Supplementary Material

1 Supplementary Figures and Tables

Puentes et al. - Variation in methyl jasmonate-induced defense among Norway spruce clones and trade-offs in resistance against a fungal and an insect pest

Tables: 7

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1.1 Supplementary Figures

Figure S1. Picture showing some of the Norway spruce (*P. abies*) plants used in the experiment. The second MeJA treatment had been applied on May 14th (one plant for each of the treatments is shown: from the left, 0, 25 and 50 mM MeJA), and the picture was taken the day of planting (May 21st) in the semi-field experiment.

Figure S2. Mean ± standard error **(a)** plant height (cm), **(b)** stem diameter (mm), and **(c)** top shoot length (mm) per clone for Norway spruce (*P. abies*) plants treated with 0, 25 or 50 mM methyl Jasmonate (MeJA). Clone number 1 did not have any plants in the 50 mM MeJA treatment (see Materials and Methods for justification and sample sizes per clone and treatment combination).

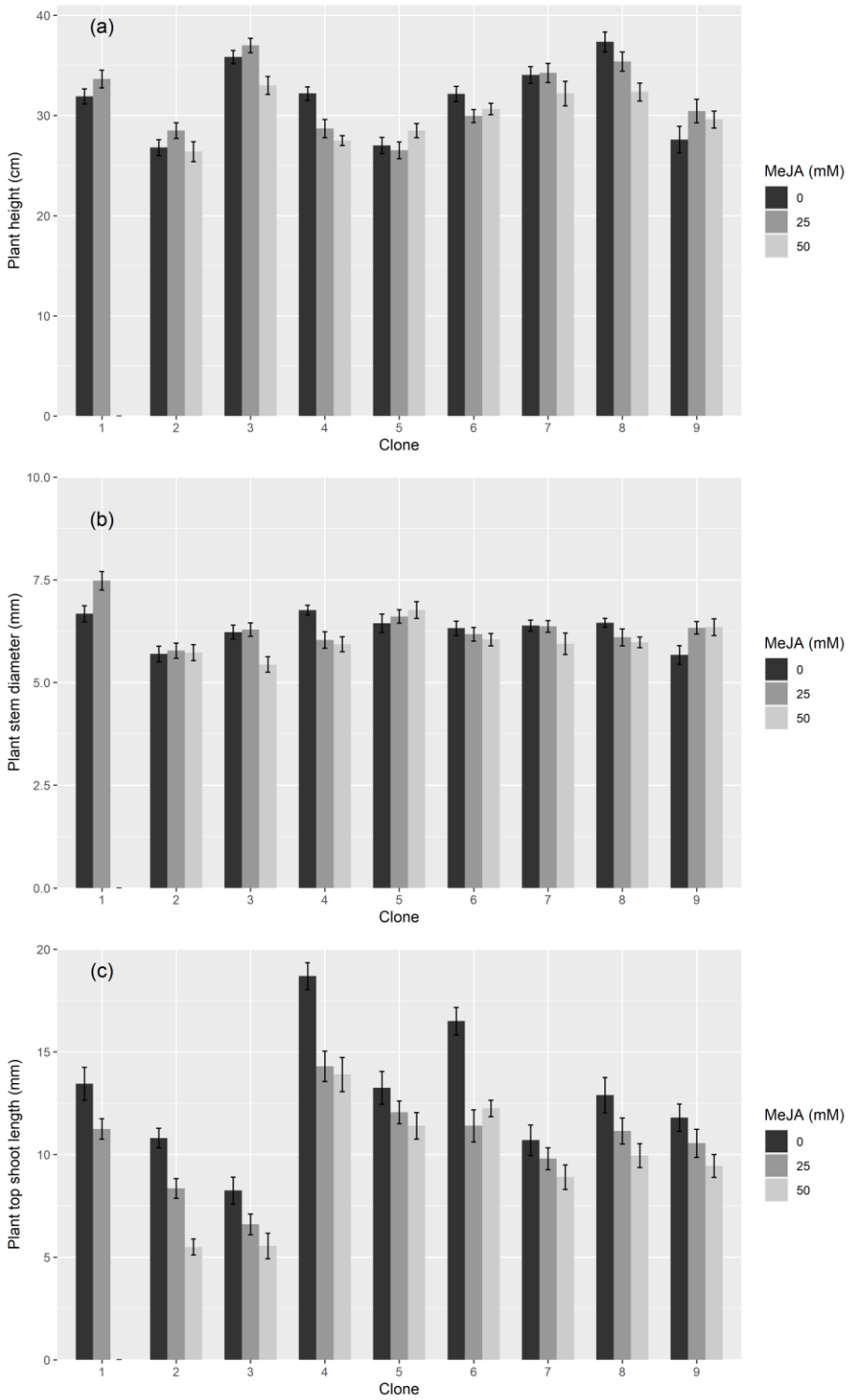


Figure S3. Principal component analysis (PCA) biplots (for non-fungus inoculated plants) of relative terpene amounts. Variation along the two main axes of variation (PC 1, PC 2; % variation explained by each axis) is shown for methyl jasmonate-treated (50 mM MeJA, blue filled triangles) and non-treated (0 mM MeJA, orange filled circles) clones of Norway spruce (*P. abies*). Mean PCA score values (± standard errors) for each clone (1-9) are shown, as well as the direction and distance of change (black arrows) in terpene chemistry between the two MeJA treatments. The position of terpenes indicates PCA loadings (vectors), which illustrate each compound's correlation with the two axes of variation (see Table S6 for loading values).

