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Università di Pisa
Corso di Dottorato di Ricerca in
Scienze Agrarie, Alimentari e Agro-ambientali
PhD Program on Agriculture, Food and Environment

Responses to ozone toxicity of two Italian *Fraxinus excelsior* provenances

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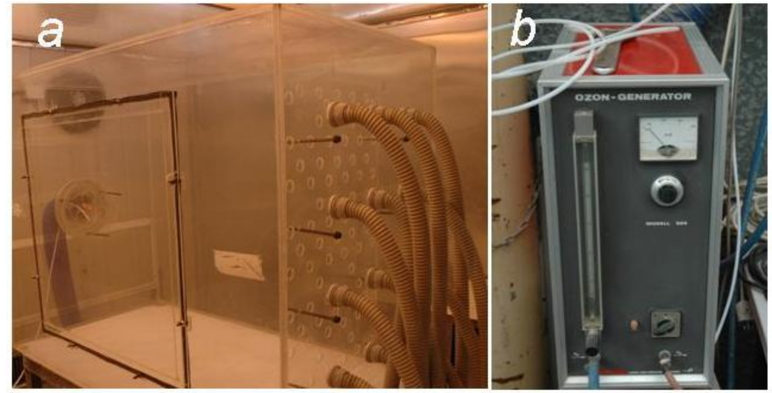
Aim of the work

- *Fraxinus excelsior*
  - 2 provenances:
    - Piedmont (North Italy)
    - Tuscany (Central Italy)

How they respond to relevant O_3 exposure?

Materials and Methods

- Exposure chamber
  - Two-year-old seedlings
  - O_3 concentration: 150 ppb, 8 h d^{-1}
  - 35 days (AOT40 = 30,800 ppb h d^{-1})



PHYSIOLOGICAL

- Visible injuries
- Leaf gas-exchanges
- Chlorophyll *a* fluorescence

BIOCHEMICAL

- Leaf pigments
- Secondary metabolites (phenolics)

Visible injuries

Piedmont

12 days
AOT40 = 10,560 ppb h

PII = 0.06

Tuscany

21 days
AOT40 = 18,480 ppb h

PII = 0.02

PII = (% of O_3-injured leaves × % of O_3-injured leaf surface per symptomatic leaf) / 100

