APPENDIX A: TREATMENT FREQUENCY INDICATOR (TFI)

Tableau A1 : TFI 'resistant variety' wine

Target	Product	Applied dose (kg or L/ha)	Legally approved dose (kg or L/ha)	Treated surface/Total surface	TFI	Biocontrol
Leafhopper, 'flavescende dorée'	Pyrevert	1,5	1,5	1	1,00	No
Leafhopper, 'flavescende dorée'	Pyrevert	1,5	1,5	1	1,00	No
Grape worm*	Bacivers	0,75	0,75	1	1,00	Yes
TOTAL TFI					**3,00**	
TOTAL TFI EXCLUDING BIOCONTROL PRODUCTS					2,00	

*Grape worm: *Lobesia botrana* and *Eupoecilia ambiguella*

Table A2 : TFI 'Standard conventional' wine

Target	Product	Applied dose (kg or L/ha)	Legally approved dose (kg or L/ha)	Treated surface/Total surface	TFI	Biocontrol
Powdery mildew	Barreur	0,12	0,25	1	0,48	No
Powdery mildew	Armicarb	3	5	1	0,60	Yes
Powdery mildew	Barreur	0,12	0,25	1	0,48	No
Powdery mildew	Barreur	0,12	0,25	1	0,48	No
Powdery mildew	Prevam	0,6	1,6	1	0,38	No
Downy mildew	Lbg	1,5	4	1	0,38	Yes
Downy mildew	Kocide	1,5	3	1	0,50	No
Powdery mildew	Prosper	0,6	0,6	1	1,00	No
Powdery mildew	Collis	0,4	0,4	1	1,00	No
Downy mildew	Privest	2,5	2,5	1	1,00	No
Grape worm	Mageos	0,1	0,1	1	1,00	No
Powdery mildew	Systhane new	1	1	1	1,00	No
Powdery mildew	Dynali	0,5	0,5	1	1,00	No
Downy mildew	Mildicut	4	4,5	1	0,89	No
Grape worm	Steward	0,125	0,125	1	1,00	No
Powdery mildew	Fluidosoufre	25	30	1	0,83	Yes
Powdery mildew	Prosper	0,6	0,6	1	1,00	No
Powdery mildew	Prosper	0,6	0,6	1	1,00	No
Grey rot	Scala	1,8	2,5	1	0,72	No
Grey rot	Armicarb	3	5	1	0,60	Yes
Downy mildew	Nordox 75 WG	2	2	1	1,00	No
Grape worm	Steward	0,125	0,125	1	1,00	No
Downy mildew	Nordox 75 WG	2	2	1	1,00	No
Grape worm	Steward	0,125	0,125	1	1,00	No
TOTAL TFI					**19,33**	
TOTAL TFI EXCLUDING BIOCONTROL PRODUCTS					16,92	

Tableau A3 : TFI 'premium conventional' wine

Target	Product	Applied dose (kg or L/ha)	Legally approved dose (kg or L/ha)	Treated surface/Total surface	TFI	Biocontrol
Weeding	Buggy plus	2	5,3	0,5	0,19	No
Dead arm 'excoriose'	Chaoline	2	3	1	0,67	No
Downy mildew /Black rot	Chaoline	3	4	1	0,75	No
Black rot	Antène	0,4	0,4	1	1,00	No
Downy mildew /Black rot	Chaoline	3	4	1	0,75	No
Black rot	Antène	0,4	0,4	1	1,00	No
Weeding	Buggy plus	2	5,3	0,5	0,19	No
Downy mildew /Black rot	Chaoline	4	4	1	1,00	No
Powdery mildew /black rot	Luna sensation	0,4	0,2	1	2,00	No
Grape worm	Reldan	1,5	1,5	1	1,00	No
Downy mildew /Black rot	Chaoline	4	4	1	1,00	No
Powdery mildew /Black rot	Milord	0,4	0,5	1	0,80	No
Downy mildew	BB RSR Disperss	1	3,75	1	0,27	No
Powdery mildew /Black rot	Milord	0,5	0,5	1	1,00	No
Downy mildew	BB RSR Disperss	1	3,75	1	0,27	No
Powdery mildew	Prosper	0,5	0,6	1	0,83	No
TOTAL TFI					**12,71**	
TOTAL TFI EXCLUDING BIOCONTROL PRODUCTS					12,71	