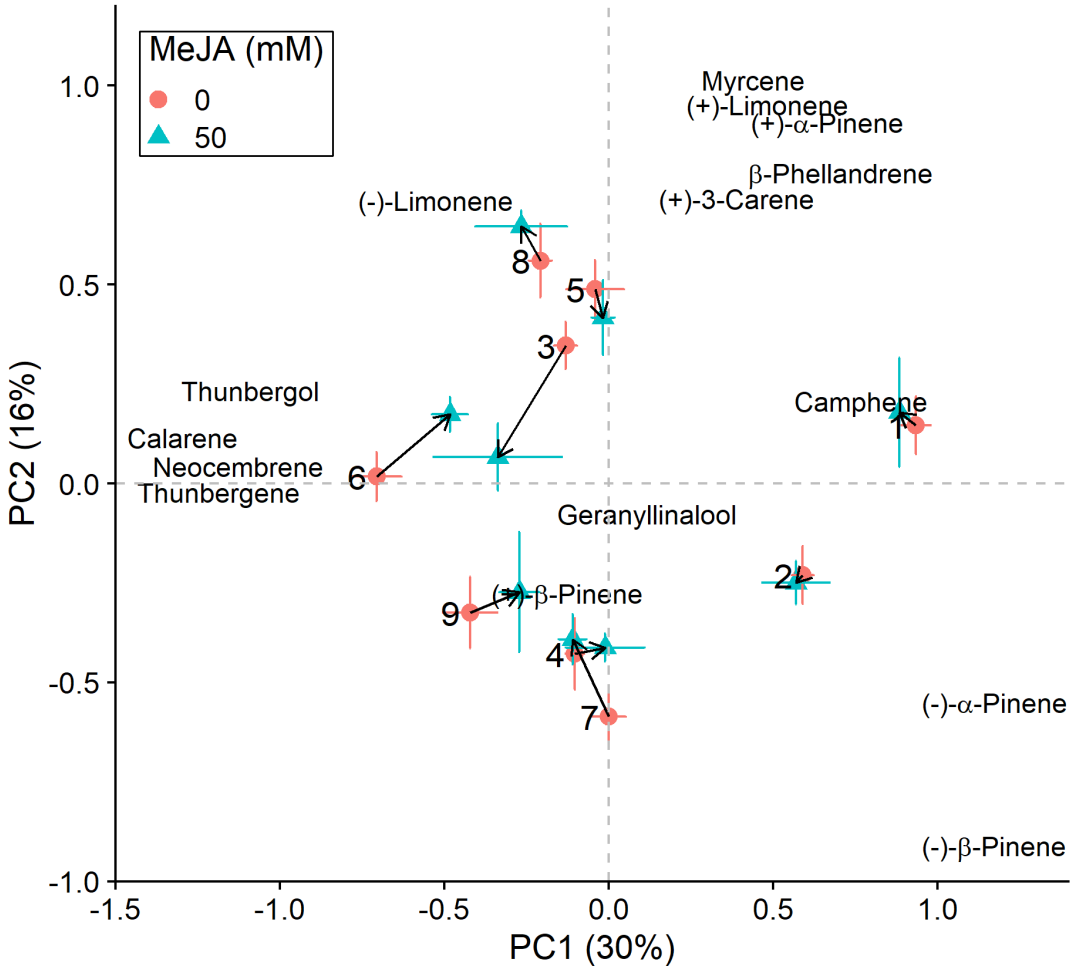
