

DISSECTING THE MOLECULAR RESPONSES POTENTIALLY INVOLVED IN THE TOLERANCE OF TWO *ULMUS MINOR* GENOTYPES DURING *OPHIOSTOMA NOVO-ULMI* COLONIZATION

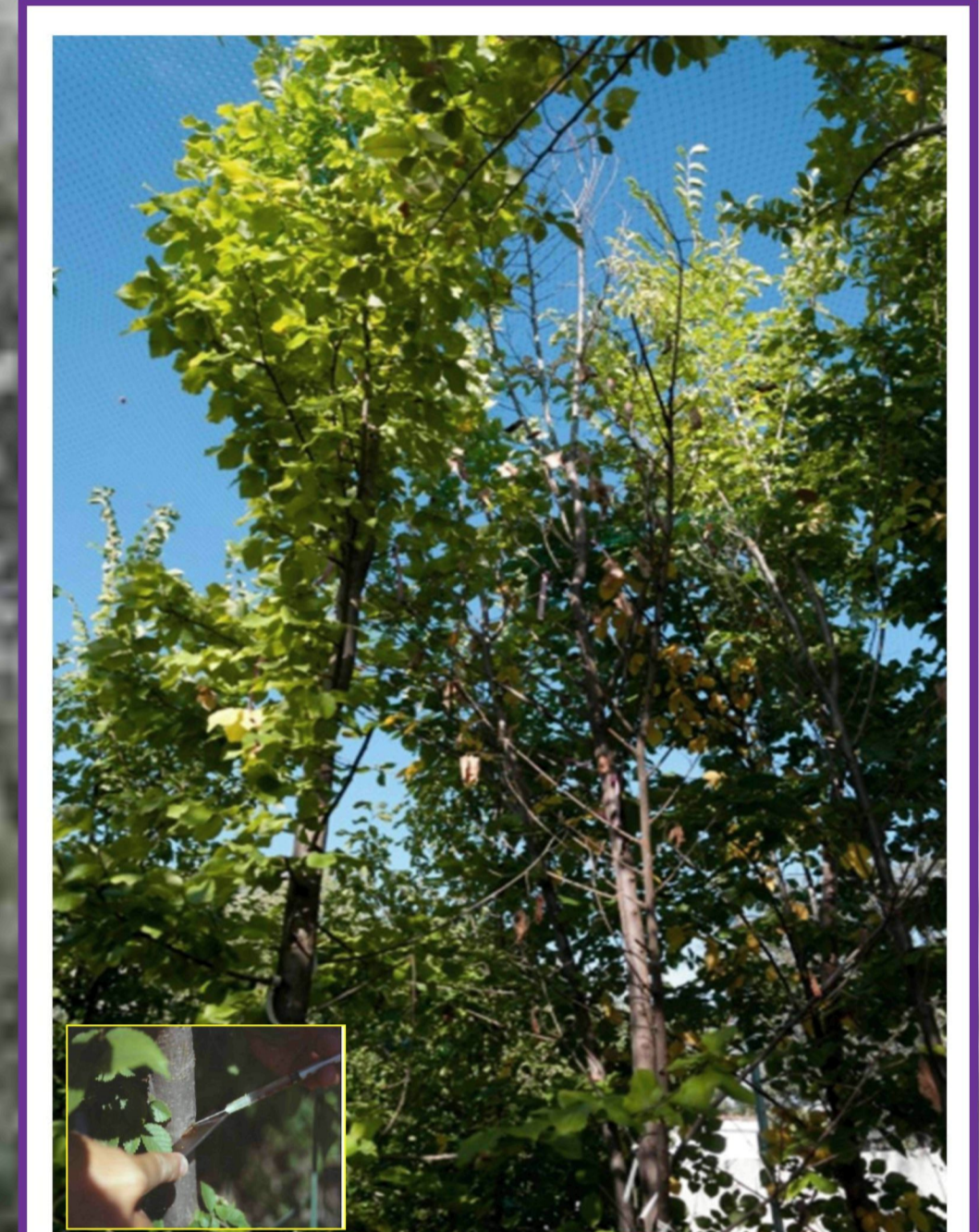
