





SEMINÁRIO
Variedades resistentes,
alternativa aos fungicidas?
REFLEXÃO







**Resíduos de pesticidas no vino.
Abordagem analítica e dados recolhidos**
(Pesticide residues in wine. Analytical approaches and occurrence data)





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Some common problems in vineyards










Are these treatments affecting the quality and/or market value of wine?


- Legal aspects (MRLs)
- Volatile profile wine
- Consumers acceptance
- Marketing

Maybe, analytical chemists are able to provide some information









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Grape producers and chemists do not use the same terms

Commercial name	Active principle
Switch	Cyprodinil, Fludioxonil
Enervin	Amectotradin, Dimetomorph
Ridomild gold	Metalaxyl, Mancozeb
Profler	Fluopicolid, Fosetyl-AI
Melody Combi	Iprovalicarb, Folpet
Fobeci	Benalaxyl, Folpet, Mancozeb
Electis	Zoxamide, Mancozeb
Teldor	Fenhexamide
Scala	Pyrimethanil
Switch	Cyprodinil, Fludioxonil
Confidor	Imidacloprid
Intrepid	Methoxyfenozide

Legislation and laboratory data are always referred to the active principle



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Laboratory tools for the analysis of phytochemical residues

Sample



Sample preparation

**SPE
extraction**

QuEChERS
Quick, Easy, Cheap,
Effective, Rugged, Safe

**LLE
extraction**

Compounds determination



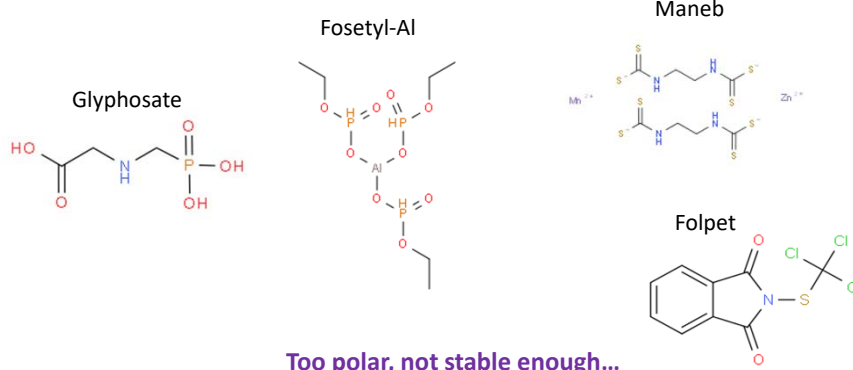
Many active chemicals can be simultaneously determined !



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Laboratory tools for the analysis of phytochemical residues

Some compounds are quite particular...they require dedicated methodologies



Too polar, not stable enough...
They require specific, complex and normally expensive approaches.



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Some compounds determined in our laboratory and their MRLs

Active compound	Use	MRL grapes (mg/Kg)	Active compound	Use	MRL grapes (mg/Kg)
Ametoctradine	Fungicide	6	Imidacloprid	Insecticide	1
Aminobenzimidazole	-	-	Iprodione	Fungicide	20
Azoxystrobin	Fungicide	3	Iprovalicarb	Fungicide	2
Benalaxyl	Fungicide	0,3	Mandipropamide	Fungicide	2
Bitertanol	Fungicide	0,01	Metalaxyl	Fungicide	1
Boscalid	Fungicide	5	Myclobutanil	Fungicide	1
Carbendazim	Fungicide	0,5	Penconazole	Fungicide	0,2
Chlorantraniliprole	Insecticide	1	Propiconazole	Fungicide	0,3
Clofentezine	Acaricide	1	Pyraclostrobin	Fungicide	2
Clothianidin	Insecticide	0,7	Pyrimethanil	Fungicide	5
Cyflufenamid	Fungicide	0,15	Quinoxifen	Fungicide	1
Cyprodinil	Fungicide	3	Tebuconazole	Fungicide	1
Difenoconazole	Fungicide	3	Tebuconazole	Fungicide	1
Dimethomorph	Fungicide	3	Tebuconazole	Insecticide	3
Diniconazole	Fungicide	0,01	Thiabendazole	Fungicide	0,05
Fenhexamide	Fungicide	15	Thiamethoxan	Insecticide	0,4
Fenpropidine	Fungicide	0,01	Thiophanate-methyl	Fungicide	3
Fludioxonil	Fungicide	4	Triadimefon	Fungicide	2
Fluopicolide	Fungicide	2	Triadimenol	Fungicide	2
Flusilazole	Fungicide	0,01	Trifloxystrobin	Fungicide	3

OIV recommendation: wine levels should not exceed 10% of MRLs for grapes.