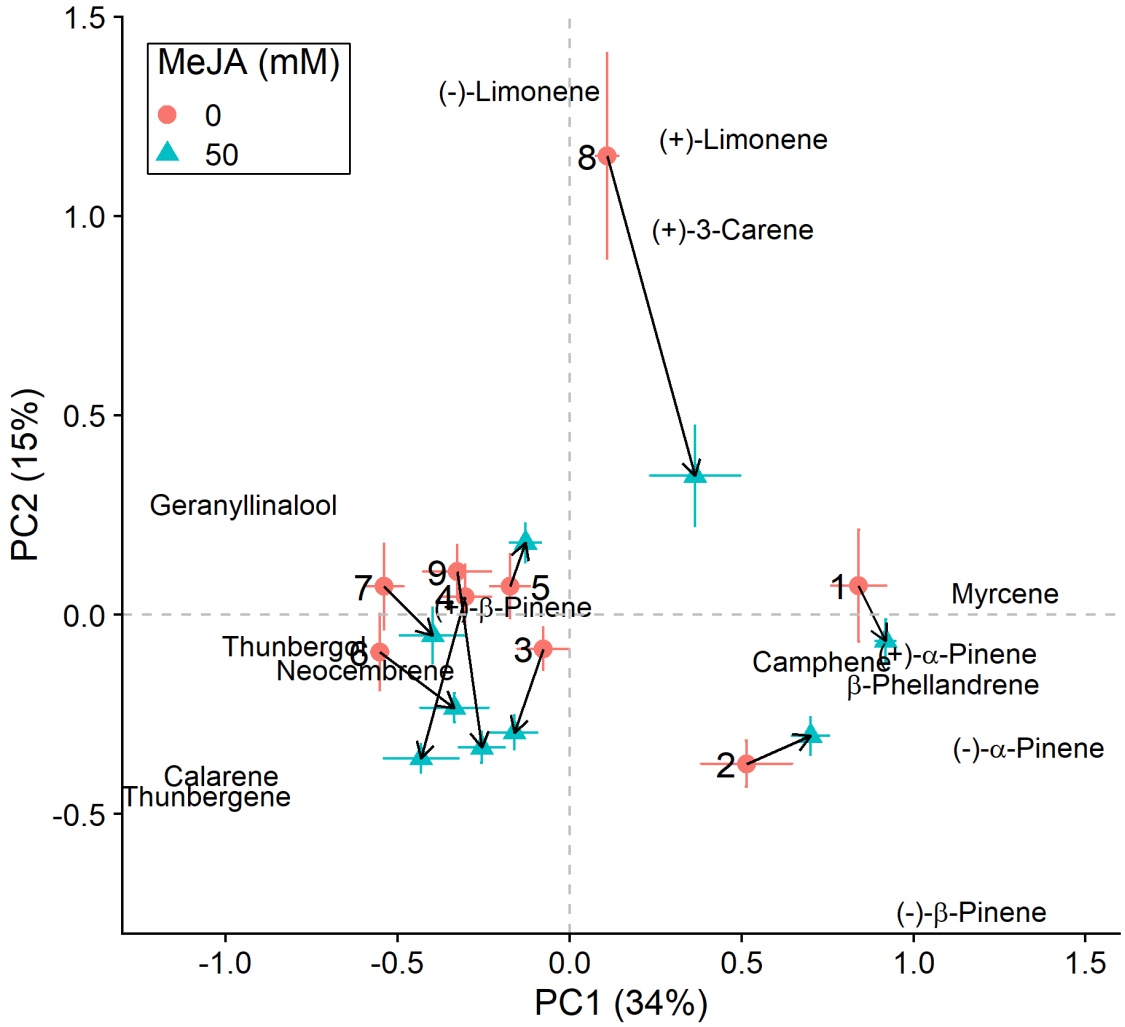


