+. However, the O2 system did not cause the overall N status of the grapevines to be low as the AA accumulation was not significantly affected by the treatments.

Biogenic amines are N-containing compounds which might pose a threat to human health when present above certain amounts in foods.[28] Some researchers have demonstrated that organic wines are more prone to their accumulation[38,39] and therefore their presence was assessed in the must samples. Amines are mainly produced during the fermentation process by microbial decarboxylation of the corresponding AA precursors or reductive amination/transamination of the corresponding aldehydes or ketones.[40,41] Yet their fate is not only determined by the presence of microorganisms as they also develop in the berries in response to abiotic factors such as heat, water stress and salt.[42] Different agricultural practices did not influence amine accumulation but the concentrations of those compounds varied according to the vintage, as also demonstrated by Martín-Álvarez *et al.*[43] By looking at the weather data, compared to the other years 2016 was characterised by lower precipitations (Supporting Information, Table S4). Even if this could hint at a water deficit situation, grapes were irrigated when needed and therefore no speculations could be made. Moreover, a correlation between amine accumulation and water status is not definitive as contrasting results are reported in the literature.[44]

2AAP is considered as the main chemical and sensorial marker of ATA.[1] It mainly forms in wine during ageing and originates from non-volatile precursors, of which IAA is the most prominent.[5] As the primary auxin (phytohormone) in plants, IAA is carefully regulated at the physiological level: it mostly occurs in its bound forms (ester conjugates of sugar moieties, amide conjugates of amino acids and methylated), since immobilisation via conjugation/methylation prevents it from being immediately used for

growth.[45] Traces of unbound IAA (<1.33 μg L^{-1}) were quantified in the musts under examination. With regard to the bound forms, some of them were evaluated in the juices and only IAA-Asp was found in minor amounts (<0.46 μg L^{-1}). Simat *et al.*[5] and Hoenicke *et al.*[46] also measured the free and bound IAA concentrations in the must samples. While they obtained similar results for the free form, they reported higher concentrations of bound IAA (35–120 μg L^{-1}) making a total quantification after an alkaline hydrolysis. When grape must is inoculated, yeast can actively transport bound IAA into the cell and following the cleavage of the bonds use the AAs for its metabolism.[5] Within the first steps of the fermentation process, IAA is then released in its free form.[47] The amounts of free IAA measured upon fermentation were considerably higher (2.09–17.5 μg L^{-1}) than those detected in the must: this was expected and found to be in agreement with the findings reported by Linsenmeier *et al.*[8] Additionally, small amounts of IAA-Asp and me-IAA (<4 μg L^{-1}) were detected in the wine samples under evaluation.

It has been suggested that the biosynthesis of IAA in plants occurs following two distinct routes: a TRP-dependent and an independent one. The first encompasses four pathways: the IAM, indole-3-pyruvic acid, YUCCA and indole-3-acetaldoxime; the second (independent) originates from the precursor indole-3-glycerol phosphate.[48,49] Moreover, the existence of a TOH pathway, which derives from the TRP metabolism and leads to IAA formation, has also been demonstrated.[50] In brief, the amount of free IAA in wine depends not only on the concentration of its bound forms present in the grapes but is also affected by the metabolic pathway of the microorganisms present during the vinification process.

Despite IAA being considered the most important precursor in the formation of 2AAP, it has been suggested that SKA and KYN might play an important role in ATA development as well.[4,51] Additionally, ILA and IAN could indirectly contribute to ATA off-flavour generation in wines.[5,52] Considering all the known intermediate compounds, to investigate the formation of the aroma fault, the concentrations of the following precursors were evaluated: ILA, IAM, IAN, TAM, TOH, SKA and KYN. Except for ILA and TOH, none of the other compounds could be detected in the samples under examination. While ILA concentrations in the musts were quite low (<1.45 μg L^{-1}), higher values (>18.7 μg L^{-1}) were detected in the corresponding wines. This was in agreement with the findings of Simat *et al.*[5] and Hoenicke *et al.*,[46] who reported an increase in ILA concentrations upon fermentation and sulfitation of the wines. TOH is the main degradation product of TRP metabolism (decarboxylation) performed by the yeast.[53] Absent in the musts, this compound was detected only in the wine samples (Supporting Information, Tables S5 and S6).

Except for the vintage 2016, the free IAA and TRP amounts quantified in our wine samples did not show any significant difference with regard to the examined management systems (Fig. 3). Moreover, compared to what is reported in the literature,[18,54] the concentrations of unbound IAA in the wines were relatively low (2.09–17.5 μg L^{-1}). It was speculated that the restricted amounts of 2AAP precursors detected in our samples resulted from the limited yield applied while pressing the grapes. Indeed, it has been demonstrated that those compounds mainly accumulate in the skins of the fruit.[23] The amounts of 2AAP generated from the accelerated ageing process were limited (0.08–1.02 μg L^{-1}) and slightly exceeded the odour threshold in vintages 2016 and 2017 (Fig. 4 and Supporting Information, Table S6). This is an important result as, no matter what grapevine management system is used, the presence of ATA-related compounds was not affected by the treatment but linked instead with the seasonal fluctuations. This result was confirmed by the PCA reported in Fig. 5.

The treatments did not cause any difference in the formation of 2AAP: besides ILA, none of the precursors detected in the wines was affected by the use of different agronomic systems. With regard to ILA, the treatments influenced its accumulation but, despite O2 being characterised by a higher concentration of this compound compared to O1, no differences in 2AAP amounts were noticed. This was expected as the conversion rate of ILA to 2AAP is only 0.95 mol%.[19] All of the 2AAP precursors detected in the wine samples greatly varied according to the vintage year (Supporting Information, Table S6) and this was confirmed by the PCA reported in Fig. 5(b). Finally, using an ANCOVA model of linearisation, it was possible to predict with a reasonable accuracy the potential development of 2AAP in the aged wines (Fig. 6).

CONCLUSIONS

In this study it was demonstrated that the use of different agronomic systems in grapevine farming did not affect the development of ATA-related compounds in wine. The occurrence of this aroma defect seems to be related to other factors that are linked to seasonal influences. Our results revealed the existence of a strong vintage effect that characterised 2018. Compared to the other vintages, this year was marked by a lower accumulation of AAs as well as reduced concentrations of ATA precursors both in musts and in wines. For our samples, 2018 could be considered a potentially ATA-free vintage as the 2AAP amounts measured after ageing (T6) were well below the odour threshold (0.5 μg L^{-1}), regardless of the agronomic system adopted.

This finding is of great relevance for winegrowers and winemakers as it demonstrates that a well-planned organic management system correctly adjusted to the climatic conditions does not pose a threat towards the development of the main ATA-related compounds in wine. Additionally, it highlights the need for further studies on the development of this off-flavour and the influence of various viticultural practices.

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CONFLICT OF INTEREST

The authors declare no competing interests.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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