Tableau A4 : TFI 'organic' wine

Target	Product	Applied dose (kg or L/ha)	Legally approved dose (kg or L/ha)	Treated surface/Total surface	TFI	Biocontrol
Powdery mildew	Microthiol	3	12,12	1	0,25	Yes
Powdery mildew	Microthiol	3	12,12	1	0,25	Yes
Powdery mildew	Microthiol	3	12,12	1	0,25	Yes
Powdery mildew	Microthiol	3	12,12	1	0,25	Yes
Powdery mildew	Microthiol	3	12,12	1	0,25	Yes
Powdery mildew	Microthiol	3	12,12	1	0,25	Yes
Powdery mildew	Fluidosoufre	10	30	1	0,33	Yes
Powdery mildew	Fluidosoufre	10	30	1	0,33	Yes
Downy mildew	Kocide 35 DF	1,5	3	1	0,50	No
Downy mildew	Kocide 35 DF	1,5	3	1	0,50	No
Insecticide	Bacivers	0,75	0,75	1	1,00	Yes
Insecticide	Bacivers	0,75	0,75	1	1,00	Yes
Insecticide	Pyrevert	1,5	1,5	1	1,00	No
TOTAL TFI					**6,15**	
TOTAL TFI EXCLUDING BIOCONTROL PRODUCTS					2,00	

APPENDIX B: ANALYSIS OF RESIDUES

For pesticide residue analyses, the ISVV used two extraction methods for each wine sample: QuEChERS extraction was performed as described in the official OIV resolution (OIV-OENO 436-2012), and Solid-Phase Extraction (SPE) was carried out using C18 cartridges following an internal procedure validated by the laboratory. Both extraction methods used three internal standards. The extracts were then injected on a C18 column for separation by reverse-phase liquid chromatography and the compounds were detected using the dMRM mode (dynamic Multiple Reaction Monitoring) on a triple quadrupole mass spectrometer (Agilent Technologies). To analyze the chromatographic data, first a screening method containing 190 compounds was used to identify the pesticide residues in the wines. The detected compounds were then quantified using a validated method and confirmed by the standard addition procedure. Besides, copper determination was carried out by atomic absorption spectrophotometry according to the official method OIV-MA-AS322-06 (OIV-OENO 377-2009 resolution).

In the analysis protocol put in place by the IIAA, the samples were extracted on reversed-phase SPE cartridges with no additional concentration or purification step. Stable isotopes of cyprodinil, metalaxyl, imidacloprid and carbendazim were used as internal standards. Determination was carried out by LC-ESI-MS/MS with a UPLC-type C18 column. Each sample was analyzed in duplicate, and the concentrations presented here are derived from their average. In Table A5, the quantitative results and limits of quantification of both laboratories are given in separate columns.

Tableau B1: Pesticide residue analyses

Wines / **Criteria**			**'Resistant variety' wine**		**'Standard conventional' wine**		**'Premium conventional' wine**		**Organic wine**		
Organoleptic quality			Standard		Standard		Superior		Standard		
Pesticide residue analyses	LOQ ISVV µg/L	LOQ IIAA µg/L	ISVV µg/L	IIAA µg/L	ISVV µg/L	IIAA µg/L	ISVV µg/L	IIAA µg/L	ISVV µg/L	IIAA µg/L	10 % of wine grape MRL µg/kg
ametoctradine	0.3	0.5	0.3*	n.d.	6	7.9	n.d.	n.d.	n.d.	n.d.	600
boscalid	1	0.3	n.d.	n.d.	9	9.6	n.d.	n.d.	n.d.	n.d.	500
carbendazim	1	0.5	n.d.	n.d.	1*	1.1*	n.d.	n.d.	n.d.	n.d.	50
fenhexamid	1.5	0.5	n.d.	1.1*	n.d	n.d	n.d	n.d	n.d	n.d	1500
myclobutanil	1	0.5	n.d.	n.d.	0.3 (LD)	n.d.	n.d.	n.d.	n.d.	n.d.	100
pyrimethanil	0.6	0.3	0.7*	0.8*	70	81.4	n.d.	n.d.	n.d.	n.d.	500
fluopyram	0.1	-	n.d.	-	n.d.	-	0.5	-	n.d.	-	150
pyriofenone	0.2	-	n.d.	-	n.d.	-	0.2*	-	n.d.	-	20
spiroxamine	0.5	-	n.d.	-	1.5	-	n.d.	-	n.d.	-	50
tetraconazole	0.9	-	n.d.	-	n.d.	-	1	-	n.d.	-	50
cuivre			28*	-	45	-	79	-	200	-	5000
Phytosanitary product residues			No residue of applied pesticide		Residues of 6 applied pesticides		Residues of 3 applied pesticides		Applied copper residues		

