Transcriptomic, morphological, and metabolomic differences in fir trees from a peri-urban forest under chronic ozone exposure

Verónica Reyes-Galindo[1], Juan Jaramillo-Correa[2], Svetlana Shishkova[3], Estela Sandoval-Zapotitla[4], Cesár Flores-Ortiz[5], Daniel Piñero[6], Lewis Spurgin[7], Claudia Martin[7], David Prince[7], Ricardo Torres-Jardón[8], Claudio Zamora-Callejas[9], and Alicia Mastretta-Yanes[10]

[1]Universidad Nacional Autónoma de México Instituto de Ecología
[2]Institute of Ecology, Universidad Nacional Autónoma de México
[3]Universidad Nacional Autónoma de México Instituto de Biotecnología
[4]Universidad Nacional Autónoma de México Instituto de Biología
[5]Universidad Nacional Autonoma de Mexico
[6]Universidad Nacional Autónoma de México
[7]University of East Anglia
[8]Universidad Nacional Autónoma de Mexico, Instituto de Ciencias de la Atmósfera y Cambio Climático
[9]Bienes Comunales Santa Rosa Xochiac
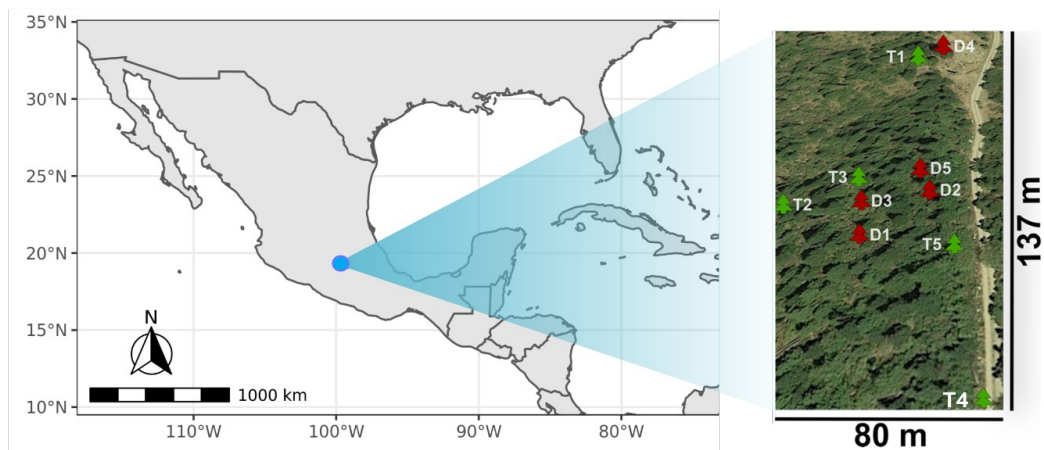[10]CONACYT Research Fellow assigned to CONABIO

July 17, 2023

Abstract

Urbanization modifies ecosystem conditions and evolutionary processes. This includes air pollution, mostly tropospheric ozone (O3), which leads to urban and peri-urban forest decline. Such is the case of fir (Abies religiosa) forests in the peripheral mountains west of Mexico City, which have been severely affected by O3 pollution since the 1970s. Interestingly, some young individuals with low O3—related damage have been observed within a zone of great O3 incidence, which might suggest rapid tolerance/adaptation to this pollutant. We compared asymptomatic and damaged individuals of the same age ([?]15 years old; n = 10) using histological, metabolomic and transcriptomic methods. Plants were sampled during days of high (170 ppb) and moderate (87 ppb) O3 concentration periods. After verifying that all individuals clustered within the same local genetic group when compared to a species-wide panel (Admixture analysis with ~1.5K SNPs), we observed thicker epidermis and more collapsed cells in the palisade parenchyma in needles from damaged than from asymptomatic individuals; such differences increased with needle age. Furthermore, damaged individuals had lower concentrations of various terpenoids (ß-Pinene, ß-Caryophylene Oxide, α-Caryophylene, ß-α-Cubebene and α-Muurolene) than asymptomatic trees, as evidenced through GC-MS. Finally, transcriptomic analyses revealed differential expression for thirteen genes related to carbohydrate metabolism, plant defense, and gene regulation. Our results indicate a rapid and contrasting phenotypic response between plants, likely modeled by standing genetic variation and/or plastic mechanisms. They open the door to future evolutionary studies for understanding how O3 tolerance develops in urban environments, and for identifying tolerant germplasm for forest restoration.
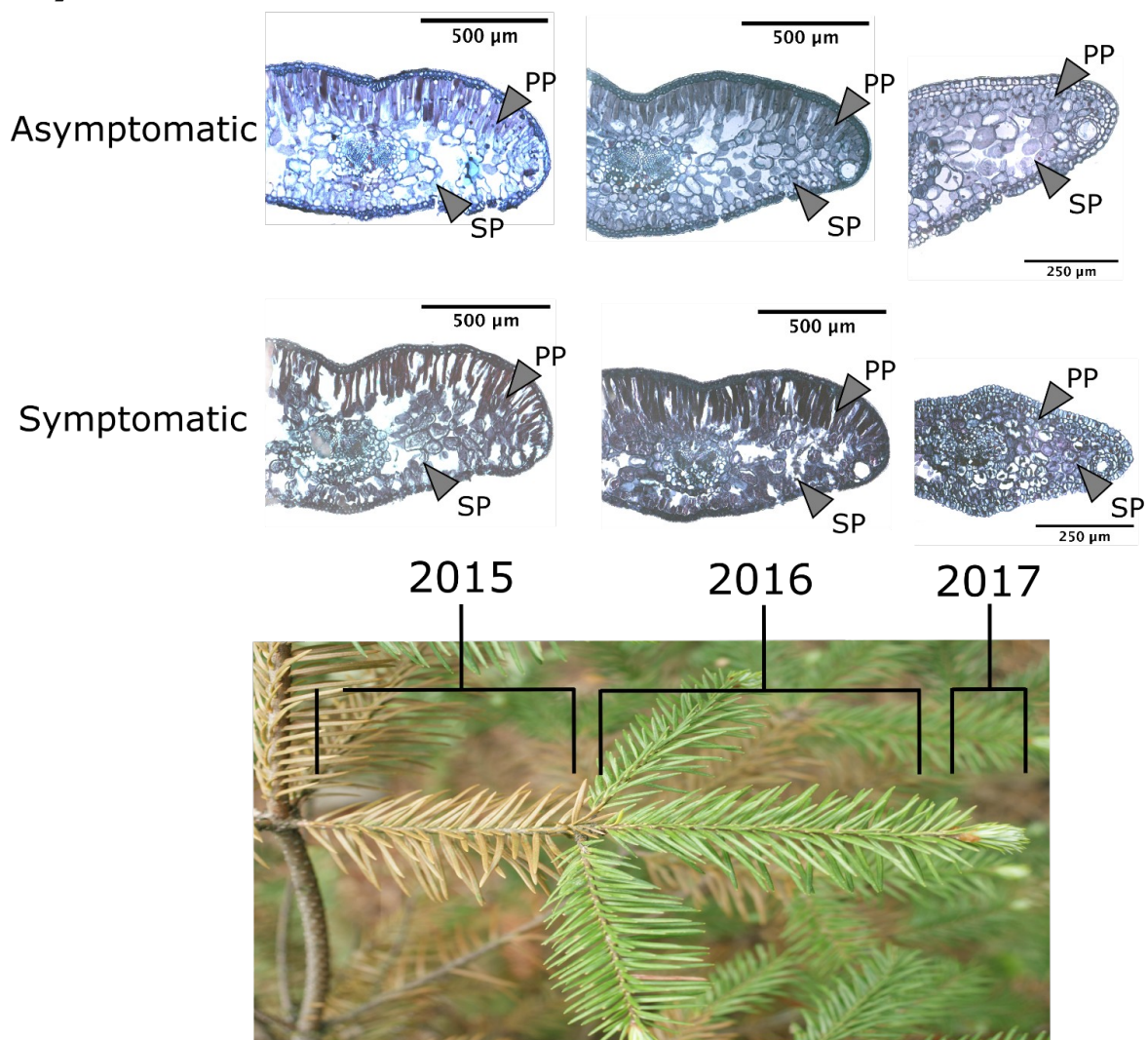
Hosted file