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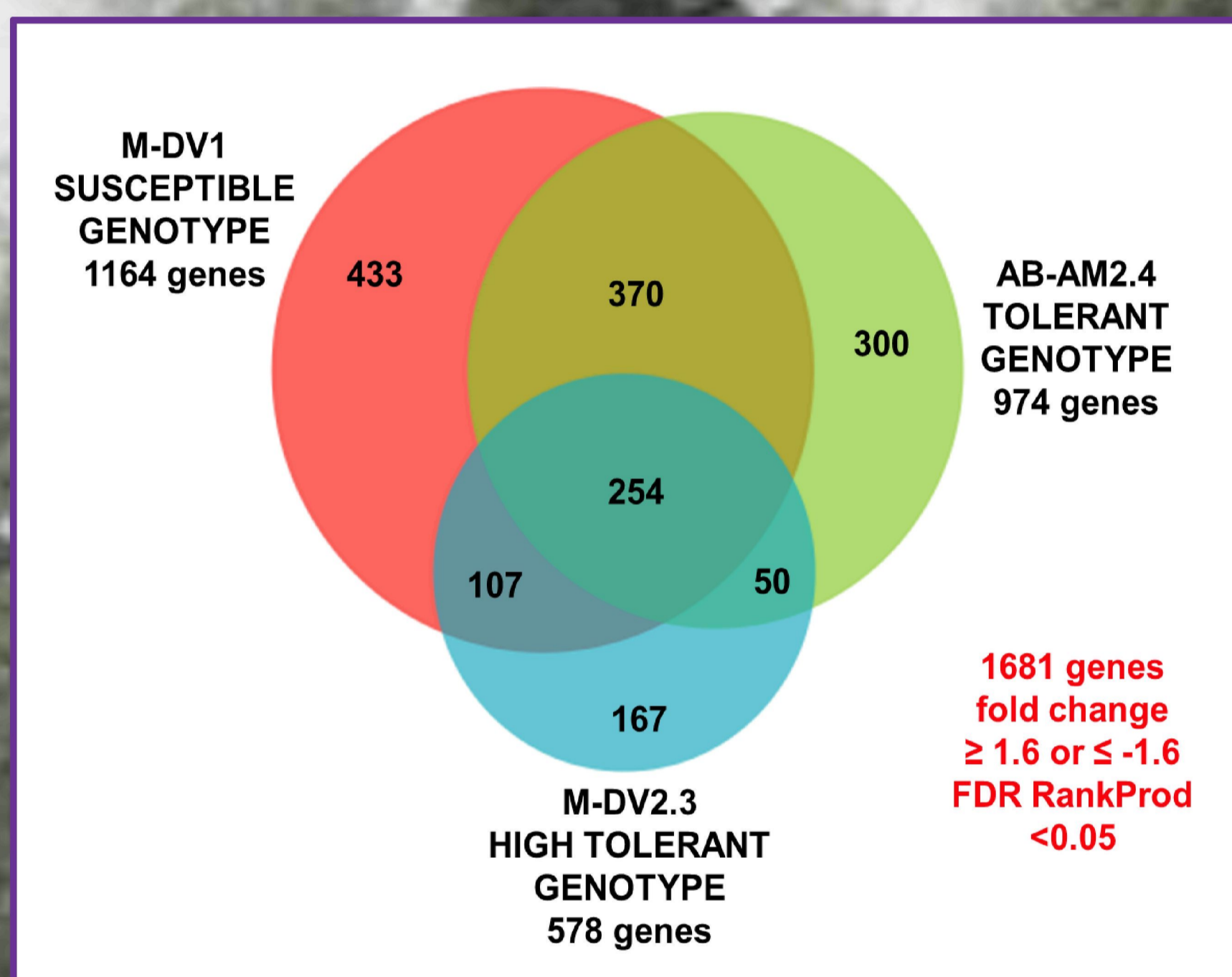
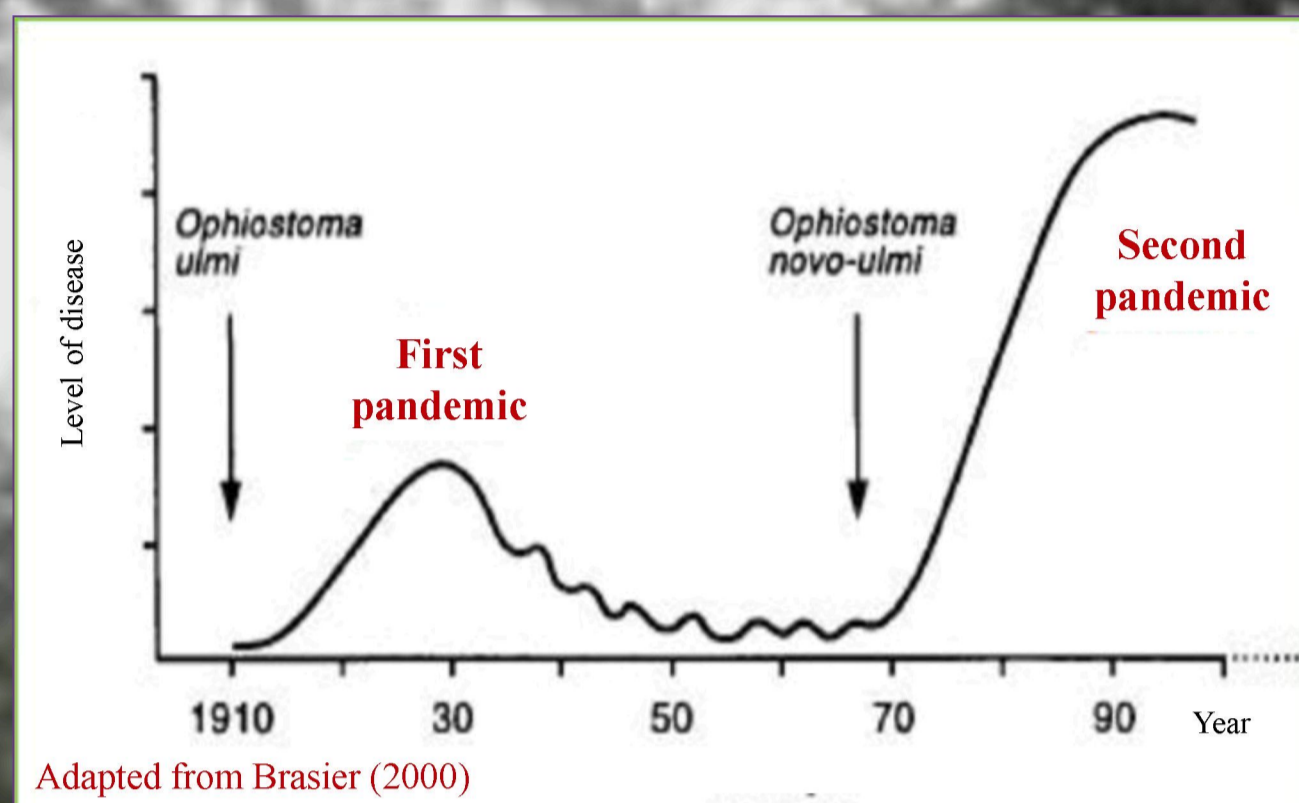
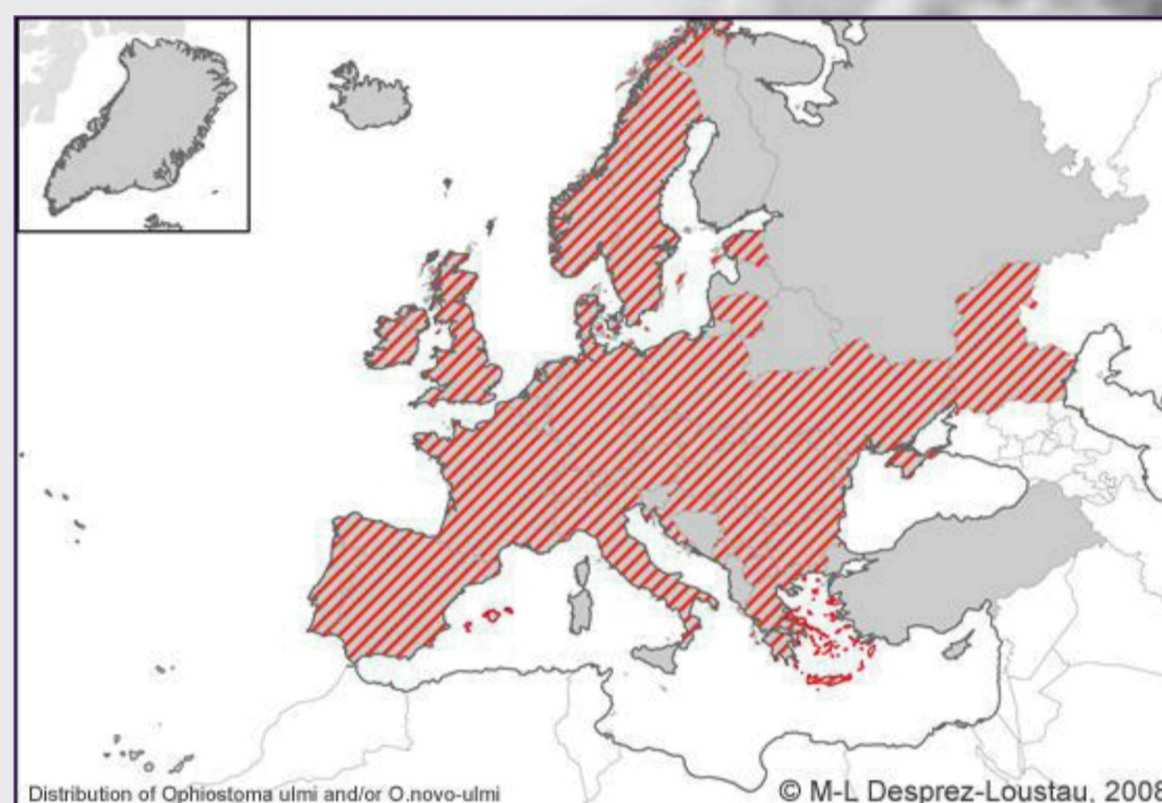
Dutch elm disease (DED) is a vascular wilt disease caused by two fungi, *Ophiostoma ulmi* and the more aggressive, *Ophiostoma novo-ulmi*. The outbreak of two pandemics during the last century severely affected North American and European elm populations. Nowadays, numerous trees are still dying due to the difficulties to control this highly virulent disease. The Spanish elm breeding programme has obtained seven highly tolerant *native U. minor* genotypes that are registered as forest reproductive material [1].