Lack of specific MRLs for fungicides in wine (UE countries).

<http://ec.europa.eu/food/plant/pesticides/eu-pesticidesdatabase/public/?event=homepage&language=EN>

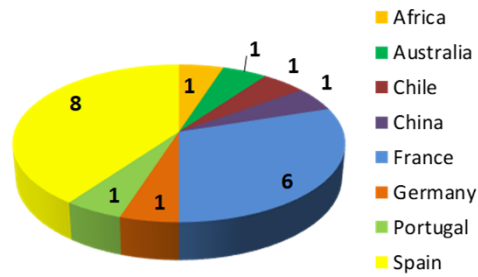


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Do fungicides and insecticides pass to elaborated wines?

SURVEY OF PESTICIDE RESIDUES IN COMMERCIAL WINES (2015-2016)

Geographic origin of conventional wines

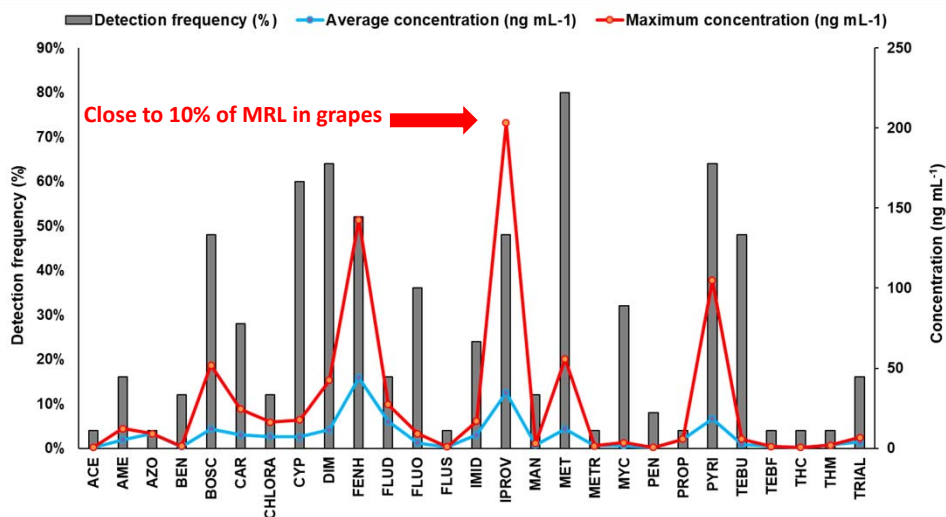


G. Castro et al., Anal. Bioanal. Chem. 410 (2018) 1139



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Data obtained for commercial wines



All wines contained residues of at least one compound !!!



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Residues of selected compounds in wines from regions adverse weather conditions (2013-2014)

Compound	Positive samples	White wine (n=14 samples)		
		< 10 ng mL ⁻¹	10- 30 ng mL ⁻¹	> 30 ng mL ⁻¹
Iprovalicarb	10	4	2	4 (49 ng mL ⁻¹)
Benalaxyl	3	3	0	0
Metalaxyl	12	3	2	7 (97 ng mL ⁻¹)
Triadimenol	6	6	0	0
Fenhexamide	9	6	0	3 (64 ng mL ⁻¹)
Myclobutanil	3	3	0	0
Pyrimethanil	10	6	1	3 (82 ng mL ⁻¹)
Cyprodinil	10	9	1	0
Fludioxonil	8	7	1	0
Tebuconazole	3	3	0	0
Procymidone	4	4	0	0
Iprodione	11	6	2	3 (73 ng mL ⁻¹)
Azoxystrobin	1	1	0	0