Ozone_MolEcol_SpecialNumber(4).docx available at https://authorea.com/users/640167/articles/655170-transcriptomic-morphological-and-metabolomic-differences-in-fir-trees-from-a-

peri-urban-forest-under-chronic-ozone-exposure

a)

b)

Asymptomatic

Symptomatic

2015 2016 2017

Figure 1 Distribution of focus trees (asymptomatic in green, T1-5; symptomatic in red, D1-5) within the study site, and location of the study site within Mexico City and Mexico (a) Transverse histological sections of needles from asymptomatic (left) and symptomatic (right) sacred fir individuals (*Abies religiosa*) for three growth periods (2015, 2016, 2017) (b) All bars = 10μm. PP, palisade parenchyma; SP, spongy parenchyma.

a)

Cerro Zamorano

San Andrés

El Chico

Tlaxco

Nev. Colima

MichoacanAlt

Cofre Perote

Sierra Manantlán

Volcán Atlitzin

Volcán Tancítaro

Malinche

Puerta Garnica

Cerro Blanco

Nev.TolucaRG

Nev. TolucaSB

Ajusco

Ixtapalucan

Nev. Toluca

Santa Rosa Xochiac

0   500 Km.

b)

West   East

K=2

K=3

K=4

K=5

Sierra Manantlán
Nevado de Colima
Volcán Tancítaro
Puerta Garnica
Michoacán
San Andrés
Cerro Zamorano
Cerro Blanco
Nevado de Toluca RG
Nevado de Toluca NT
Nevado de Toluca SB
Santa Rosa Xochiac D
Santa Rosa Xochiac T
Ajusco
Ixtapalucán
El Chico
Tlaxco
Volcán Atlitzin
Malinche
Cofre de Perote
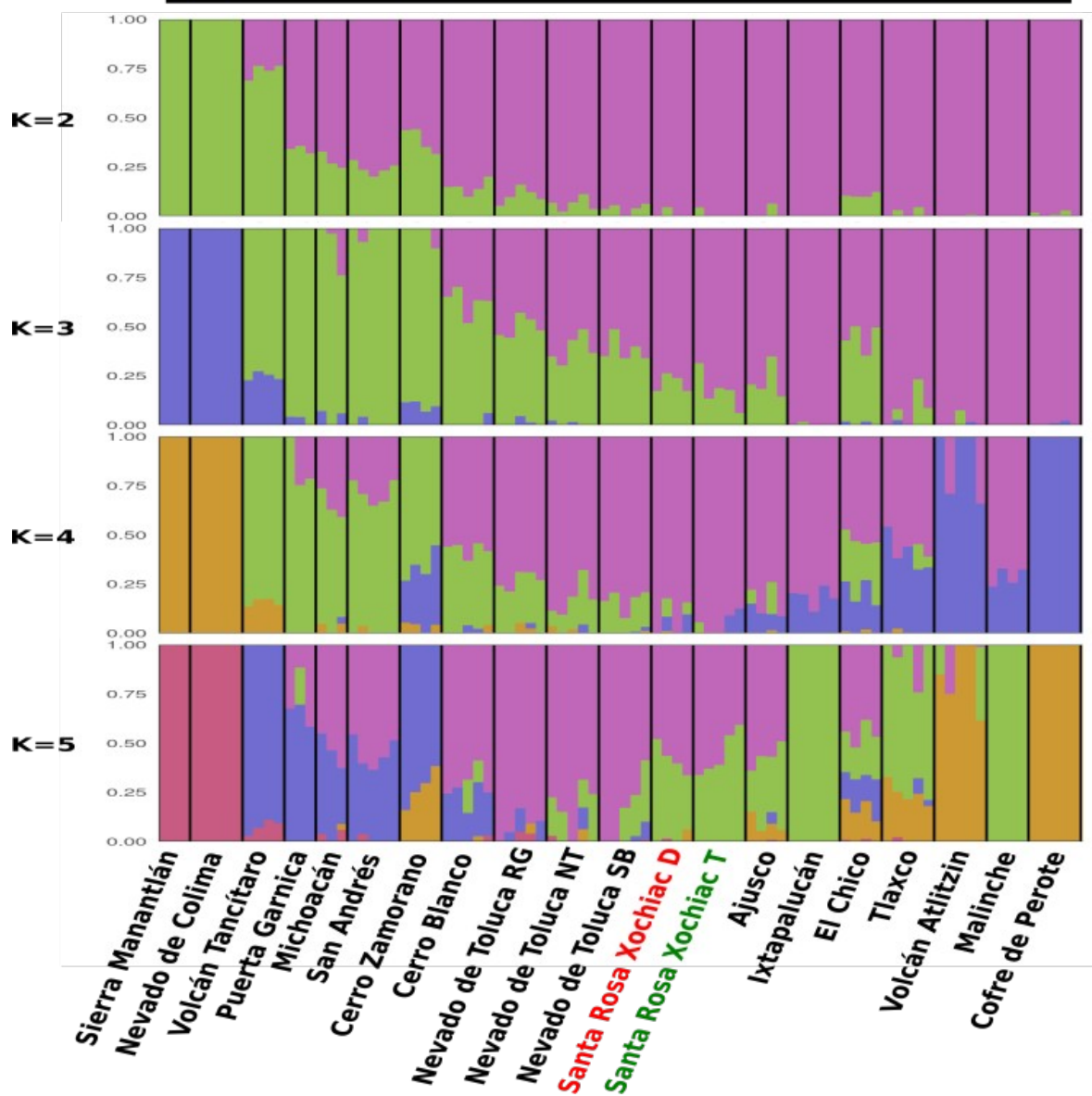
Figure 2. Assignment of focus individuals to sacred fir (*Abies religiosa*) genetic clusters based on admixture results (based on 1,550 SNPs). Symptomatic trees indicated in red below figure; asymptomatic trees in green. Plots are shown for k = 2 to k = 5, all of which denote identical cluster assignments for both types of trees. Individuals (n= 88) are shown as vertical bars colored in proportion to their estimated ancestry for each cluster. Black lines separate populations listed from West to East along the species distribution.
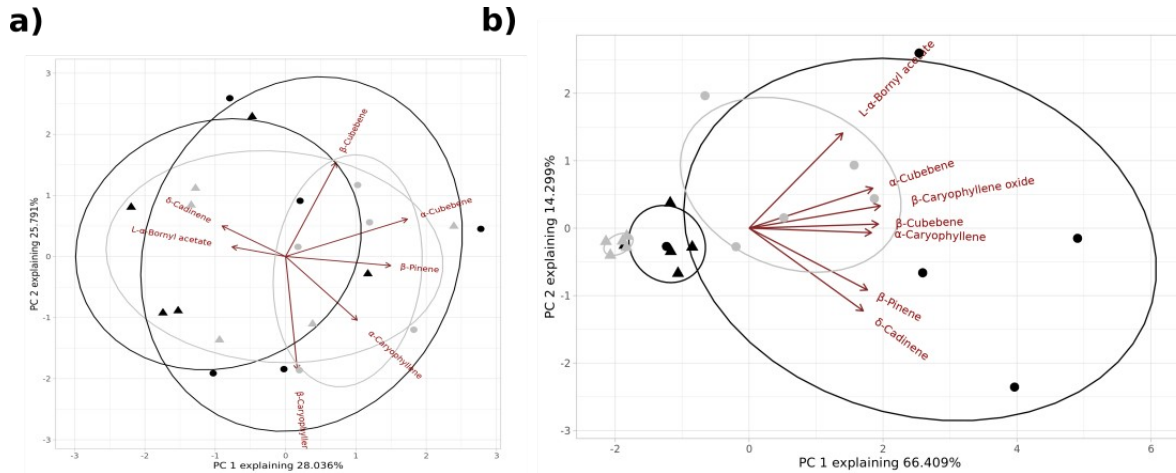
Figure 3 Principal component analysis (PCA) of terpenoid profiles for asymptomatic (circles) and symptomatic (squares) sacred fir (*Abies religiosa*) individuals from a heavily polluted forest near Mexico City for two periods with contrasting O3 concentration: (a) high O_3 concentration (170ppb; May 17th); (b) moderate O_3 concentration (87ppb; April 15th). Two- and three-years old needles can be differentiated according to color code (see inset).
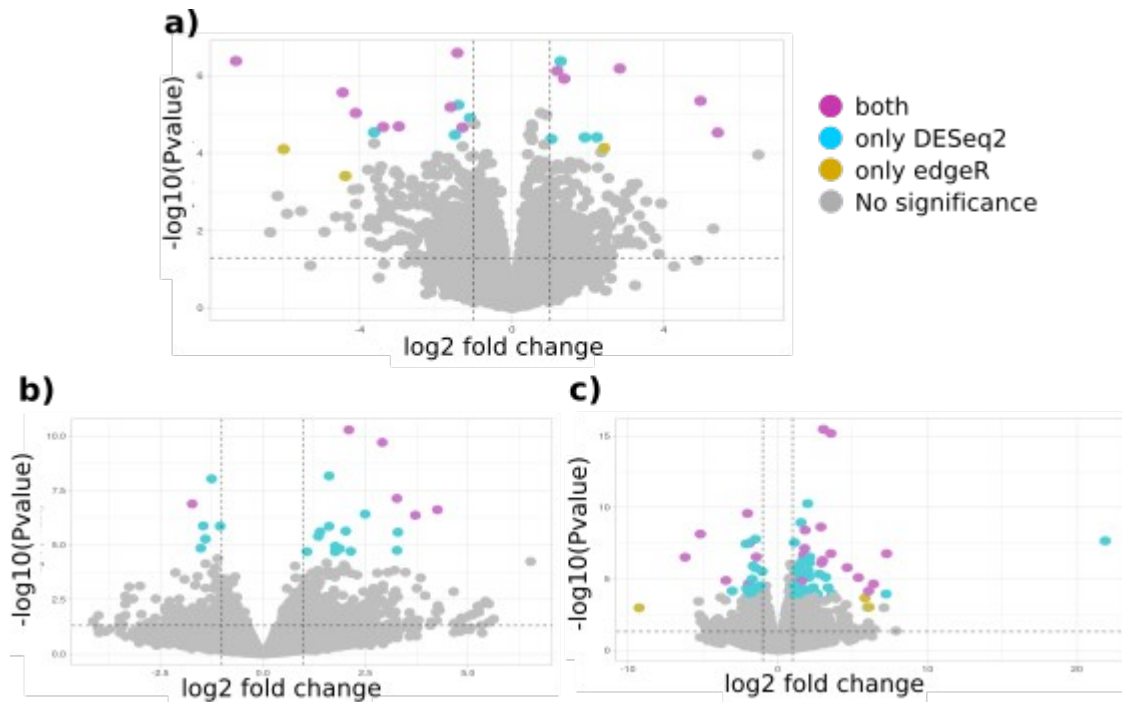
Figure 4 Differential Expression Analysis of RNA transcripts with two methods (DESeq2 in blue; edgeR in yellow; retained transcripts were those detected by both methods, in purple; $p < 0.005$). Volcano plots depicting the fold difference in transcripts level for asymptomatic vs symptomatic trees during the high O_3 period (a); high vs moderate O_3 concentration periods for symptomatic individuals (b); and high vs moderate O_3 concentration periods for asymptomatic trees (c). DETs were selected with thresholds of fold change > 1 (represented by two dotted black vertical lines) and $p < 0.005$ (represented by dotted black horizontal lines).