Ecophysiological measurements

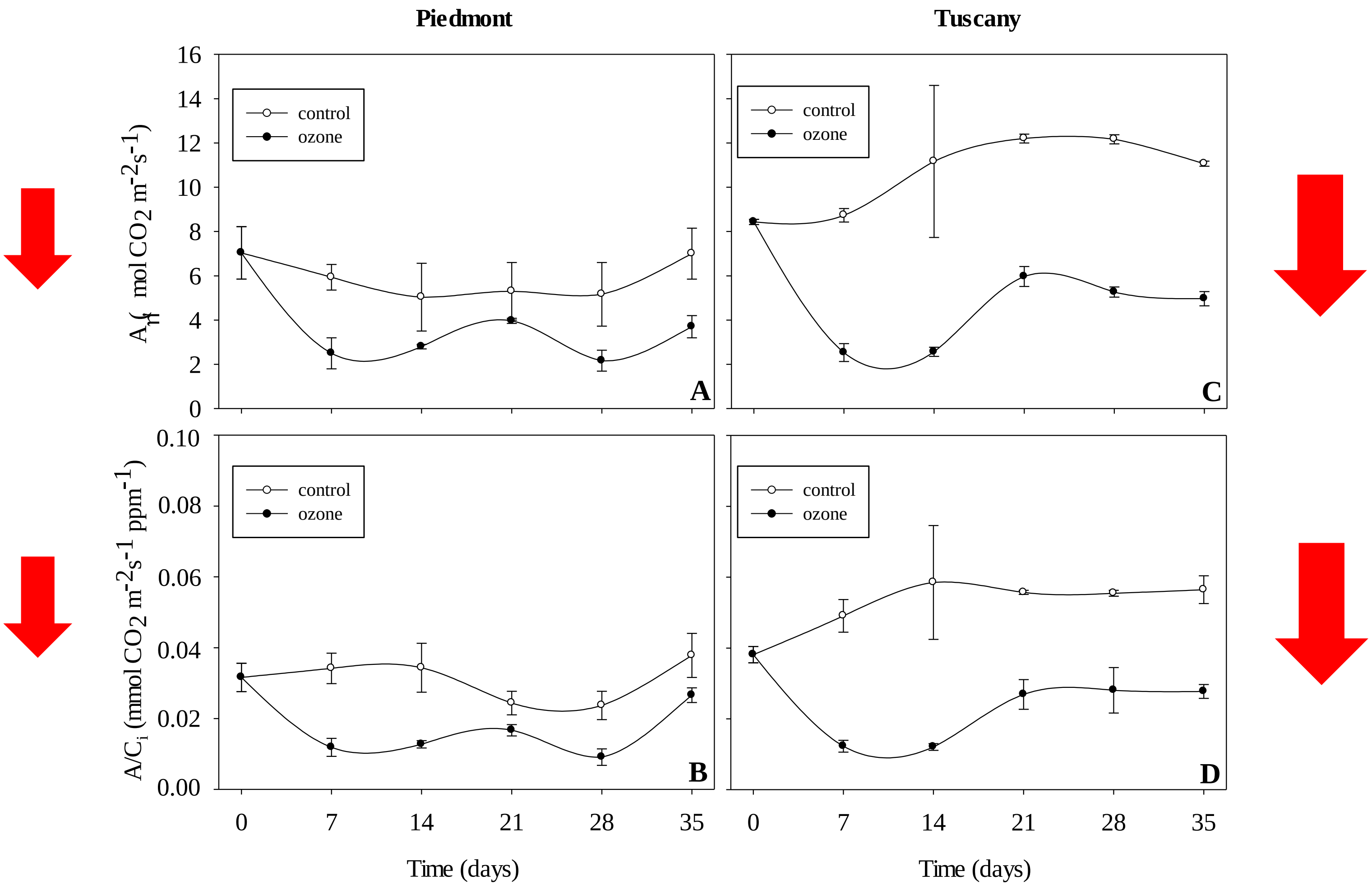
Two-way repeated measures ANOVA

Effects	**d.f.**	**A**	**A/C_i**	**F_v/F_m**
Ozone	1	***	***	***
Provenance	1	***	***	***
Time	5	***	***	***
Ozone x Provenance	1	***	***	***
Ozone x Time	5	***	***	***
Provenance x Time	5	***	***	***
Ozone x Provenance x Time	5	***	*	*
		net photosynthesis	carbossilation efficiency	photochemical maximum PSII efficiency

Differential responses to O_3 between provenances

In collaboration with:
Dott. Elena Paoletti
CNR Firenze (Italy)