Figure S4. Principal component analysis (PCA) biplots (for *E. polonica*-inoculated plants) of relative terpene amounts. Variation along the two main axes of variation (PC 1, PC 2; % variation explained by each axis) is shown for methyl jasmonate-treated (50 mM MeJA, blue filled triangles) and non-treated (0 mM MeJA, orange filled circles) clones of Norway spruce (*P. abies*). Mean PCA score values (± standard errors) for each clone (1-9) are shown, as well as the direction and distance of change (black arrows) in terpene chemistry between the two MeJA treatments. The position of terpenes indicates PCA loadings (vectors), which illustrate each compound's correlation with the two axes of variation (see Table S6 for loading values).



1.2 Tables

Table S1. Krutzch index indicating the developmental stage of Norway spruce (*P. abies*) clones before MeJA treatment was conducted. The index ranges between 0 to 8 and describes terminal bud development on the uppermost whorl of branches. 0 = Dormant buds, 1 = Buds slightly swollen, needles below buds bent backwards and outwards, 2 = Buds swollen, green to grey-green in color, bud scales still closed, 3 = Burst of bud scales, tips of needles emerging. See Krutzch (1973) for the description of remaining index values (4-8).

Clone #	Index
1	3
2	0
3	0
4	3
5	2
6	3
7	1
8	0
9	0

Table S2. Results from contrasts between treatments, obtained from models examining the effect of MeJA (MJ: 0, 25 or 50 mM) on area debarked (mm^2) by pine weevils (*H. abietis*) or lesion lengths (mm) inflicted by the blue-stain fungus (*E. polonica*) on Norway spruce (*P. abies*) clones. Models included the main effect and interaction of MeJA and clone, experimental enclosure or sub-plot for lesion length, and plant height as a covariate (for area debarked only). Estimate of differences between treatment means (Estimate), standard error of the difference (SE), degrees of freedom (DF), t-ratio, and p-value (P) are presented. Note that for clone 1, no plants in the 50 mM MeJA treatment were included in the experiment (see Materials and Methods).

Table in next page.