Supplementary Images
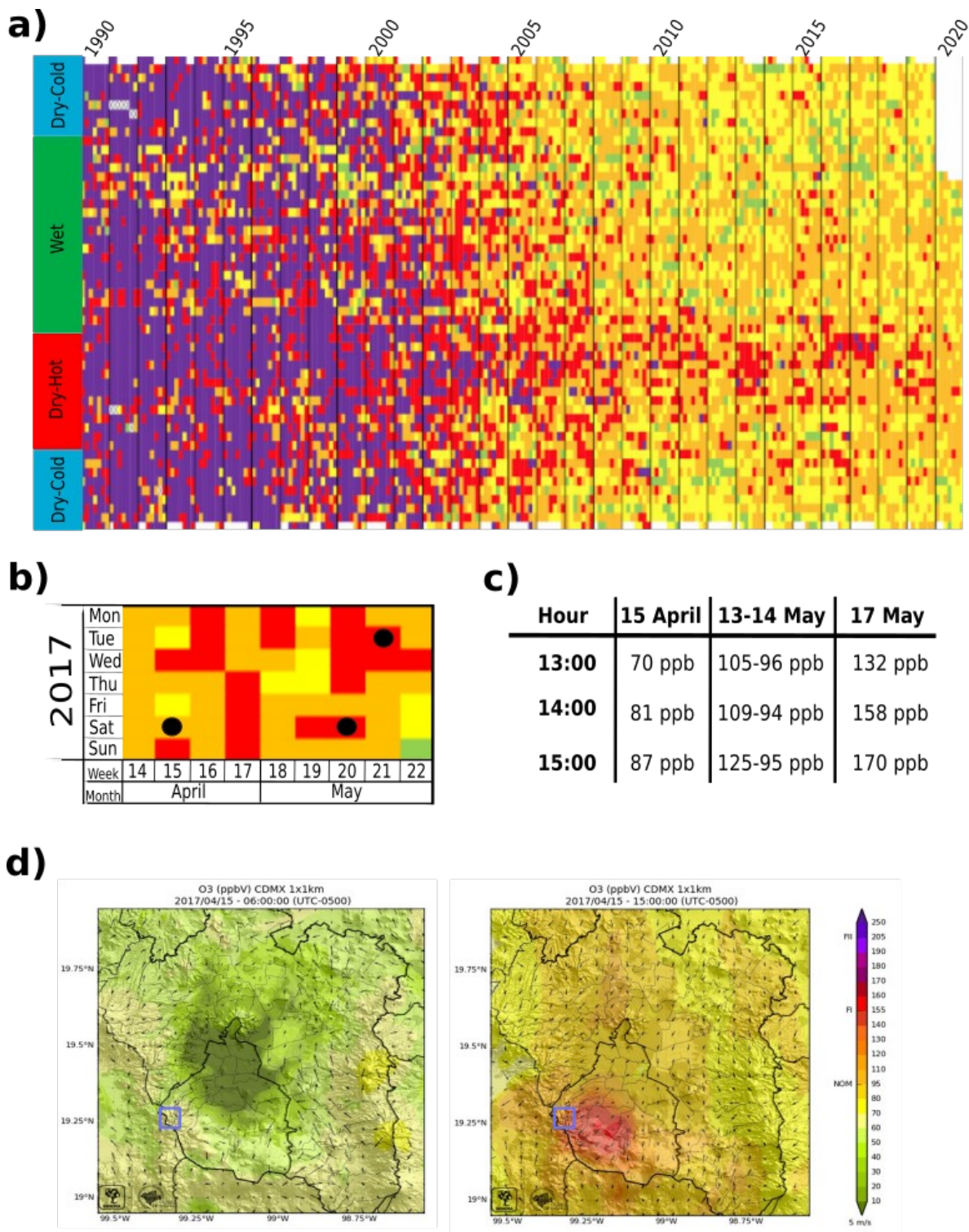
**Figure S1** Change of O₃ concentration in the Mexico City (CDMX) metropolitan area since 1990 (a) Air quality is represented by colors: green, good (0-70ppb); yellow, regular (71-95ppb); orange, bad (96-154ppb); red, very bad (155-204ppb)

and purple, extremely bad (> = 205). (b) average O_3 concentration during the study period (april and may, 2017). Black circles show collection days. (c) O_3 concentration as measured at the nearby station to the sampling site (PEDREGAL) during the sampling period. All graphs modified from SEDEMA (2020). (d) Wind direction and O_3 concentration in CDMX at 6:00 am (~ 50 ppb; left). and at 15:00 pm (~ 130 ppb; middle; see colorimetric scale at right) on a regular day between April and may. Modified from SEDEMA 2018. Blue boxes indicate the location of the study site. Arrow size indicates wind speed; vector at right (below colored bar) shows 5 m / s.