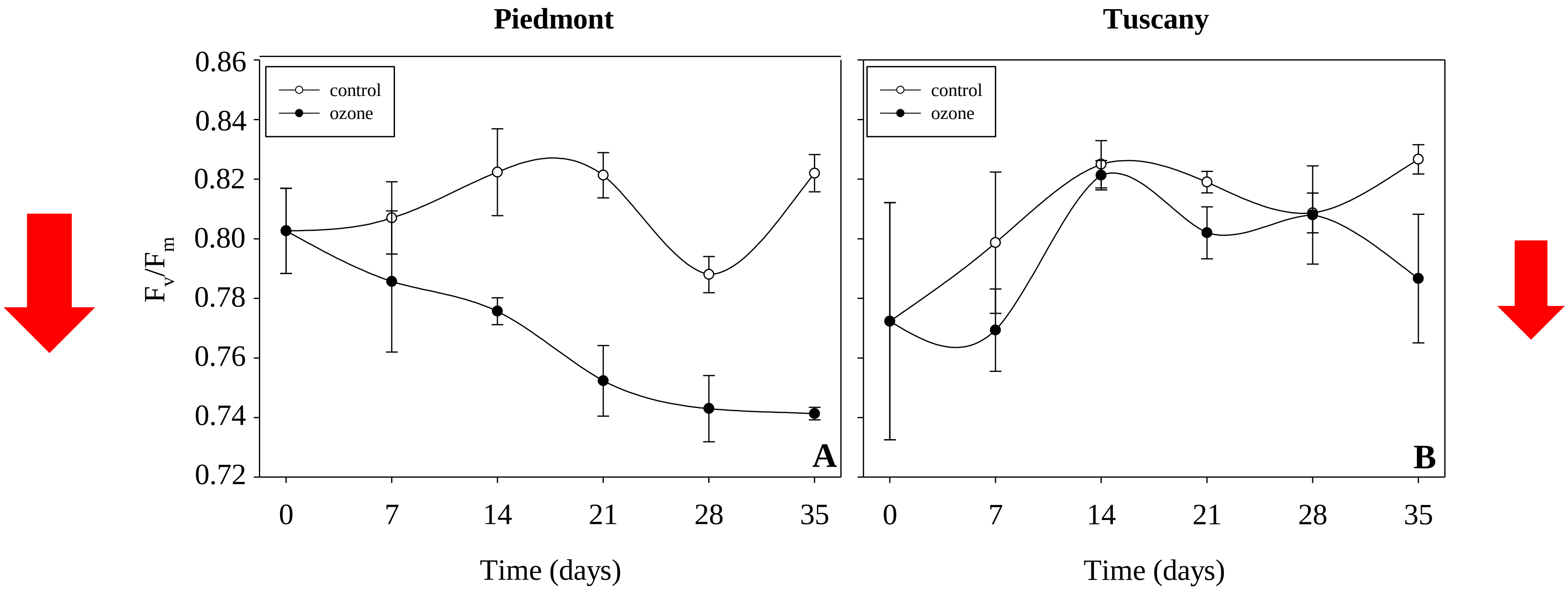
Gas-exchanges



Correlation between A and G_w		
	Control	Ozone
Piedmont	$R^2 = 0.55$	$R^2 = 0.84$
Tuscany	$R^2 = 0.56$	$R^2 = 0.72$

A: net photosynthesis
A/C_i: carbossilation efficiency
G_w: stomatal conductance

Chlorophyll *a* fluorescence



Photodamage

F_v/F_m: photochemical maximum PSII efficiency

Leaf pigments

No differential responses between provenances (two-way ANOVA)

Leaf pigments	Piedmont			Tuscany		
	Control	Ozone	*P*	Control	Ozone	*P*
	μg mg^{-1} FW			μg mg^{-1} FW		
Chlorophyll *a*	12.84 ±0.525	9.21 ±0.697 ↓	**	7.68 ±0.892	5.56 ±0.955 ↓	*
Chlorophyll *b*	5.77 ±0.541	3.90 ±0.975 ↓	*	2.35 ±0.361	1.28 ±0.372 ↓	*
Chlorophyll (*a*+*b*)	18.61 ±1.023	13.10 ±0.687 ↓	**	10.03 ±0.592	6.84 ±0.665 ↓	**
β-carotene	7.05 ±0.599	4.59 ±0.197 ↓	**	4.19 ±0.405	2.38 ±0.509 ↓	**
Lutein	0.32 ±0.023	0.22 ±0.029 ↓	*	0.18 ±0.021	0.09 ±0.025 ↓	*
Neoxanthin	0.79 ±0.114	0.40 ±0.013 ↓	**	0.91 ±0.111	0.48 ±0.081 ↓	**
Violaxanthin	1,55 ±0,162	1,24 ±0,126	ns	0.81 ±0.153	0.44 ±0.029 ↓	*
Anteraxanthin	0.22 ±0.017	0.15 ±0.008 ↓	*	0.10 ±0.028	0.05 ±0.007 ↓	*
Total carotenoids	9.93 ±0.847	6.60 ±0.292 ↓	**	6.18 ±0.701	3.44 ±0.553 ↓	**

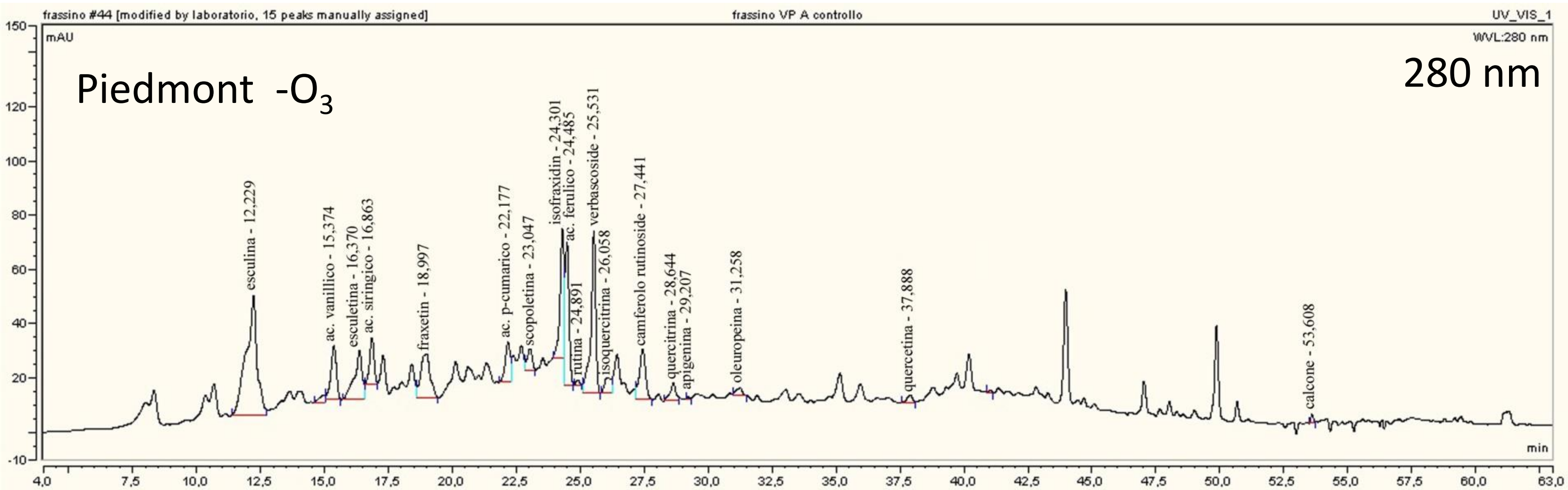
Photodamage and / or photoprotection?