Year	2011	2012	2011	2012	2011	2012
Time	% Wilting 30 d.p.i		% Wilting 60 d.p.i		% Wilting 90 d.p.i	
M-DV2.3	1.7 ± 4.8	3.1 ± 7.7	1.4 ± 4.9	1.7 ± 5.4	3.3 ± 5.4	3.5 ± 5.4
AB-AM2.4	36.5 ± 4.8	34.8 ± 7.7	31.9 ± 4.9	28.6 ± 5.4	32.3 ± 5.4	26.9 ± 5.4
M-DV1	82.3 ± 4.8	58.9 ± 8.9	86.7 ± 4.9	80 ± 6.3	87.7 ± 5.4	81.7 ± 6.2

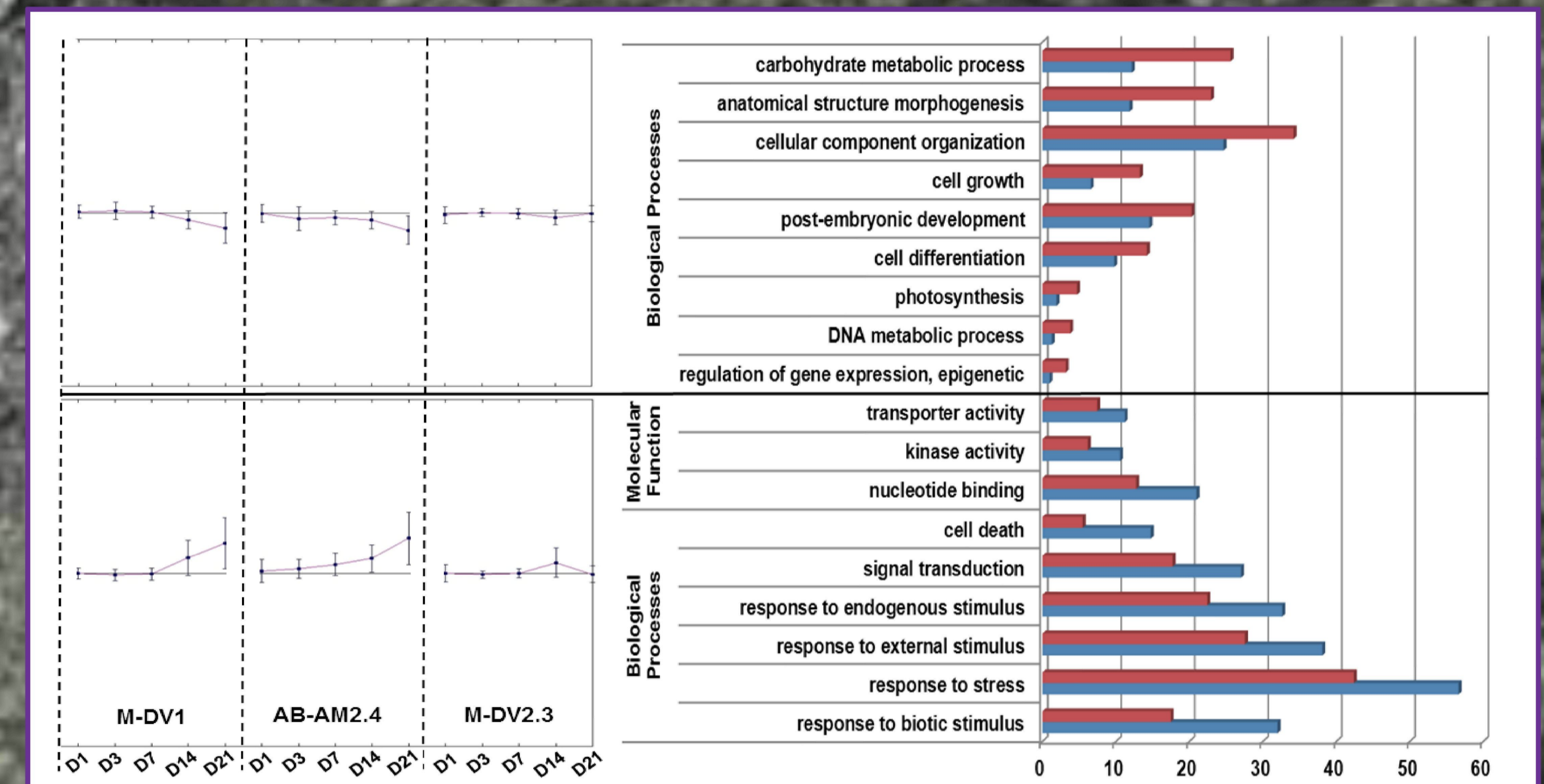
Three Spanish elms with contrasted tolerance to DED were selected for this study (Table above shows canopy wilting percentage in a previous experiment). Inoculation was carried out about 15-30 days after full leaf development. Symptoms were visually assessed during four months after inoculation by estimating the percentage of leaf wilting (shown in Table below the image). Inoculated plants from susceptible genotypes showed the first symptoms in the crown at 14 days post inoculation, reaching 10-15% of wilting leaves. By 21 days after inoculation, tolerant genotypes inoculated with *Ophiostoma novo-ulmi* showed a low percentage of wilting (10-15%) whereas susceptible genotypes reached values exceeding 50%.