(a)
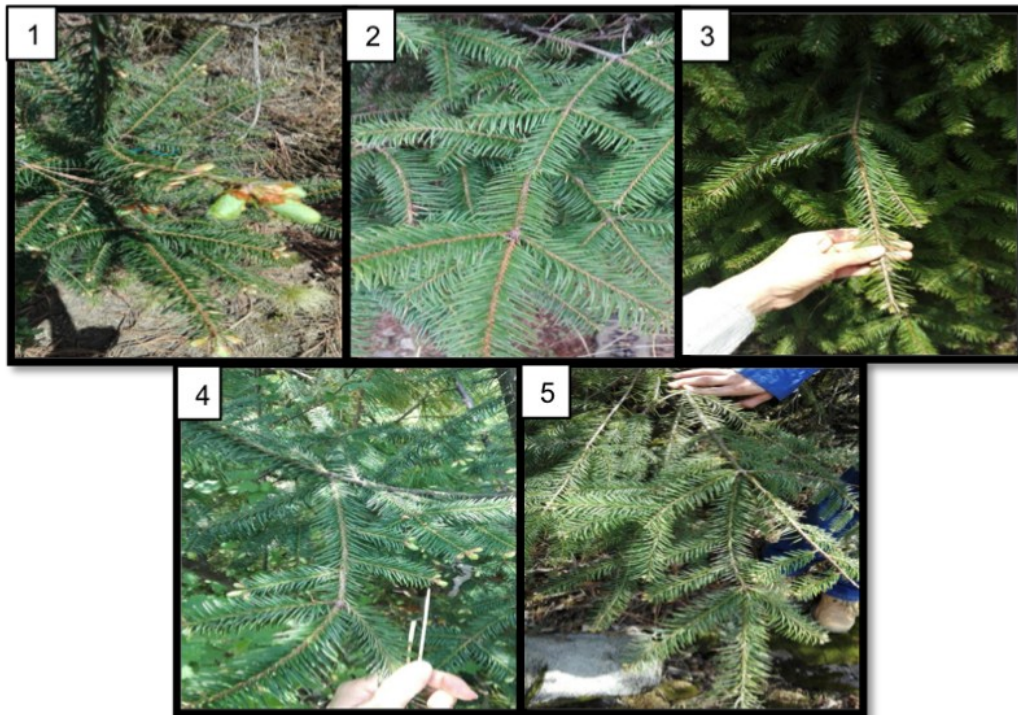
(b)
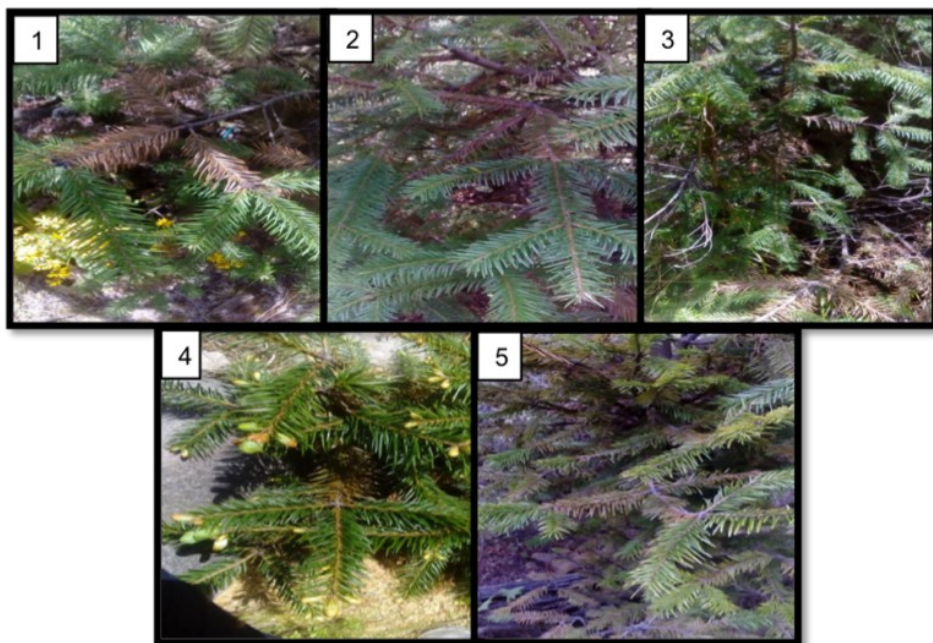
Figure S2 Branch photographs for each sampled tree. **(a)** asymptomatic trees **(b)** symptomatic trees.
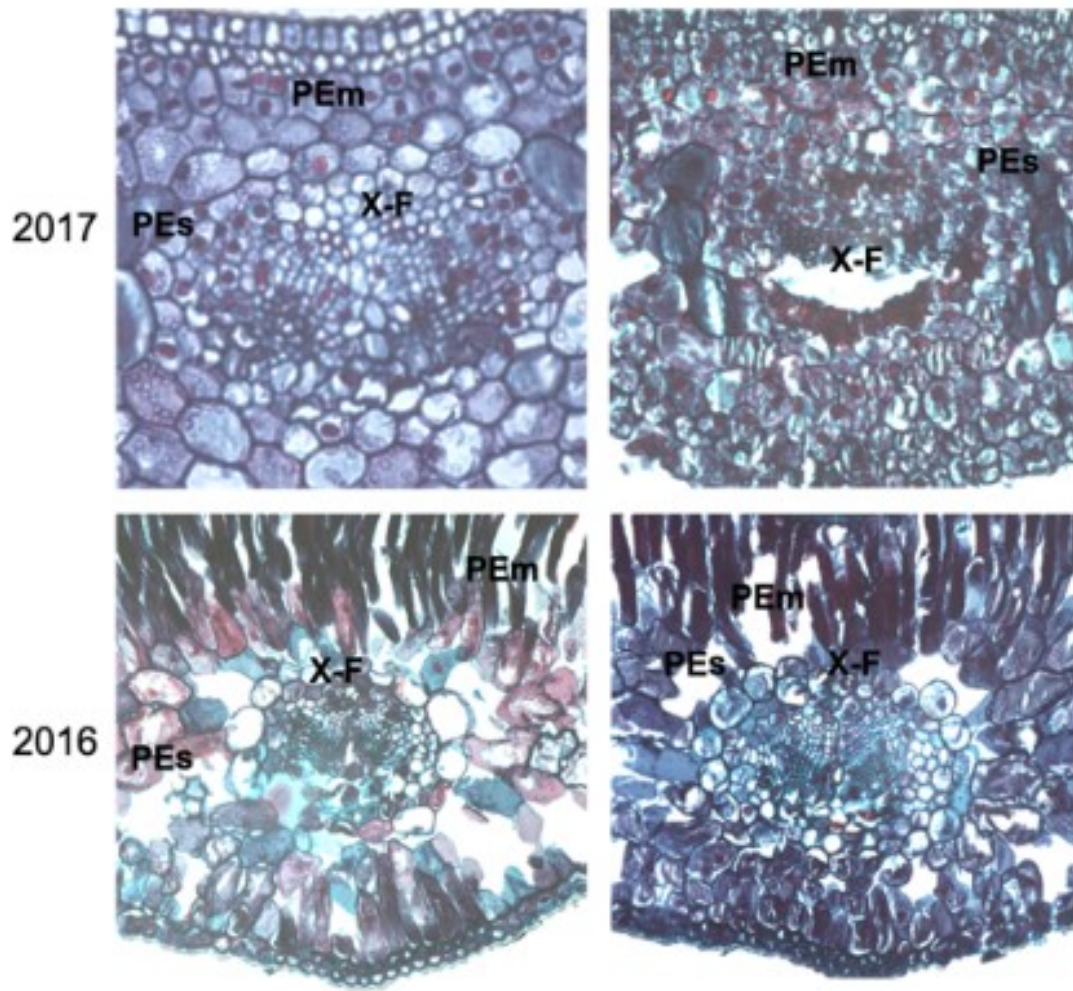
Figure S3 Histological sections of needles from asymptomatic (left) and symptomatic (right) sacred (*Abies religiosa*) individuals from two growing seasons (2017 top; 2015 bottom). All bars = 10µm. PEm, palisade parenchyma; PEs, spongy parenchyma; X-P, xylem and phloem.

a)

Damaged-2015 | Damaged-2016
Tolerant-2015 | Tolerant-2016

Categories: å-Muurolene, ð-Cadinene, å-Cubebene, ß-Cubebene, å-Caryophylene, ß-Caryophylene oxide, L-å-Bornyl acetate, ß-Pinene

g/100g Tissue

b)