LOQ: limit of quantification

n.d.: not detected

*: not applied in the vineyard

The pesticide residue levels found in the samples are very low and almost exclusively below 10 µg/L except for pyrimethanil in the 'standard conventional' wine. However, for the latter, the measured concentration is at least six times lower than 10% of the wine grape MRL (ie less than 1.6% of the wine grape MRL).

Both laboratories found similar results concerning the 33 molecules initially selected for quantification.

In the 'standard conventional' wine, only four compounds could be quantified: ametoctradine, boscalid, carbendazim and pyrimethanil and one compound (myclobutanil) was found at trace level. Carbendazim concentration was low, close to the limit of quantification. The use of this compound is forbidden in France but thiophanate-methyl, which is allowed, is known to decompose in carbendazim. In the 'resistant variety' wine, pyrimethanil was quantified at a level close to the limit of quantification. ISVV also found traces of ametoctradine (close to the limit of quantification) and IIA found traces of fenhexamid. For 'organic' and 'premium conventional' samples no trace of any of the 33 compounds was detected.

Following the screening of the wine samples (190 compounds sought), the ISVV could detect and quantify spiroxamine in the 'standard conventional' sample and low levels of fluopyram, pyriofenone and tetraconazole in the 'premium conventional' sample.

Knowledge of the phytosanitary treatment schedule confirms the use of all compounds found in the 'standard conventional' sample except carbendazim or thiophanate-methyl. However some active compounds applied were not found (cyazofamid, cycflufenamid, difenoconazole, indoxacarb, kresoxim-methyl and tetraconazole). For the 'resistant variety' wine none of the compounds found in the sample at trace level were used. The detected residues may be due to contamination in the vineyard as both vine plots are close or in the cellar, however no fenhexamid was used. 'Resistant variety' and 'organic' wines did not receive phytosanitary treatments with any molecules included in the analytical methods. Concerning the 'premium conventional' wine phytosanitary treatments include, in particular, fluopyram, spiroxamine, tebuconazole, tetraconazole and trifloxystrobin. Traces of fluopyram and tetraconazole were detected but no spiroxamine, tebuconazole or trifloxystrobin. Moreover, no pyriofenone was applied to the vines. In addition, some compounds used in the vineyards are not included in the analysis method (alpha-cypermethrin, chlorpyrifos-methyl, glyphosate, fosetyl-aluminum, pyrethrins, sulfur-based products, potassium bicarbonate, potassium phosphonate, disodium phosphonate, sweet-orange essential oil, microorganisms).

Copper was applied in all vineyards that were used to produce the wine samples except 'resistant variety' wine. Analyses showed that 'resistant variety' and 'standard conventional' wines contain less copper than 'organic' and 'premium conventional' wines. The copper content of the 'resistant variety' sample may again result from a drift of the phytosanitary treatment in the vineyard or a contamination during the winemaking process in the cellar.

From the knowledge of the phytosanitary treatment schedule for the production of the four sampled wines it could be noted that metiram was used once for 'standard conventional' wine and five times for 'premium conventional' wine. This compound is well known to degrade into ethylenethiourea (ETU). Using an analytical method under development, the IIAA detected a weak signal for ETU, although below the LOQ (15 µg/L), only on the 'standard conventional' sample.