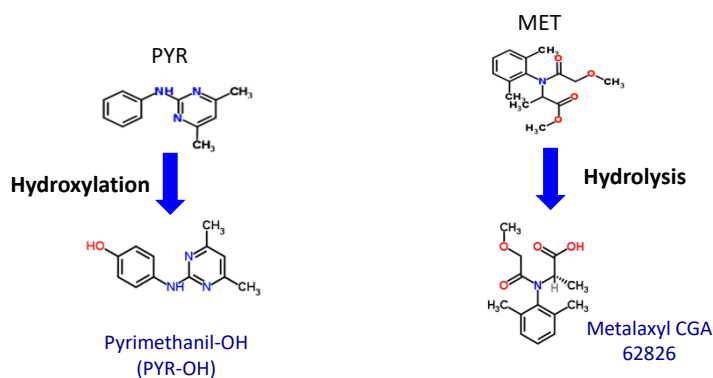
T. Rodríguez-Cabo et al., J. Chromatogr. A 1442 (2016) 107

With the current UE legislation, it is hard to violate the 10% of MRLs set for vinification grapes



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Fungicides are also transformed after field application



Transformation products (TPs) are not regulated.
 Their toxicity has not been investigated (in most cases).
 They might appear in wine and/or remain in the vineyard environment.
 Conventional analytical methods are blind to TPs.



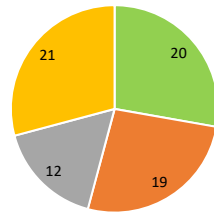
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Screening of selected TPs in commercial wines

Number of positive samples for parent fungicides and TPs.
Total 22 wines

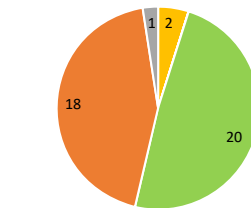
Parent pesticides

■ PYR ■ CYP ■ IMID ■ MET



TPs

■ MET- CGA 62826 ■ PYR-OH
■ CYP-OH ■ IMID-olef

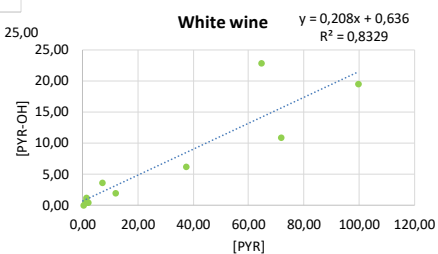
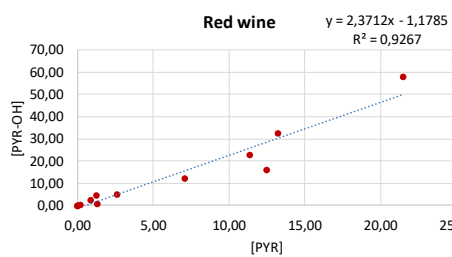


I. Rodríguez et al., in preparation



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Correlation between TPs and parent fungicide concentrations



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Do lab data match with grape producers records?

Data for experimental wines obtained in micro-vinification experiments

	Wine 1 (ng mL ⁻¹)			Wine 2 (ng mL ⁻¹)		
	Lab 1	Lab 2	Recorded by producer	Lab 1	Lab 2	Recorded by producer
Pyrimethanil	4	2	NO	1,2	0,6	NO
Cyprodinil	1,7	0,9	NO	0,4	0,2	NO
Imidacloprid	17,5	12	YES	37,8	40	YES
Metalaxyl	1,7	1,1	NO	1,4	1,1	NO
Fenhexamid	68,2	49	YES	22	16	YES
Tebuconazole	4,6	4	YES	0,4	0,1	YES
Iprovalicarb	399	311	YES	146	140	YES
Iprodione	16	7	NO	85	46	YES
Fluopicolid	19	25	YES	16,6	23	YES
Dimethomorph	0,6	0,4	NO		0,3	NO

- Both labs found compounds not applied in the experimental vineyards.
- Contamination at caves is unlikely in micro-vinification experiments.
- Levels of non applied compounds are not relevant for conventional wines.



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Are we able to produce commercial wines free of pesticides?