Damaged-2015 | Damaged-2016
Tolerant-2015 | Tolerant-2016

Categories: å-Muurolene, ð-Cadinene, å-Cubebene, ß-Cubebene, å-Caryophylene, ß-Caryophylene oxide, L-å-Bornyl acetate, ß-Pinene

g/100g Tissue

Figure S4 Sesquiterpene relative concentrations (mg / 100g dry weight) in needles from symptomatic (red) and asymptomatic (green) sacred fir (*Abies religosa*) during two periods with contrasting O_3 concentration (87ppb, S4a; 170 ppb, S4b). Measures taken from two-(light colors) and three-years old (darker colors) needles.
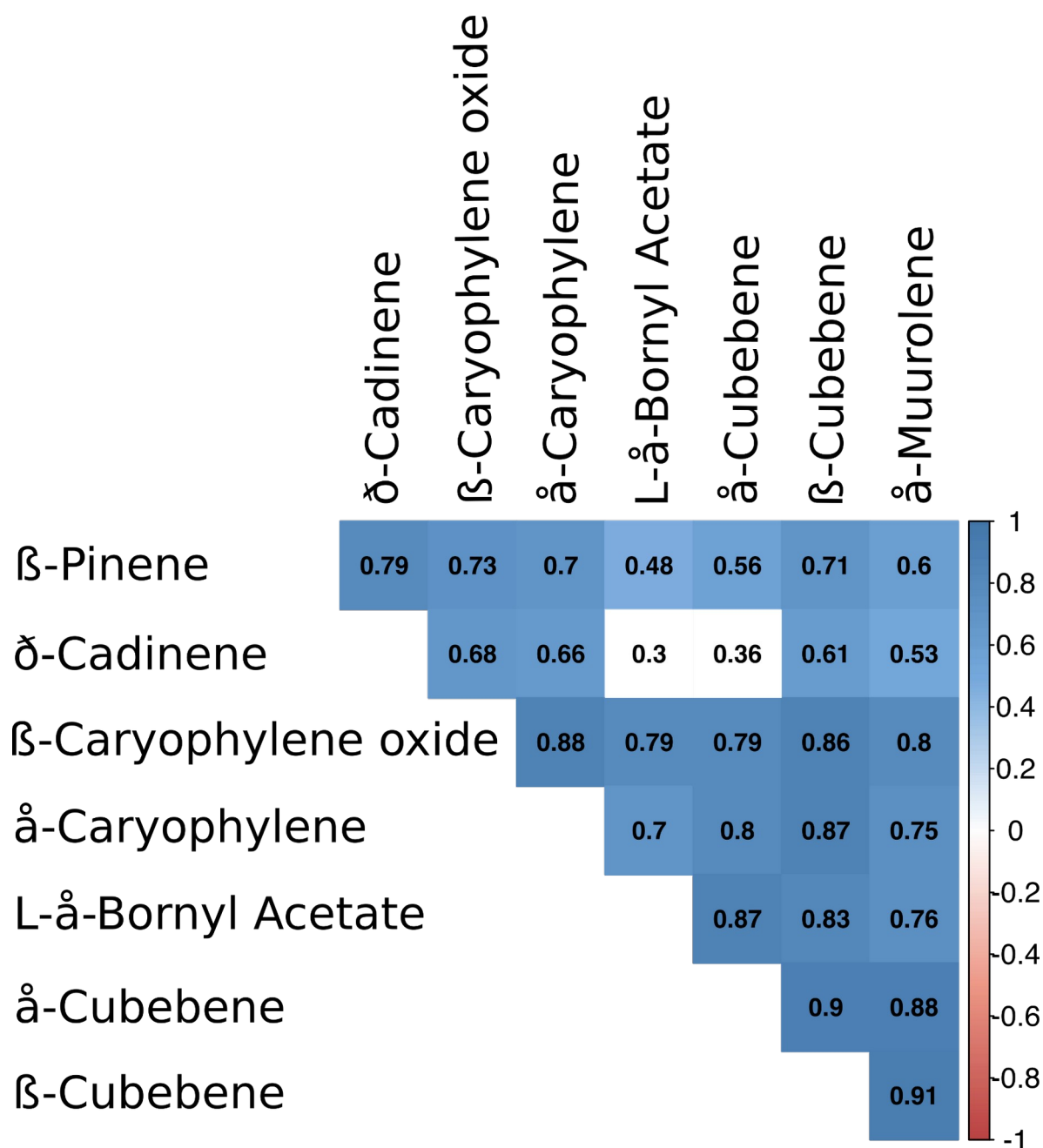
Figure S5 Correlation coefficients between sesquiterpenes detected in needles of sacred fir (*Abies religiosa*) individuals from a heavily polluted site near Mexico City. β-cubebene was dismissed from posterior analysis.
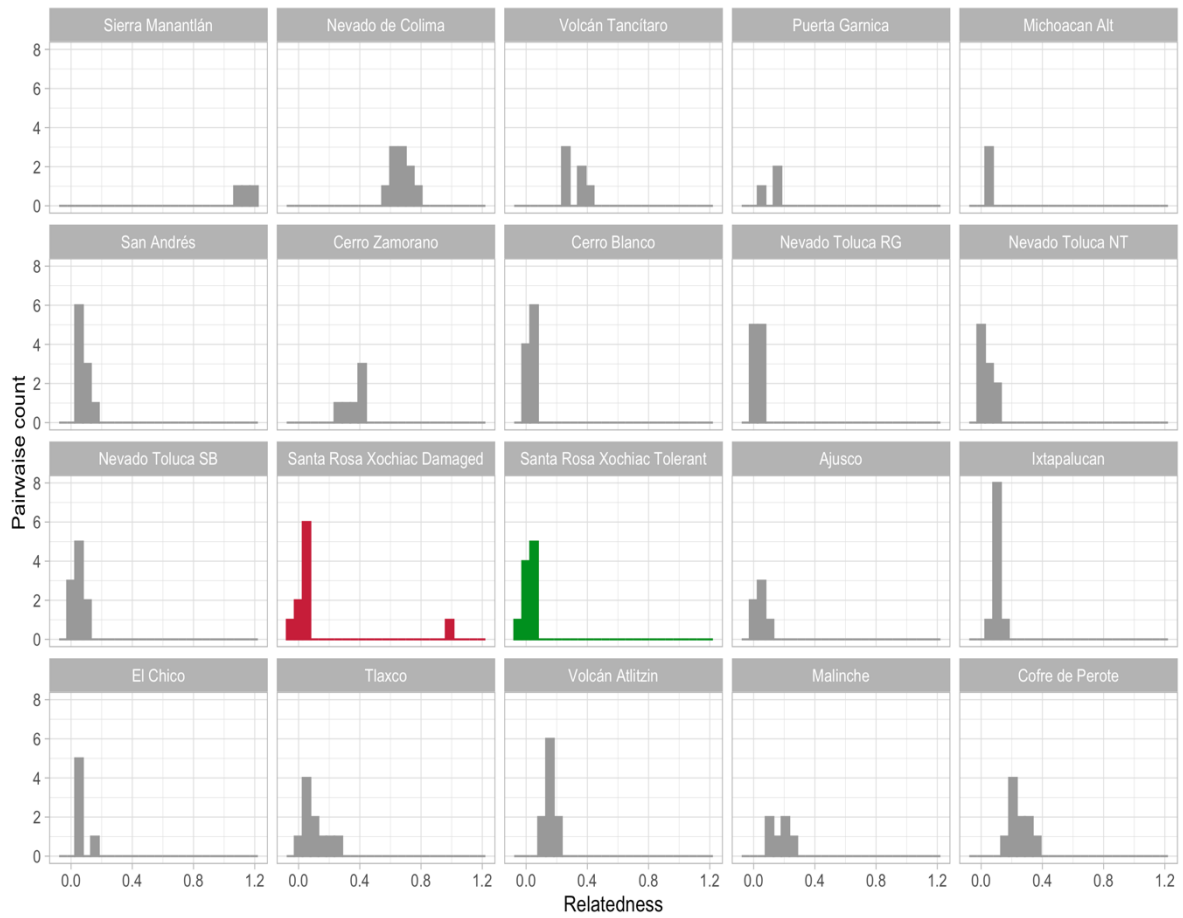
Figure S6 Relatedness between sacred fir (*Abies religiosa*) individuals used for genetic assignment analyses. Asymptomatic individuals from study sites in green, symptomatic trees in red.