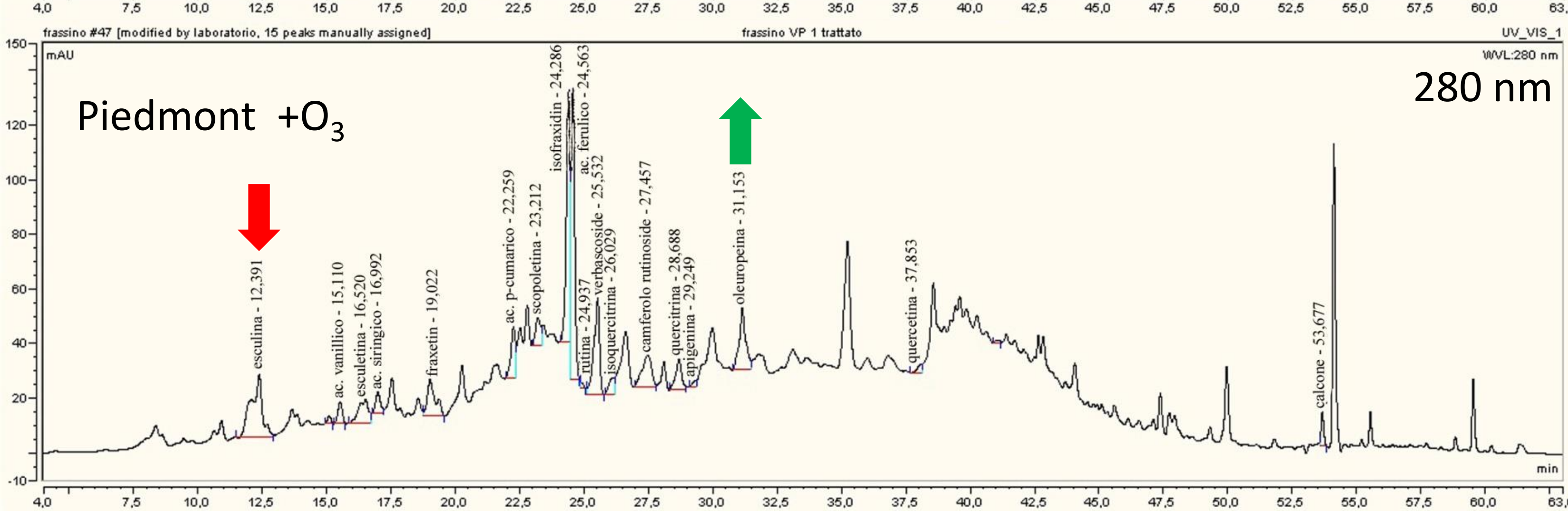
Phenolics – spectrophotometer analyses

		Total phenols (Gallic acid equivalents mg g^{-1} FW)	**Flavonoids** (Quercetin equivalents mg g^{-1} FW)	**Tannins** (Catechin equivalents mg g^{-1} FW)	**Lignins** (Lignin equivalents mg g^{-1} FW)	**Anthocyanins** (Cianidin 3-glucoside equivalent mg g^{-1} FW)
Piedmont	Control	11.79±*0.996* a	1.80±*0.224*	2.84±*0.267* a	27.06±*5.333* a	0.19±*0.011*
	Ozone	16.69±*0.558* b	2.37±*0.186*	3.58±*0.284* b	25.32±*2.646* a	0.15±*0.003*
Tuscany	Control	10.87±*0.136* a	2.25±*0.180*	3.36±*0.242* b	24.23±*1.574* a	0.19±*0.003*
	Ozone	11.57±*1.199* a	2.76±*0.439*	2.72±*0.130* a	37.61±*2.272* b	0.16±*0.014*
Ozone		***	*	ns	*	***
Provenance		***	*	ns	*	ns
Ozone x Provenance		**	ns	***	**	ns