Pesticides in commercial ECO wines

Compound	Spain	Spain	Spain	Spain	Spain
	E1	E2	E3	E4	E5
ACE					
AME					
AZO					
BEN					
BOSC					
CAR	4.3				
CHLORA					
CYP	3.4				
DIM		6.1			
FENH		8.3			
FLUD					
FLUO					
FLUS					
IMID					
IPROV		3.5			
MAN					
MET	5.9	4.8	1.2	0.9	n.d.
METR					
MYC		0.7			
PEN					
PROP					
PYRI		15.3			
TEBU	1.9				
TEBF					
THC					
THM					
TRIAL		0.5			
Total conc. (ng mL⁻¹)	15.5	39.2	1.2	0.9	

Metalaxyl residues in experimental wines, from grapes not treated with this a.i.

Code	Concentration (ng mL ⁻¹)
G1	0,6
G2	n.d.
G3	1,7
G4	1,4
CAT1	0,9
CAT2	0,8
CAT3	0,6
CAT4	n.d.
CAT5	0,2
CAT6	0,3
POR1	3
POR2	1
POR3	1



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So,...

- Analytical chemists never report/guarantee a “zero concentration level”.
- Analytical tools are able to detect and to measure lower and lower concentration levels.
- Several parameters might lead to grapes contamination
 - Transport of pesticides in the atmosphere...
 - Existence of pesticide residues in soils...
 - Fungicides free grapes can be contaminated at caves..

Will be interesting/possible to force legislators to fix a threshold value above the LOQs of analytical methods?



If your wine contain less than X ng mL⁻¹, then it can be considered as “pesticide” free ...



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Known transfer factors from grapes to wine

Compuesto	Abrev.	Factor de transferencia (%)	Ref.
Acetamiprid	ACE	98	[5]
Azoxystrobin	AZO	68	[6]
Boscalid	BOSC	10	[7]
Carbendazim	CAR	82	[5]
Chlorpyrifos	CHLOR	4	[6]
Cyprodinil	CYP	19	[8]
Dimetomorph	DIM	14	[7]
Fenhexamid	FENH	60	[7]
Fludioxonil	FLUD	30	[8]
Imazalil	IMA	10	[5]
Imidacloprid	IMID	98	[5]
Iprovalicarb	IPROV	56	[9]
Metalaxyl	MET	69	[10]
Penconazole	PEN	52	[6]
Prochloraz	PROC	20	[7]
Pyraclostrobin	PYRA	< 2.5	[9]
Pyrimethanil	PYRI	92	[8]
Quinoxifen	QUIN	3	[10]
Tebuconazole	TEBU	38	[8]
Thiabendazole	THIAB	30	[5]

When several a.i. are available, it might be interesting to consider the TFs before application



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Are pesticides accumulated in vine soils?