Table S2

Variable	Clone	Contrast	Estimate	SE	DF	t-ratio	P
Area debarked (mm^2)	1	0 vs MJ25	99.0	21.2	337	4.6	<0.0001
	2	0 vs MJ25	75.4	19.9	448	3.8	0.0002
		0 vs MJ50	76.1	19.8	448	3.8	0.0001
		MJ25 vs MJ50	0.7	6.2	448	0.1	0.9
	3	0 vs MJ25	68.7	16.4	448	4.2	<0.0001
		0 vs MJ50	73.1	16.6	448	4.4	<0.0001
		MJ25 vs MJ50	4.4	5.3	448	0.8	0.4
	4	0 vs MJ25	58.8	15.4	448	3.8	0.0002
		0 vs MJ50	49.2	16.2	448	3.0	0.003
		MJ25 vs MJ50	-9.6	7.9	448	-1.2	0.2
	5	0 vs MJ25	41.5	15.7	448	2.6	0.009
		0 vs MJ50	35.3	16.2	448	2.2	0.03
		MJ25 vs MJ50	-6.1	10.5	448	-0.6	0.6
	6	0 vs MJ25	56.5	13.6	448	4.2	<0.0001
		0 vs MJ50	55.3	13.6	448	4.1	0.0001
		MJ25 vs MJ50	-1.2	4.00	448	-0.3	0.76
	7	0 vs MJ25	36.9	12.4	448	3.0	0.003
		0 vs MJ50	38.4	12.4	448	3.1	0.002
		MJ25 vs MJ50	1.6	6.9	448	0.2	0.8
	8	0 vs MJ25	15.2	8.4	448	1.82	0.07
		0 vs MJ50	14.4	8.7	448	1.7	0.09
		MJ25 vs MJ50	-0.8	6.1	448	-0.1	0.90
	9	0 vs MJ25	10.7	7.8	448	1.4	0.2
		0 vs MJ50	8.3	8.0	448	1.0	0.3
		MJ25 vs MJ50	-2.4	5.6	448	-0.4	0.7
Lesion length (mm)	1	0 vs MJ25	2.8	1.5	66	1.9	0.07
	2	0 vs MJ25	1.8	0.9	88	1.9	0.05
		0 vs MJ50	1.2	0.9	88	1.2	0.2
		MJ25 vs MJ50	-0.7	0.8	88	-0.9	0.4
	3	0 vs MJ25	2.5	1.7	88	1.4	0.16
		0 vs MJ50	3.6	1.7	88	2.1	0.03
		MJ25 vs MJ50	1.1	1.4	88	0.8	0.42
	4	0 vs MJ25	5.5	1.8	88	3.1	0.003
		0 vs MJ50	4.6	1.8	88	2.5	0.01
		MJ25 vs MJ50	-0.9	1.0	88	-0.9	0.4
	5	0 vs MJ25	6.4	1.8	88	3.5	0.0008
		0 vs MJ50	6.0	1.8	88	3.3	0.001
		MJ25 vs MJ50	-0.4	0.9	88	-0.4	0.7
	6	0 vs MJ25	1.4	1.4	88	1.0	0.3
		0 vs MJ50	3.2	1.3	88	2.5	0.01
		MJ25 vs MJ50	1.8	1.1	88	1.7	0.09
	7	0 vs MJ25	1.5	0.9	88	1.6	0.1
		0 vs MJ50	0.7	1.0	88	0.7	0.5
		MJ25 vs MJ50	-0.8	0.8	88	-0.9	0.4
	8	0 vs MJ25	10.6	2.4	88	4.4	<0.0001
		0 vs MJ50	10.6	2.4	88	4.4	<0.0001
		MJ25 vs MJ50	-0.02	0.8	88	-0.02	0.9
	9	0 vs MJ25	13.3	2.8	88	4.7	<0.0001
		0 vs MJ50	12.7	2.9	88	4.4	<0.0001
		MJ25 vs MJ50	-0.6	0.9	88	-0.7	0.5

Table S3. Estimated means (from models presented in Table 1 in the main text) ± standard error for area debarked (mm^2) by pine weevils (*H. abietis*) and lesion lengths (mm) inflicted by the blue-stain fungus (*E. polonica*) on Norway spruce (*P. abies*) clones (1-9) that were treated with 0 mM and 50 mM methyl jasmonate (MeJA). These estimated means are depicted in Figure 1 in the main text.