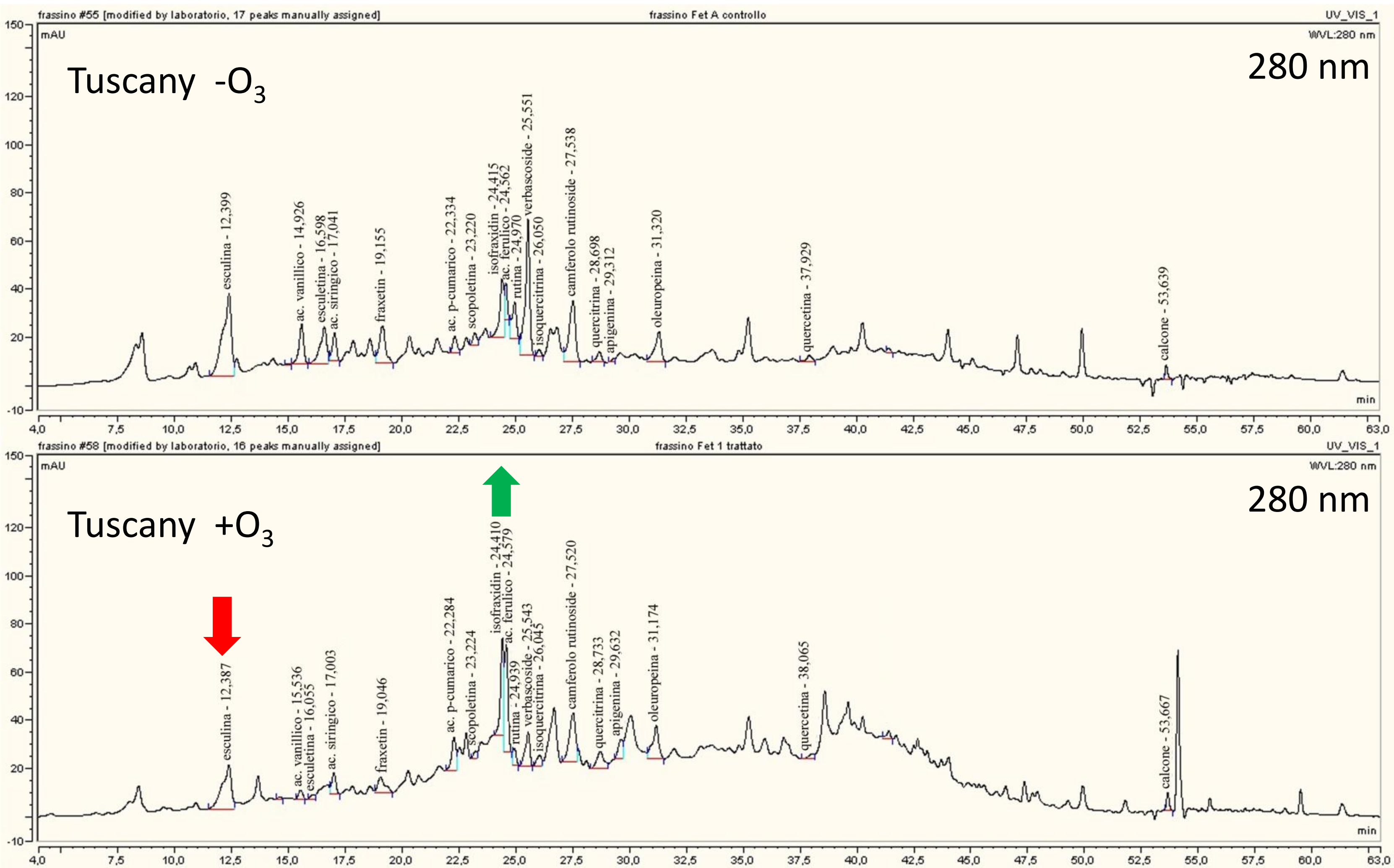
In the literature species-specific differences are known, they could be the same for provenances