TABLES

Table S1 Differentially expressed transcripts between symptomatic and asymptomatic sacred fir (*Abies religiosa*)

Contig ID	Log$_2$ fold change [a]	Query length, nts	Score[b] / Max query cover in the 1st 5 hits, %	Annotation	Notes
AB_000588_T.1	7.257	707	L / 40	Hypothetical protein KI387_017072, partial [*Taxus chinensis*]	The only hit returned by the ncbi Blastx
AB_045531_T.1	4.450	1192	M / 68	Hypothetical protein	Mostly bacterial hits
AB_015092_T.1	1.614	1944	H / 89	*Nuclear fusion defective 4-like,* N*odulin-like*	*Nuclear fusion defective 4* in *A. thaliana* is involved in response to salt stress (Sottosanto et al. 2007).
AB_036475_T.1	1.437	650	H / 78	*Chitinase* class VII / II / IV / or EP3-like / 4 / 5	Chitinases are involved in responses to various abiotic and biotic stresses. Acidic chitinase is **upregulated after ozone exposure** in tobacco (Ernst et al. 1992)
AB_018867_T.1	1.302	409	L / 37	Unknown [*Picea sitchensis*]	Four hits in 2 unknown proteins of *P. sitchensis* (Could be conifer-specific protein)
AB_029334_T.1	-1.187	2594	H / 72	*Probable L-type lectin-domain containing receptor kinase S.5*	L-type lectin receptor kinases are involved in defense response to bacteria and oomycetes (Bouwmeester and Govers 2009).
AB_029013_T.1	-1.371	1214	VL / 21	Hypothetical protein	Three hits in two different OFRs

AB_035458_T.1	-2.8306	928	H / 99	*Leucine-rich repeat (LRR) receptor-like serine/threonine -protein kinase*	A large family of LRR receptor-like kinases (RLK) participate in all aspects of plant development, in response to abiotic stresses, in defense processes and in plant-micbrobe interactions. Loss of the LRR-RLK GHR1 **resulted in O3 sensitivity** in *A. thaliana*, likely mediated by the associated disruption of stomatal function (Sierla et al. 2018).
AB_038616_T.1	- 4.951	752	H / 88		
AB_027319_T.1	-7.549	895	L / 39	Tetratricopeptide repeat (TPR)-like / patatin-like phospholipase domain protein / oidium resistance required protein 1/ TOM1-like protein 2	Members of TPR protein superfamily includes ones with potential to interact with Hsp90/Hsp70 as co-chaperones in nucleus and cytoplasm, thus participating in response to biotic stresses; RNA binding proteins involved in mRNA edition in plastid and mitochondria, are involved in plant development. Patatin-like phospholipase domain proteins involved in plant development, synthesis of secondary metabolites, cell death, defense responses, response to abiotic stresses (Lebeda et al. 2014).

AB_038562_T.1	-23.104	951	No hit	No hit	No significant similarity either in BLASTn search in NCBI nr database, neither in congenie.

[a] Positive value: up regulated in symptomatic trees; Negative value: down regulated in symptomatic trees;

[b] H: high (>200); M: medium (80-200); L: low (50-80); VL: very low (40-50).

Table 2 Differentially expressed transcripts in symptomatic sacred fir trees high vs. moderate O_3 concentration dates.

ID Locus	Log$_2$ fold change [a]	Query length, nts	Score[b] / Max query cover in the 1st 5 hits, %	Annotation	Notes
AB_002157_T.1	4.255	609	VL / 30	Hypothetical protein [*Acinetobacter baumannii*]	NCBI BLASTn returns five hits of mRNA sequences of *Picea glauca* with 81.49% to 87.93% identity
AB_028063_T.1	3.717	1034	No hit	No hit	
AB_029211_T.1	3.265	1193	H / 44 (H / 76)	No Apical Meristem, (NAC) transcription factor (Unannotated protein [*Picea sitchensis*])	Members of the huge family of NAC transcription factors are involved in many aspects of plant development, defense response to bacteria and other organisms, response to water deprivation and to abscisic, secondary metabolic processes. ANAC013, ANAC016, ANAC017, ANAC053 and ANAC078 regulate oxidative stress in *A. thaliana* (De Clercq et al. 2013). At4g28530 of *A. thaliana* is an **ozone-repressed** gene (Mahalingam et al. 2006).
AB_023740_T.1	2.911	1320	H / 62	Xyloglucan endotrans glucosylase (XET) /hydrolase; Glycosyl hydrolase family 16	XET enzymes participate in cell wall remodeling, thus modulating its expansion and strength. The contig covers complete XET CDS. Xyloglucan endo-β-transglucosylases/hydrolases was down-regulated in rice **sensitive to ozone**, this alters the cell wall composition (Le Gall et al. 2015). Expression of XET

					coding gene XTR9 **increased in response to O$_3$** (Zhang et al. 2017).
AB_015079_T.1	2.094	1291	M / 24	Linker histone H1	Linker (H1) histones are the most variable histones; H1.3 variant of *A. thaliana* is involved in adaptive responses to abiotic stress (Rutowicz et al. 2015).
AB_008838_T.1	-1.7	1312	H / 89	UDP-glucosyl transferase (UGT) 7-deoxyloganetin glucosyltransferase-	The enzymes of the UGT family act on a variety of substrates and participates in many metabolic processes, including flavonol (e.g. UGT78D1/At1g30530), tetrapyrrole (e.g UGT85A1/AT1G22400) or terpenoid (e.g. UGT89B1/ AT1G73880) biosynthesis. Some UGTs involved in response to abiotic and biotic stresses (Rehman et al. 2018). Transcription of UGT78D2/At5g17050 gene was **decreased after O$_3$ exposure** for 2 days (Booker et al. 2012).

[a] Positive value: up regulated during high O$_3$ concentration dates; Negative value: down regulated during high O$_3$ concentration dates;
[b] H: high (>200); M: medium (80-200); L: low (50-80); VL: very low (40-50).

Table 3 Differentially expressed transcripts in asymptomatic sacred fir trees during high vs. moderate O_3 concentration dates.

ID Locus	Log$_2$ fold change [a]	Query length, nts	Score[b] / Max query cover in the 1st 5 hits, %	Annotation	Notes
AB_010244_T.1	7.274	2007	H / 59	Metal tolerance protein (MTP) 5, 11 Cation diffusion facilitator (CDF) efflux family proteín	Plant MTPs from CDF family are involved in enhancing resistance to heavy metal tolerance
AB_022453_T.1	6.398	613	M / 56	Pathogenesis-related (PR) thaumatin family protein	PR thaumatin family proteins are involved in defense response, response to fungus, response to osmotic stress, response to water deprivation, response to wounding, regulation of metabolism and plant development (e.g. AT4G36010 and AT1G20030 in *A. thaliana*) regulation of metabolism and plant development (many in *A. thaliana*).
AB_040533_T.1	6.07	561	H / 90	Disease resistance-responsive dirigent-like protein	Many dirigent-like proteins are involved in defense response; some in response to wounding, cell wall biogenesis and metabolic processes.
AB_025629_T.1	5.388	1582	H, M / 88	LRR and NB-ARC domain disease resistance protein; disease	NB-ARC domain disease resistance (R) proteins in plants are involved in pathogen recognition and subsequent activation of innate immune responses. Besides, Glyma12g01420

				resistance protein RPP13, RPM1, RGA2, RGA4	was **upregulated in response to elevated ozone** in Glycine max (Leisner et al. 2014).
AB_022256_T.1	4.635	1436	H / 82 --	S-adenosyl methionine (SAM) synthase	Small family of plant S-adenosylmethionine synthases, or methionine adenosyltransferase (MAT) produces SAM from methionine and ATP. Methyl group of SAM can be transferred to a variety of molecules that includes nucleic acids, proteins, lipids and secondary metabolites. Therefore, the methylation rates for a variety of substrates affects multiple aspects of plant fitness. Besides, in plants SAM is a precursor of ethylene and polyamines. Histone and DNA methylation is highly important for the regulation of gene expression (Sekula et al. 2020).
AB_013716_T.1	3.549	1989	H / 74	3-ketoacyl (oxoacyl)-CoA synthase	Members of the 3-ketoacyl-CoA synthase family are involved in the biosynthesis of very long chain fatty acids (VLCFA), therefore, in cuticle development and wax and suberin synthesis. They also have an important role in response to cold, to light stimulus, to osmotic stress and to wounding
AB_043005_T.1	3.549	1193	M / 63	B-box-type Zinc finger and CCT domain protein CONSTANS-LIKE (COL)	COL transcription factors are involved in regulation of plant growth and development, control of flowering time and responses to stresses (Khatun et al. 2021).
AB_000610_T.1	3.054	1461	H / 68	beta-1,3-glucanase, or glucan endo-1,3-beta-glucosidase	Beta-1,3-glucanases degrade plant callose and components of plants, fungi and bacteria, therefore, are involved in defense response. Some of them are also involved in response to cold, heat and wounding.