Clone	0 mM MeJA		50 mM MeJA	
	Area debarked (mm^2)	Lesion length (mm)	Area debarked (mm^2)	Lesion length (mm)
1	42.47 ± 1.26	5.61 ± 1.24	8.07 ±1.39	2.84 ± 1.23
2	32.33 ± 1.31	4.69 ± 1.23	4.15 ± 1.53	3.60 ± 1.23
3	27.53 ± 1.29	8.48 ± 1.25	2.82 ± 1.56	5.04 ± 1.28
4	25.75 ± 1.30	8.70 ± 1.26	8.37 ± 1.41	4.44 ± 1.26
5	22.61 ± 1.34	9.71 ± 1.23	9.73 ± 1.40	4.13 ± 1.23
6	20.76 ± 1.32	6.32 ± 1.23	2.34 ± 1.62	3.41 ± 1.28
7	17.64 ± 1.33	4.20 ± 1.26	4.88 ± 1.47	3.61 ± 1.28
8	9.18 ± 1.38	13.39 ± 1.23	4.70 ± 1.46	3.57 ± 1.24
9	7.64 ± 1.45	16.01 ± 1.23	4.34 ± 1.49	4.27 ± 1.23

Table S4. Analysis of variance (SS: Sum of Squares, DF: degrees of freedom, F: F-value, P: p-value) results from models examining the effects of MeJA (MJ: 0, 25 or 50 mM) on plant height (cm), top shoot length (mm) and plant diameter (mm) of Norway spruce (*P. abies*) clones. Models included the main effect and interaction of MeJA and clone, and experimental enclosure (Block) as explanatory factors. Clone 1 was not included in these models since plants in the 50 mM MeJA treatment were not included in the experiment (see Materials and Methods). Significant effects are in bold ($P < 0.05$).

Variable	Source	SS	DF	F	P
Plant height (cm)	MJ	233.1	2	7.7	**0.0005**
	Clone	4365.1	7	41.3	**<0.0001**
	MJ x clone	695.3	14	3.3	**<0.0001**
	Block	93.5	3	2.1	0.1
	Residuals	6800.0	450		
Top shoot length (mm)	MJ	902.0	2	56.9	**<0.0001**
	Clone	3334.2	7	60.1	**<0.0001**
	MJ x clone	248.5	14	2.2	**0.006**
	Block	136.8	3	5.8	0.0007
	Residuals	3568.3	450		
Diameter (mm)	MJ	4.4	2	3.5	**0.03**
	Clone	25.3	7	5.7	**<0.0001**
	MJ x clone	28.8	14	2.8	**0.0005**
	Block	9.2	3	4.9	0.002
	Residuals	283.8	450		

Table S5. Multivariate analysis of variance (MANOVA) results (DF: degrees of freedom, SS: Sum of Squares, MS: Mean Squares, F: F-value, R^2: adjusted R-squared, P: p-value). Models examined the effect of Norway spruce (*P. abies*) clone, MeJA treatment (MJ: 0, 50 mM) and their interaction, on relative terpene amounts when plants had not been (No fungus exposure) and had been exposed to *E. polonica* (Exposed to fungus) following MeJA treatment. Significant effects are in bold ($P < 0.05$).

	Source	DF	SS	MSS	F	R^2	P
No fungus exposure	MJ	1	12.49	12.49	1.59	0.01	0.1
	Clone	8	682.07	85.26	10.85	0.52	**0.001**
	Clone x MJ	8	60.57	7.58	0.96	0.05	0.5
	Residuals	70	549.87	7.86		0.42	
	Total	87	1305.00			1.00	
Exposed to fungus	MJ	1	39.79	39.80	6.16	0.03	**0.001**
	Clone	8	715.53	89.44	13.85	0.55	**0.001**
	MJ x clone	8	89.07	11.13	1.72	0.07	**0.004**
	Residuals	69	445.60	6.46		0.34	
	Total	86	1290.00			1.00	

Table S6. Loadings for each compound in Principal components 1 and 2 (PC 1, PC 2) from the PCAs that examined variation in relative terpene chemistry (see Figures S3 and S4) among Norway spruce (*P. abies*) clones treated with 0 and 50 mM MeJA. Separate analyses were conducted for plants not exposed (No fungus exposure) and those exposed to *E. polonica* (Exposure to fungus).