Phenolics – HPLC analyses





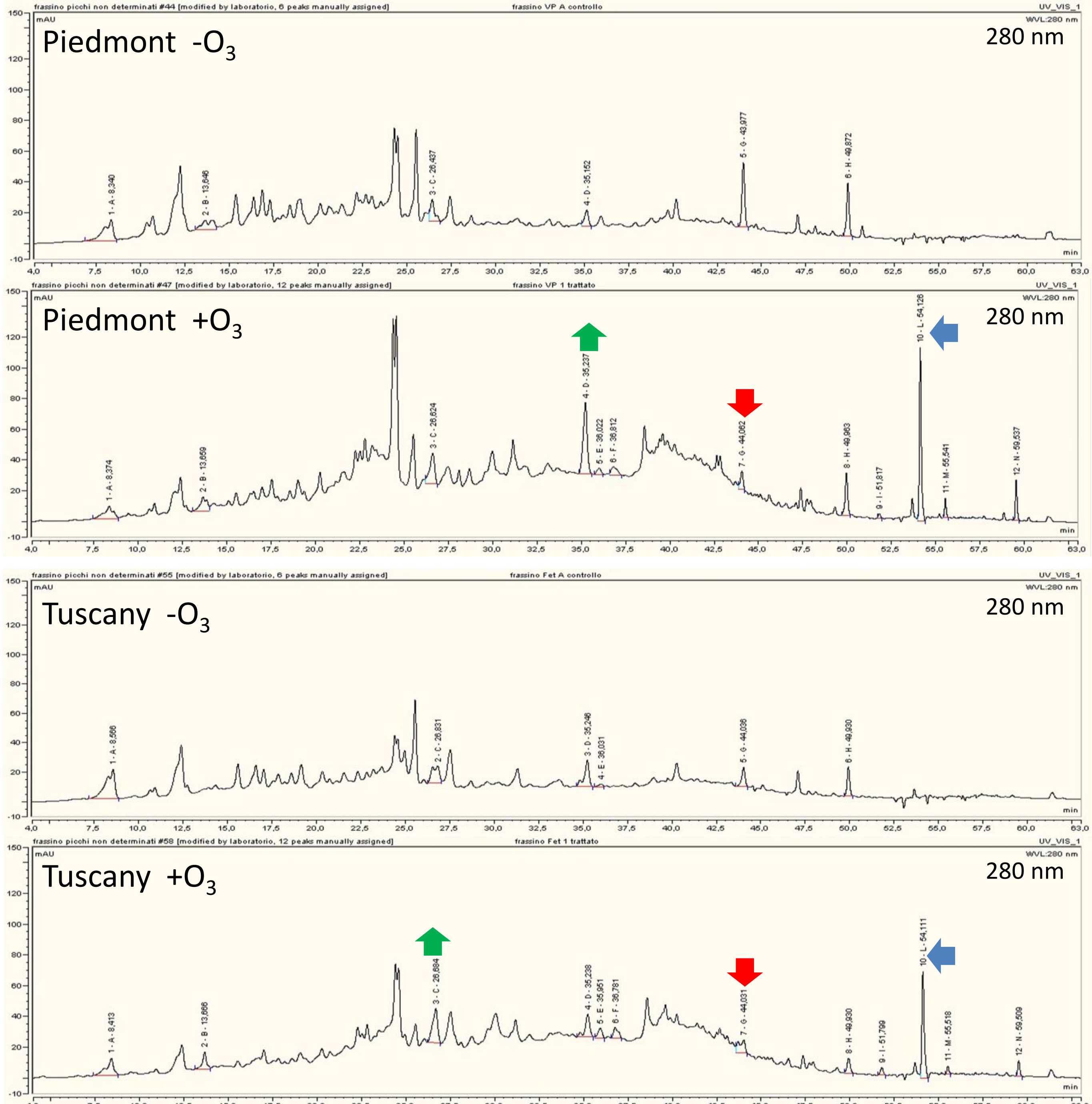
Phenolics – HPLC analyses



Phenolics – HPLC analyses

		Hydroxybenzoic acids		Hydroxycinnamic acids		Hydroxycoumarins				
		Vanillic acid (μg g^{-1} FW)	**Syringic acid** (μg g^{-1} FW)	**ρ-coumaric acid** (μg g^{-1} FW)	**Ferulic acid** (μg g^{-1} FW)	**Esculin** (μg g^{-1} FW)	**Esculetin** (μg g^{-1} FW)	**Fraxetin** (μg g^{-1} FW)	**Scopoletin** (μg g^{-1} FW)	**Isofraxidin** (μg g^{-1} FW)
Piedmont	Control	0.0729 *±0.01050*	0.0330 *±0.00633*	0.0278 *±0.00300* a	0.1573 *±0.00979* c	1.9308 *±0.09373* d	0.1342 *±0.01343*	0.3408 *±0.07393*	0.0579 *±0.02736*	0.3323 *±0.00993* b
	Ozone	0.0236 *±0.00313*	0.0173 *±0.00066*	0.0332 *±0.00168* c	0.2767 *±0.00513* d	0.9901 *±0.03119* b	0.0813 *±0.00760*	0.2137 *±0.01465*	0.0495 *±0.02004*	0.5466 *±0.09967* c
Tuscany	Control	0.0559 *±0.00854*	0.0202 *±0.00683*	0.0128 *±0.00067* a	0.0335 *±0.00069* a	1.3651 *±0.01639* c	0.0870 *±0.02257*	0.2271 *±0.01854*	0.0233 *±0.00344*	0.1855 *±0.00916* a
	Ozone	0.0104 *±0.00155*	0.0153 *±0.00385*	0.0289 *±0.00267* b	0.1171 *±0.01639* b	0.7357 *±0.02176* a	0.0082 *±0.00256*	0.1009 *±0.01170*	0.0110 *±0.00239*	0.2433 *±0.03142* ab
Ozone		***	**	***	***	***	***	***	ns	**
Provenance		***	*	***	****	***	***	***	**	***
Ozone x Provenance		ns	ns	**	***	***	ns	ns	ns	*