AB_021997_T.1	2.999	2144	H / 81 Phosphoenol pyruvate phosphomut ase	Isocitrate lyase	Isocitrate lyase is a glyoxylate cycle enzyme; it is involved in plant salt tolerance (Yuenyong et al. 2019).
AB_002147_T.1	2.926	1211	H 7 82	Peroxidase 72 class III peroxidase	*A. thaliana* Peroxidase 72 (AT5G66390) is involved in lignin biosynthesis and in response to oxidative stress; many class III peroxidases are located in cell wall and involved in cell wall modification; some may play a role in generating H_2O_2 during defense response. **Near-ambient concentrations of ozone can induce ascorbate peroxidase** APX1 gene expression in *A. thaliana* and tobacco (Kubo et al. 1995, Wang et al. 1999). At least part of the induction of heat shock proteins during light stress in Arabidopsis is mediated by H_2O_2 that is scavenged by APX1.
AB_000596_T.1	2.883	475	No hit	No hit	
AB_013152_T.1	1.832	1494	H / 65	Carboxyleste rase 15; alpha/beta hydrolase fold	Carboxylesterases hydrolyze esters of short-chain fatty acids and involved in metabolism of jasmonic acid and salicylic acid and in systemic acquired resistance. They belong to the larger alpha/beta hydrolase fold superfamily of enzymes.
AB_028624_T.1	1.798	967	H / 40	Early nodulin-like (ENODL) with cupredoxin/p lastocyanin domain	Cupredoxins contain type I copper centers and are involved in inter-molecular electron transfer reactions. ENODLs extracellular proteins are anchored in the plasma membrane. AtENODL1 (AT5G53870) transcript is up-regulated in leaves of *A. thaliana* subjected to a combination of drought and heat stress. AtENODL2 (AT4G27520) is involved on responses to water deprivation, abscisic

					acid, salt stress, light and temperature stimuli (Rizhsky et al. 2004).
AB_031334_T.1	1.736	752	M / 40	Zinc finger Ran-binding domain-containing protein 2; RNA-binding protein c17h9.04c; UPF0481 protein	Mammalian zinc finger Ran-binding domain-containing protein 2 is an RNA-binding protein involved in alternative splicing.
AB_015079_T.1	1.73	1291	M / 24	Histone H1	Linker (H1) histones are the most variable histones; H1.3 variant of *A. thaliana* is involved in adaptive responses to abiotic stress (Rutowicz et al. 2015).
AB_039330_T.1	1.601	974	L (M) / 25	Hypothetical protein (plants), Set1 complex component ash2	The Set1 complex specifically methylates Lys-4 of histone H3 (H3K4). H3K4 is an epigenetic modification involved in the **regulation (induction) of gene expression.**
AB_013119_T.1	1.429	465	No hit		Two Picea BLASTn hits suggest that it could be conifer-specific polyA RNA.
AB_018867_T.1	-1.431	409	L / 37	Unknown protein [*Picea sitchensis* only]	Could represent a conifer-specific proteín
AB_000811_T.1	-1.949	1592	H / 61	Flavonol synthase 2OG-Fe(II) oxygenase GA2ox9, GA2ox10	Some 2OG-Fe(II) oxygenases (as AT5G24530 in *A. thaliana*) participates in flavonoid biosynthesis; therefore, they may be involved in response to salicylic acid and defense response to bacteria, oomycetes and fungus. A homology to GA2ox9 that contribute to cold stress tolerance and involved in response to water deprivation and wounding (Lange et al. 2020), is also revealed/present.

AB_029470_T.1	-3.459	1182	H / 69	(Iso)eugenol synthase 1, isoflavone reductase, propenylphenol synthase 1 NmrA-like protein	The inferred protein posess similarity to several classes of enzymes with Rossman fold. Among them are the isoflavone reductases Involved in response to oxidative stress and to wounding, as well as the propenylphenol synthases involved in synthesis of phenylpropanoid compounds, propenyl-phenols (Wibe et al. 1997), presumed to serve mainly in defense against herbivores and parasites.
AB_008960_T.1	-5.169	1226	H / 80 NmrA-like family NAD(P)H-binding NAD dependent epimerase/dehydratase family		
AB_000071_T.1	-6.206	1408	H / 60	Ferritin, desiccation-related protein PCC13-62	Arabidopsis ferritins are essential to protect cells against oxidative damage (Ravet et al. 2009).

[a] Positive value: up regulated during high O_3 concentration dates; Negative value: down regulated during high O_3 concentration dates;
[b] H: high (>200); M: medium (80-200); L: low (50-80); VL: very low (40-50).

Table S4 Three-way ANOVA. Interactions between Condition (asymptomatic or damaged), Exposure Year (2015 or 2016) and Period (high or moderate). *(***) Significant at the 0.0001 probability level. (**) Significant at the 0.001 probability level. (*) Significant at the 0.05 probability level. (.) Significant at the 0.1 probability level. (ns) nonsignificant.*

Metabolite		Df	Sum Sq	Mean Sq	F value	Pr (>F)	sig
β-pinene	Period	1	3.480	3.480	7.093	0.01201	*
	Condition	1	4.706	4.706	9.594	0.00404	**
	Exposure Year	1	0.824	0.824	1.679	0.20433	ns
	Period: Condition	1	3.571	3.571	7.279	0.01104	*
	Period: Exposure Year	1	1.732	1.732	3.531	0.06937	.
	Condition: Exposure Year	1	0.903	0.903	1.842	0.18425	ns
	Period: Condition: Exposure Year	1	1.056	1.056	2.154	0.15200	ns
	Residuals	32	15.698	0.491			
L-α-bornyl acetate	Period	1	12.014	12.014	22.328	4.41e-05	***

		Df	Sum Sq	Mean Sq	F value	Pr(>F)	
	Condition	1	10.554	10.554	19.616	0.0000104	***
	Exposure Year	1	0.350	0.350	0.651	0.425854	ns
	Period: Condition	1	13.014	13.014	24.187	2.52e-05	***
	Period: Exposure Year	1	0.039	0.039	0.072	0.790364	ns
	Condition: Exposure Year	1	0.572	0.572	1.063	0.310371	ns
	Period: Condition: Exposure Year	1	0.505	0.505	0.938	0.340058	ns
	Residuals	32	17.218	0.538			
β-caryophyllene oxide	Period	1	40.60	40.60	49.994	5.10e-08	***
	Condition	1	25.17	25.17	30.991	3.82e-06	***
	Exposure Year	1	2.48	2.48	3.056	0.090	.
	Period: Condition	1	22.96	22.96	28.273	7.89e-06	***
	Period: Exposure Year	1	2.92	2.92	3.597	0.067	.
	Condition: Exposure Year	1	0.46	0.46	0.570	0.456	ns
	Period: Condition: Exposure Year	1	0.20	0.20	0.246	0.624	ns
	Residuals	32	25.98	0.81			
α-caryophyllene	Period	1	0.4886	0.4886	12.412	0.00131	**
	Condition	1	0.3166	0.3166	8.042	0.00786	**
	Exposure Year	1	0.1317	0.1317	3.347	0.07668	.
	Period: Condition	1	0.2543	0.2543	6.461	0.01607	*
	Period: Exposure Year	1	0.1809	0.1809	4.596	0.03974	*
	Condition: Exposure Year	1	0.1062	0.1062	2.697	0.11033	ns
	Period: Condition: Exposure Year	1	0.0686	0.0686	1.744	0.19601	ns
	Residuals	32	1.2597	0.0394			
β-cubebene	Period	1	1.5426	1.5426	22.981	3.61e-05	***
	Condition	1	0.8936	0.8936	13.312	0.000929	***
	Exposure Year	1	0.1360	0.1360	2.026	0.164339	
	Period: Condition	1	0.7971	0.7971	11.875	0.001611	**
	Period: Exposure Year	1	0.1751	0.1751	2.608	0.116137	ns
	Condition: Exposure Year	1	0.0315	0.0315	0.470	0.497991	ns
	Period: Condition: Exposure Year	1	0.0334	0.0334	0.497	0.485712	ns
	Residuals	32	2.1480	0.0671			
α-cubebene	Period	1	1.0663	1.0663	16.388	0.000306	***
	Condition	1	0.8664	0.8664	13.316	0.000928	***
	Exposure Year	1	0.2654	0.2654	4.079	0.051861	.
	Period: Condition	1	0.7493	0.7493	11.516	0.001855	**
	Period: Exposure Year	1	0.2611	0.2611	4.012	0.053701	.
	Condition: Exposure Year	1	0.1652	0.1652	2.538	0.120943	ns
	Period: Condition: Exposure Year	1	0.1623	0.1623	2.495	0.124049	ns
	Residuals	32	2.0821	0.0651			