Compound	No fungus exposure		Exposure to fungus	
	PC1	PC2	PC1	PC2
(-)-α-Pinene	0.36	-0.23	0.38	-0.15
(+)-α-Pinene	0.20	0.39	0.32	-0.04
Camphene	0.23	0.08	0.21	-0.05
(+)-β-Pinene	-0.04	-0.12	-0.05	0.006
(-)-β-Pinene	0.36	-0.38	0.33	-0.33
Myrcene	0.13	0.42	0.36	0.02
(+)-3-Carene	0.12	0.31	0.14	0.42
(-)-Limonene	-0.16	0.30	-0.04	0.57
(+)-Limonene	0.15	0.40	0.14	0.52
β-Phellandrene	0.22	0.32	0.31	-0.05
Thunbergene	-0.36	-0.005	-0.30	-0.19
Calarene	-0.40	0.05	-0.29	-0.18
Neocembrene	-0.34	0.02	-0.17	-0.06
Geranyllinalool	0.03	-0.03	-0.27	0.12
Thunbergol	-0.33	0.09	-0.23	-0.03

Table S7. Results from Pearson product-moment correlations (*r* = Pearson correlation coefficient; P = p-value) between estimated means per clone (n = 9) of area debarked (mm^2) (or lesion length, mm) and absolute amounts of each terpene compound (or the sum of all terpenes) ($\mu g\ g^{-1}$ dry wt). Estimates of area debarked and lesion lengths for each clone were obtained from models presented in Table 1 in the main text. These estimates were correlated to the average absolute terpene amounts of each compound (or the sum of all terpenes) per clone, both under the constitutive and induced state and (0 and 50 mM MeJA respectively) and without fungal inoculation. Significant correlations are in bold ($P < 0.05$).

Compound	0 mM MeJA				50 mM MeJA			
	Area debarked (mm^2)		Lesion length (mm)		Area debarked (mm^2)		Lesion length (mm)	
	r	**P**	***r***	**P**	***r***	**P**	***r***	**P**
(-)-α-Pinene	-0.44	0.24	**0.75**	**0.02**	-0.55	0.12	-0.16	0.69
(+)-α-Pinene	0.04	0.93	0.22	0.56	-0.36	0.34	-0.52	0.15
Camphene	0.05	0.90	0.64	0.06	**-0.82**	**0.007**	-0.14	0.72
(+)-β-Pinene	-0.31	0.41	-0.18	0.65	-0.12	0.76	-0.21	0.60
(-)-β-Pinene	-0.09	0.82	0.55	0.13	-0.55	0.13	-0.17	0.67
Myrcene	0.05	0.90	**0.79**	**0.01**	**-0.74**	**0.02**	-0.3	0.43
(+)-3-Carene	-0.2	0.61	0.57	0.11	0.09	0.82	-0.64	0.06
(-)-Limonene	**-0.81**	**0.009**	**0.93**	**0.00**	-0.21	0.59	-0.05	0.89
(+)-Limonene	-0.31	0.42	**0.91**	**0.001**	-0.28	0.47	-0.43	0.25
β-Phellandrene	0.18	0.65	**0.72**	**0.03**	**-0.74**	**0.02**	-0.29	0.44
Thunbergene	-0.46	0.21	0.45	0.22	**-0.78**	**0.01**	0.24	0.54
Calarene	-0.48	0.19	0.52	0.15	**-0.80**	**0.009**	0.31	0.41
Neocembrene	-0.41	0.27	0.54	0.13	-0.55	0.13	-0.13	0.75
Geranyllinalool	0.09	0.83	-0.16	0.68	-0.35	0.35	0.09	0.81
Thunbergol	-0.36	0.34	**0.79**	**0.01**	**-0.68**	**0.04**	0.23	0.55
Sum of terpenes	-0.36	0.35	**0.71**	**0.03**	**-0.80**	**0.01**	-0.02	0.97