GENOTYPES	Days post inoculation (dpi)								
	1	3	7	14	21	28	60	120	
HIGH TOLERANT	M-DV2.3	0	0	0	0	15	22.5	20	12.5
TOLERANT	AB-AM2.4	0	0	0	0	7.5	20	20	20
SUSCEPTIBLE	M-DV1	0	0	0	5	67.5	90	85	92.5



A selection of unigenes from a biotic stress library of *Ulmus minor* [2] was included in the microarray design (Agilent 8 x 60K, Agilent Technologies, CA, USA). A total of 1,681 unigenes significantly modified their level of transcripts in at least one sampling point in one genotype during *Ophiostoma novo-ulmi* infection. 300 genes were exclusively identified in AB-AM2.4, 167 genes in M-DV2.3 and 50 genes in both genotypes



Differentially expressed genes follow two main trends; increase or decrease in transcript levels. Graphics on the left show the average trend for each genotype. An enrichment analysis indicated an increase of Gene Ontology (GO) terms related to "response to biotic stimulus", "response to stress" or "kinase activity" in genes upregulated (blue bars) whereas downregulated genes showed significant enrichment in GO terms related to anatomical structure morphogenesis, cell growth or cell differentiation (red bars).

Top upregulated genes exclusively found in AB-AM2.4 genotype at short and long-term after inoculation with *Ophiostoma novo-ulmi*

ID_ISOTIG	DESCRIPCIÓN BLAST2GO	D1	D3	D7	D14	D21
isotig18444	acidic endochitinase-like					14,73
isotig08555	udp-glucuronosyl udp-glucosyl transferase family protein	-1,64				9,59
isotig21148	thioredoxin-like protein				3,68	7,30
isotig07125	basic form of pathogenesis-related protein 1					7,07
isotig08310	(-)-isopiperitenol (-)-carveol mitochondrial-like					5,94
isotig13236	---NA---					5,63
isotig20653	udp-glycosyltransferase 75d1-like					4,99
isotig13410	probable glutathione s-transferase					4,98
isotig02505	probable lrr receptor-like serine threonine-protein kinase at1g53430-like					4,60
isotig02150	ethylene-responsive transcription factor rap2-12-like	-2,59			2,59	4,45
isotig07680	at5g56980 mh17_10					4,33
isotig19684	cationic amino acid transporter 1-like					4,00
isotig16541	probable s-adenosylmethionine-dependent methyltransferase at5g38100					3,90
isotig10720	light-inducible protein cprf2-like					3,88
isotig15975	multiple c2 and transmembrane domain-containing protein 1-like	1,86				3,80
isotig12538	cellulose synthase-like protein g3-like					3,78
isotig04982	arabinogalactan peptide 20-like	2,08				3,74
isotig18079	transcription factor bhlh36-like	1,91				3,73
isotig05901	conserved hypothetical protein	2,10			2,10	3,27
contig08923	cell wall-associated partial				2,14	
isotig04660	cell wall-associated hydrolase				1,95	
isotig19859	outer envelope pore protein 16- chloroplastic-like				1,74	
isotig14781	ethylene-responsive transcription factor erf109-like	5,81		-1,73		
isotig18662	1-phosphatidylinositol- -bisphosphate	2,78				
isotig01174	probable xyloglucan endotransglucosylase hydrolase protein 23-like	2,50				
isotig13783	abscisic acid receptor pyl4-like	2,40				
isotig13774	u-box domain-containing protein 21-like	2,35				
isotig16863	homeobox-leucine zipper protein athb-12-like	2,32				
isotig13964	e3 ubiquitin-protein ligase pub23-like	2,16				
isotig19668	hypothetical protein POPTR_0019s10350g	2,07				

Significant increases in level of transcripts of specific defence genes or transcription factors were identified specifically in tolerant genotypes. These results suggest that *U. minor* tolerance to *O. novo-ulmi* is related to the differential expression of these genes.

Top upregulated genes exclusively found in M-DV2.3 genotype at short and long-term after inoculation with *Ophiostoma novo-ulmi*

ID_ISOTIG	DESCRIPCIÓN BLAST2GO	D1	D3	D7	D14	D21
isotig00311	hypothetical protein POPTR_0002s22940g					2,30
isotig04987	two-component response regulator arr9-like					2,06
isotig20904	protease inhibitor seed storage lipid transfer family protein	1,91				1,94
isotig09369	glutaredoxin family protein	-2,00				1,94
isotig20747	two-component response regulator arr5-like					1,84
isotig17060	protein glutamine dumper 3-like					1,79
isotig08725	expansin a1					1,79
isotig17614	heavy metal-associated isoprenylated plant protein 26-like					2,07
isotig06888	probable rna-dependent rna polymerase 1					2,63
isotig01332	laccase-14-like	-1,60	-1,65			2,31
isotig04713	PREDICTED: uncharacterized protein LOC103436664					2,26
isotig18152	probable calcium-binding protein cml10-like	1,73				2,04
isotig21152	---NA---	2,94				
isotig17069	probable xyloglucan endotransglucosylase hydrolase protein 23	2,30				
isotig18160	copper transporter 1-like	2,29				
isotig06364	udp-glycosyltransferase 85a2-like	2,00				
isotig18411	transcription factor bhlh30-like	1,96				
isotig20057	u-box domain-containing protein 19-like	1,88				
isotig20807	hypothetical protein POPTR_0001s10440g	1,83				
isotig10818	two-component response regulator-like prr95-like isoform x3	1,80				

References

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- Perdiguero P, Venturas M, Cervera MT, Gil L, Collada C. (2015). Massive sequencing of *Ulmus minor*'s transcriptome provides new molecular tools for a genus under the constant threat of Dutch elm disease., *Frontiers in Plant Sciences*, 6:541.

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