γ-cadinene	Period	1	3.363	3.363	7.835	0.00861	**
	Condition	1	2.012	2.012	4.688	0.03794	*
	Exposure Year	1	1.387	1.387	3.231	0.08172	.
	Period: Condition	1	3.230	3.230	7.525	0.00989	**
	Period: Exposure Year	1	0.258	0.258	0.602	0.44363	ns
	Condition: Exposure Year	1	0.060	0.060	0.140	0.71047	ns
	Period: Condition: Exposure Year	1	0.086	0.086	0.201	0.65655	ns
	Residuals	32	13.737	0.429			
α-muurolene	Period	1	0.3238	0.3238	9.643	0.00396	**
	Condition	1	0.2355	0.2355	7.012	0.01247	*
	Exposure Year	1	0.0661	0.0661	1.969	0.17016	ns
	Period: Condition	1	0.1995	0.1995	5.941	0.02053	*
	Period: Exposure Year	1	0.0431	0.0431	1.283	0.26584	ns
	Condition: Exposure Year	1	0.0075	0.0075	0.223	0.64028	ns
	Period: Condition: Exposure Year	1	0.0242	0.0242	0.721	0.40217	ns
	Residuals	32	1.0745	0.0336			

Booker F, Burkey K, Morgan P, Fiscus E, Jones A (2012) Minimal influence of G-protein null mutations on ozone-induced changes in gene expression, foliar injury, gas exchange and peroxidase activity in Arabidopsis thaliana L.: Minimal influence of G-proteins on ozone responses. Plant Cell Environ 35:668–681.

Bouwmeester K, Govers F (2009) Arabidopsis L-type lectin receptor kinases: phylogeny, classification, and expression profiles. J Exp Bot 60:4383–4396.

De Clercq I, Vermeirssen V, Van Aken O, Vandepoele K, Murcha MW, Law SR, Inzé A, Ng S, Ivanova A, Rombaut D, van de Cotte B, Jaspers P, Van de Peer Y, Kangasjärvi J, Whelan J, Van Breusegem F (2013) The Membrane-Bound NAC Transcription Factor ANAC013 Functions in Mitochondrial Retrograde Regulation of the Oxidative Stress Response in Arabidopsis. Plant Cell 25:3472–3490.

Ernst D, Schraudner M, Langebartels C, Sandermann H (1992) Ozone-induced changes of mRNA levels of β-1,3-glucanase, chitinase and 'pathogenesis-related' protein 1b in tobacco plants. Plant Mol Biol 20:673–682.

Khatun K, Debnath S, Robin AHK, Wai AH, Nath UK, Lee D-J, Kim C-K, Chung M-Y (2021) Genome-wide identification, genomic organization, and expression profiling of the CONSTANS-like (COL) gene family in petunia under multiple stresses. BMC Genomics 22:727.

Kubo A, Saji H, Tanaka K, Kondo N (1995) Expression of arabidopsis cytosolic ascorbate peroxidase gene in response to ozone or sulfur dioxide. Plant Mol Biol 29:479–489.

Lange T, Krämer C, Pimenta Lange MJ (2020) The Class III Gibberellin 2-Oxidases AtGA2ox9 and AtGA2ox10 Contribute to Cold Stress Tolerance and Fertility. Plant Physiol 184:478–486.

Le Gall H, Philippe F, Domon J-M, Gillet F, Pelloux J, Rayon C (2015) Cell Wall Metabolism in Response to Abiotic Stress. Plants 4:112–166.

Lebeda A, Mieslerová B, Petřivalský M, Luhová L, Špundová M, Sedlářová M, Nožková-Hlaváčková V, Pink DAC (2014) Resistance mechanisms of wild tomato germplasm to infection of Oidium neolycopersici. Eur J Plant Pathol 138:569–596.

Leisner CP, Ming R, Ainsworth EA (2014) Distinct transcriptional profiles of ozone stress in soybean (Glycine max) flowers and pods. :13.

Mahalingam R, Jambunathan N, Gunjan SK, Faustin E, Weng H, Ayoubi P (2006) Analysis of oxidative signalling induced by ozone in Arabidopsis thaliana. Plant Cell Environ 29:1357–1371.

Ravet K, Touraine B, Boucherez J, Briat J-F, Gaymard F, Cellier F (2009) Ferritins control interaction between iron homeostasis and oxidative stress in Arabidopsis. Plant J 57:400–412.

Rehman HM, Nawaz MA, Shah ZH, Ludwig-Müller J, Chung G, Ahmad MQ, Yang SH, Lee SI (2018) Comparative genomic and transcriptomic analyses of Family-1 UDP glycosyltransferase in three Brassica species and Arabidopsis indicates stress-responsive regulation. Sci Rep 8:1875.

Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R (2004) When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant Physiol 134:1683–1696.

Rutowicz K, Puzio M, Halibart-Puzio J, Lirski M, Kroteń MA, Kotliński M, Kniżewski Ł, Lange B, Muszewska A, Śniegowska-Świerk K, Kościelniak J, Iwanicka-Nowicka R, Żmuda K, Buza K, Janowiak F, Jõesaar I, Laskowska-Kaszub K, Fogtman A, Zielenkiewicz P, Tiuryn J, Kollist H, Siedlecki P, Ginalski K, Świeżewski S, Koblowska M, Archacki R, Wilczyński B, Rapacz M, Jerzmanowski A (2015) A specialized histone H1 variant is required for adaptive responses to complex abiotic stress and related DNA methylation in Arabidopsis. Plant Physiol:pp.00493.2015.

Sekula B, Ruszkowski M, Dauter Z (2020) S-adenosylmethionine synthases in plants: Structural characterization of type I and II isoenzymes from Arabidopsis thaliana and Medicago truncatula. Int J Biol Macromol 151:554–565.

Sierla M, Hõrak H, Overmyer K, Waszczak C, Yarmolinsky D, Maierhofer T, Vainonen JP, Salojärvi J, Denessiouk K, Laanemets K, Tõldsepp K, Vahisalu T, Gauthier A, Puukko T, Paulin L, Auvinen P, Geiger D, Hedrich R, Kollist H, Kangasjärvi J (2018) The Receptor-like Pseudokinase GHR1 Is Required for Stomatal Closure. Plant Cell 30:2813–2837.

Sottosanto JB, Saranga Y, Blumwald E (2007) Impact of AtNHX1, a vacuolar Na+/H+ antiporter, upon gene expression during short- and long-term salt stress in Arabidopsis thaliana. BMC Plant Biol 7:18.

Wang J, Zhang H, Allen RD (1999) Overexpression of an Arabidopsis Peroxisomal Ascorbate Peroxidase Gene in Tobacco Increases Protection Against Oxidative Stressl. :8.

Wibe A, Borg-Karlson A-K, Norin T, Mustaparta H (1997) Identification of plant volatiles activating single receptor neurons in the pine weevil (Hylobius abietis). J Comp Physiol A 180:585–595.

Yuenyong W, Sirikantaramas S, Qu L-J, Buaboocha T (2019) Isocitrate lyase plays important

roles in plant salt tolerance. BMC Plant Biol 19:472.

Zhang L, Xu B, Wu T, Wen M, Fan L, Feng Z, Paoletti E (2017) Transcriptomic analysis of Pak Choi under acute ozone exposure revealed regulatory mechanism against ozone stress. BMC Plant Biol 17:236.