		Chalcons	Flavonols					Flavons	Phenylethanoid glycosides	Secoiridoids
		Trans-chalcone (μg g^{-1} FW)	**Quercetin** (μg g^{-1} FW)	**Rutin** (μg g^{-1} FW)	**Isoquercetrin** (μg g^{-1} FW)	**Quercetrin** (μg g^{-1} FW)	**Kaempferol -rutinoside** (μg g^{-1} FW)	**Apigenin** (μg g^{-1} FW)	**Verbascoside** (μg g^{-1} FW)	**Oleuropein** (μg g^{-1} FW)
Piedmont	Control	0.0029 *±0.00036* a	0.0123 *±0.00227*	0.0166 *±0.00915* a	0.0535 *±0.01600*	0.0617 *±0.02455*	0.2124 *±0.05747*	0.0007 *±0.00048* a	0.6199 *±0.17229* b	0.1963 *±0.05010* a
	Ozone	0.0109 *±0,00114* c	0.0128 *±0.00228*	0.0112 *±0.00502* a	0.0584 *±0.00553*	0.0776 *±0.05940*	0.2270 *±0.03095*	0.0024 *±0.00064* b	0.6164 *±0.09985* b	1.5877 *±0.10381* c
Tuscany	Control	0.0065 *±0.00090* b	0.0117 *±0.00097*	0.1456 *±0.01438* c	0.0230 *±0.01015*	0.0450 *±0.00702*	0.3280 *±0.04005*	0.0014 *±0.00032* ab	0.6481 *±0.24375* b	0.8477 *±0.33103* b
	Ozone	0.0071 *±0.00009* b	0.0110 *±0.00296*	0.0646 *±0.00916* b	0.0579 *±0.01724*	0.0991 *±0.01055*	0.2685 *±0.03893*	0.0109 *±0.00132* c	0.1945 *±0.01258* a	1.0862 *±0.12635* b
Ozone		***	ns	***	*	ns	ns	***	*	***
Provenance		ns	ns	***	ns	ns	*	***	ns	ns
Ozone x Provenance		***	ns	***	ns	ns	ns	***	*	***



Piedmont -O3
280 nm
Piedmont +O3
280 nm
Tuscany -O3
280 nm
Tuscany +O3
280 nm

Phenolics – HPLC analyses

Peaks	R.t. (VP treated)	Piedmont			Tuscany		
		Control	Ozone	*P*	Control	Ozone	*P*
		mAU			mAU		
A	8,374	6.09 ±*2.335*	3.70 ±*1.645*	ns	10.22 ±*2.068*	5.62 ±*0.486*	*
B	13,659	4.53 ±*1.261*	3.82 ±*0.384*	ns	n.d.	3.03 ±*0.163*	
C	26,624	5.12 ±*1.755*	6.77 ±*0.325*	ns	5.69 ±*0.386*	7.15 ±*0.677*	*
D	35,237	2.43 ±*1.019*	11.58 ±*0.786*	***	5.08 ±*1.018*	4.19 ±*0.174*	ns
E	36,022	n.d.	1.01 ±*0.121*		0.42 ±*0.102*	1.68 ±*0.086*	***
F	36,812	n.d.	2.33 ±*0.364*		n.d.	2.31 ±*0.271*	
G	44,062	7.32 ±*0.502*	2.53 ±*0.312*	***	2.96 ±*0.041*	3.58 ±*0.120*	**
H	49,963	5.67 ±*0.334*	4.52 ±*0.362*	*	3.16 ±*0.327*	1.84 ±*0.074*	**
I	51,817	n.d.	0.39 ±*0.053*		n.d.	0.83 ±*0.056*	
J	54,126	n.d.	14.50 ±*0.139*		n.d.	10.49 ±*0.401*	
K	55,541	n.d.	1.37 ±*0.038*		n.d.	0.067 ±*0.048*	
L	59,537	n.d.	3.24 ±*0.290*		n.d.	1.31 ±*0.053*	