Sampling date:
31th March 2018

Upper 5 cm soil layer

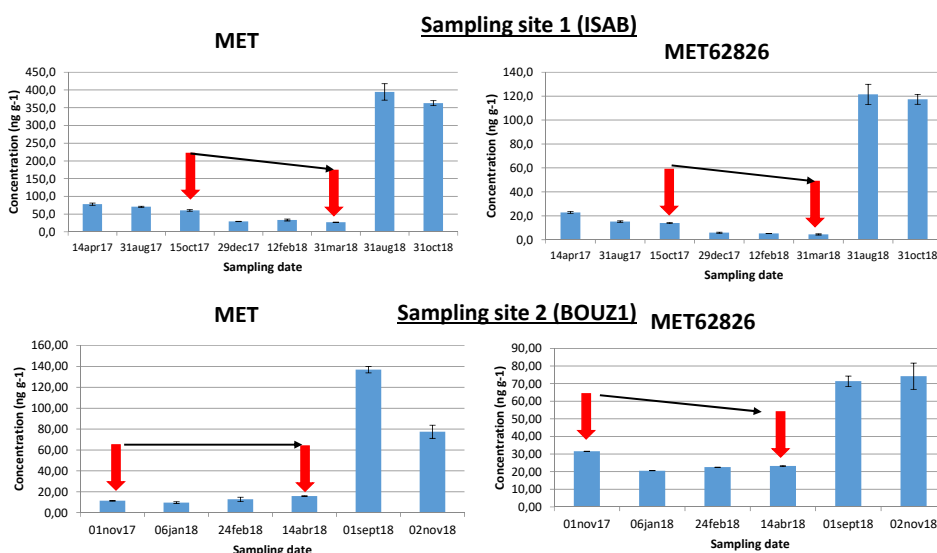
Concentrations
ng/g

Compound	SAMPLING CODES					
	ISAB	ISAA	PEP	BOU1	BOU2	CAMP
Carbendazim	201	8		357	118	16
Pyrimethanil	145					
Metalaxyl	27	36		16	19	9
MET62826	4	14		23	6,00	
Penconazole	52					65
Boscalid	32					
Dimethomorph 1	12			8		
Dimethomorph 2	502			27	6	
Myclobutanil		9	31		2	406
Pyraclostrobin		81				
Triadimenol			31			
Imidacloprid				67	58	
Fenhexamide				13	22	
Fludioxonil				9	24	316
Iprovalicarb				5	9	
Benalaxyl				9	10	73
Cyprodinil						78
Propiconazole						13
TOTAL (ng/g)	977	148	61	532	274	976

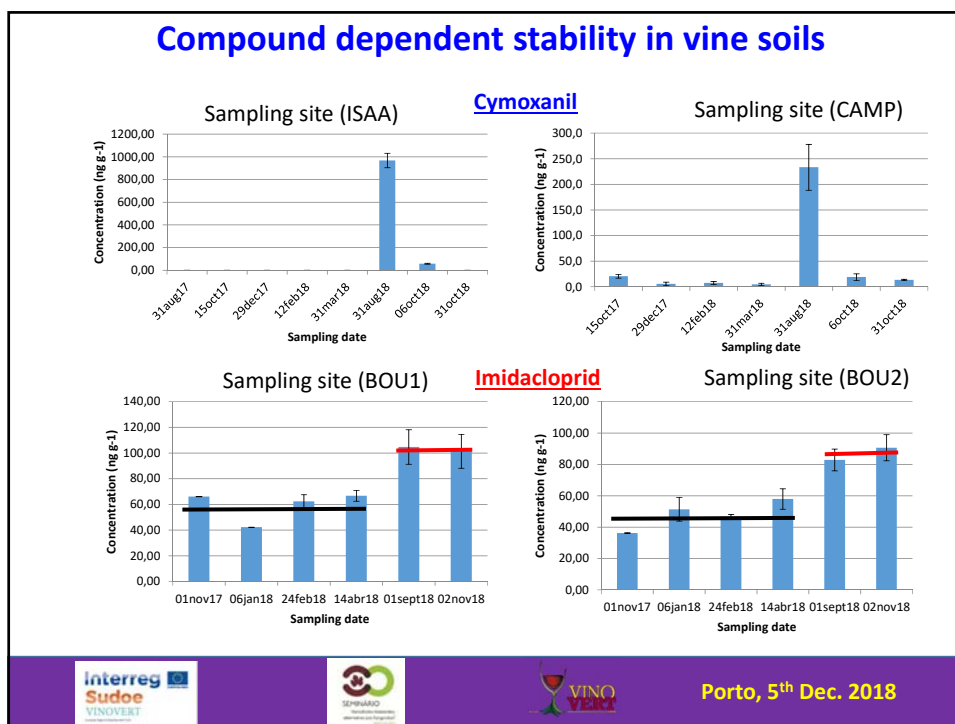


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Time-course of Metalaxyl in soil



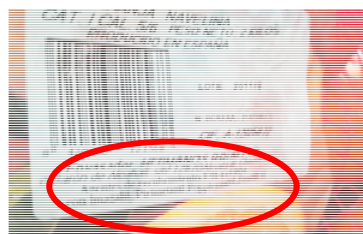
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Conclusions

- Fungicides and insecticides are partially transferred from grapes to wine.
- It is really difficult to get wines totally free of fungicide residues.
- With the UE legislation very few wines are above the 10% of MRLs for grapes.
- Environmental impact in wine production areas is likely relevant.
- Consumers are still sensible to words as “chemical residues”.
- **Wine analysis for pesticide residues does not improve, does not damage wine quality. It just provides data.**

What is the contribution of wine consumption to total human exposure to fungicides?





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**Obrigado pela sua
atenção !**



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