Phytoalexins: secondary metabolites with antimicrobial activity are synthesized *de novo* after biotic and abiotic stresses

		Piedmont	Tuscany
✓	Leaf foliar symptoms	YES	YES
✓	Carbon dioxide assimilation rate	↓	↓
	Stomatal limitation	YES	YES
✓	Biochemical limitation	YES	YES
✓	Photodamage	YES	YES

	Photosynthetic pigments	↓	↓
	Accessory pigments	↓	↓
✓	Total phenols	↑	-
	Flavonoids	↑	-
✓	Lignins	-	↓
✓	Tannins	↑	↓
	Anthocyanins	↓	↓
✓	Alteration of the phenolic profile	YES	YES

✓ = differential responses between provenances

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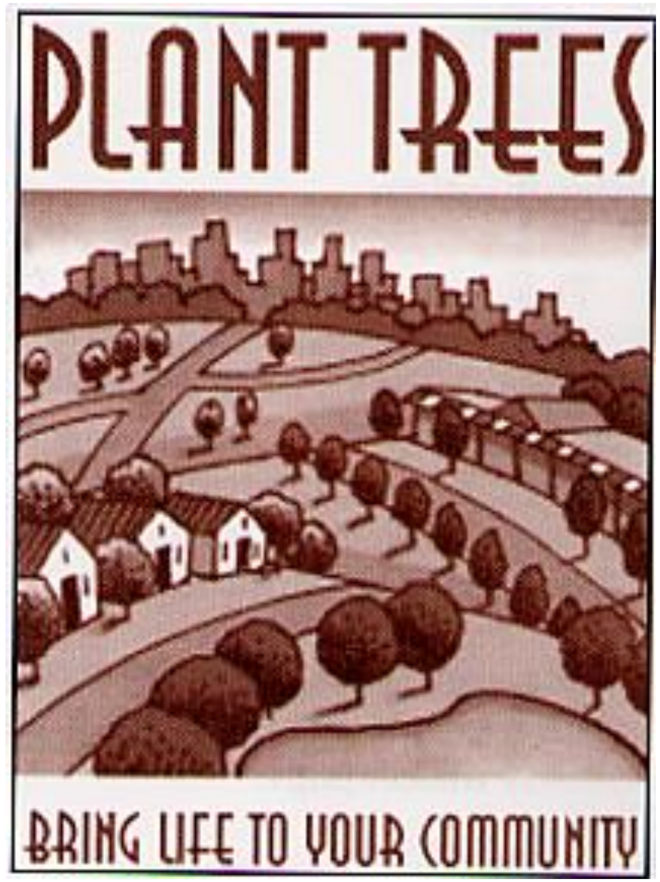

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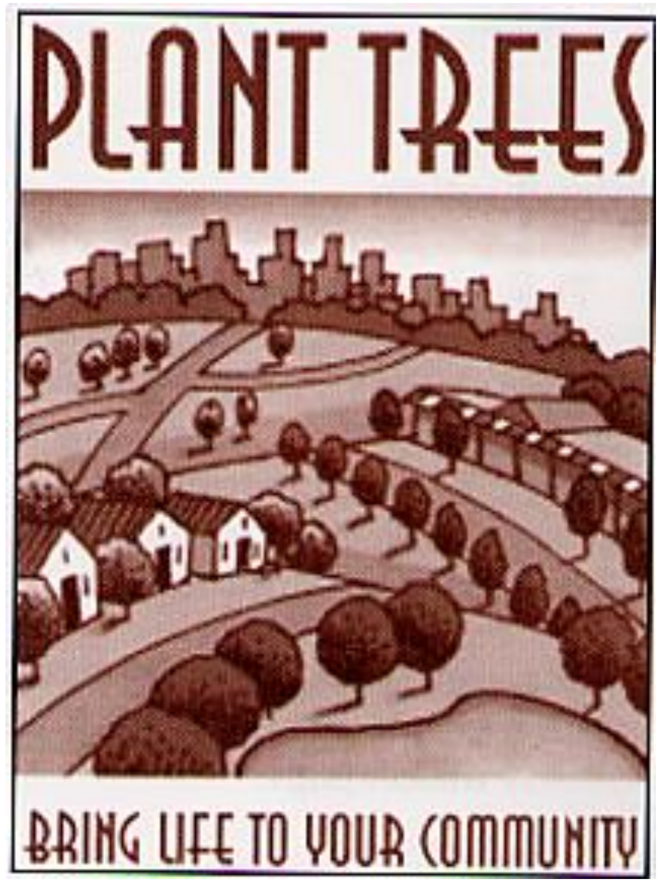

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