SUPPORTING INFORMATION (SI) APPENDIX

Neonicotinoid insecticides induce salicylate-associated plant defense responses

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K.A.F. and J.E.C. contributed equally to this work. K.A.F., J.E.C., D.C., K.A.D., R.S., E.M.B., and M.C.W. designed experiments. K.A.F., D.C., A.G.G., R.A.O., K.A.D and E.M.B. performed experiments. J.E.C. and M.C.W. wrote the paper. D.C. and M.C.W assessed and analyzed gene expression and powdery mildew growth. SA in plant extracts was assessed by LC/MS/MS (K.A.F., A.G.G., and J.E.C.) and HPLC with fluorescence detection (A.G.G., R.A.O., and M.C.W). For detection of 2-HOCPA in plant extracts, A.G.G., R.A.O., and M.C.W. performed 2-HOCPA extractions and HPLC analyses, with LC/MS analysis done by K.A.F. and J.E.C. E.M.B. and R.S. synthesized 2-HOCPA. R.A.O. and M.C.W. assessed inhibition of PBS3 activity. K.A.D. modeled ligand binding to SABP2.

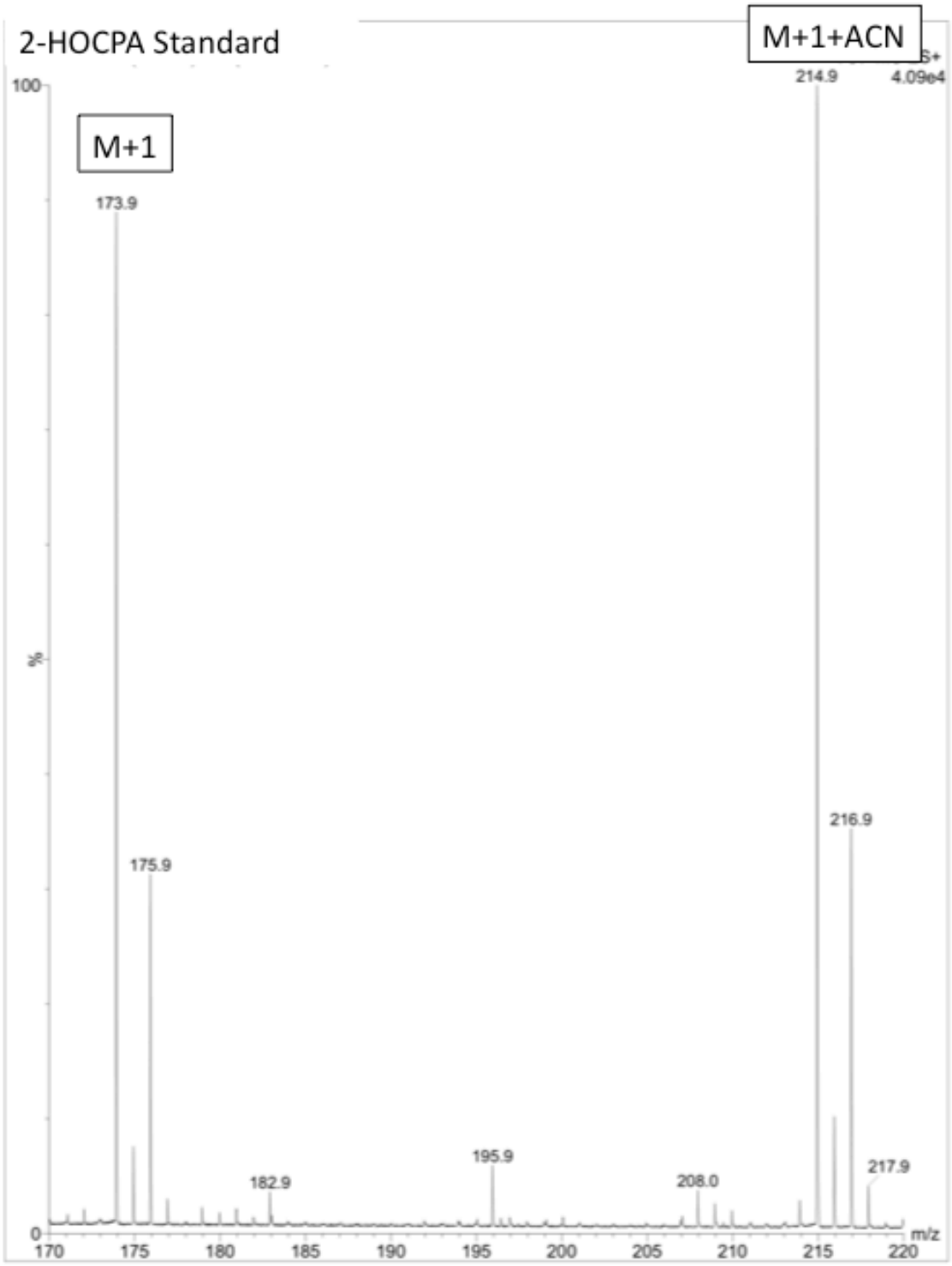
Supporting Information Appendix

SI Method 3. Synthesis of 6-chloro-2-hydroxypyridinyl-3-carboxylic acid (10 pages)

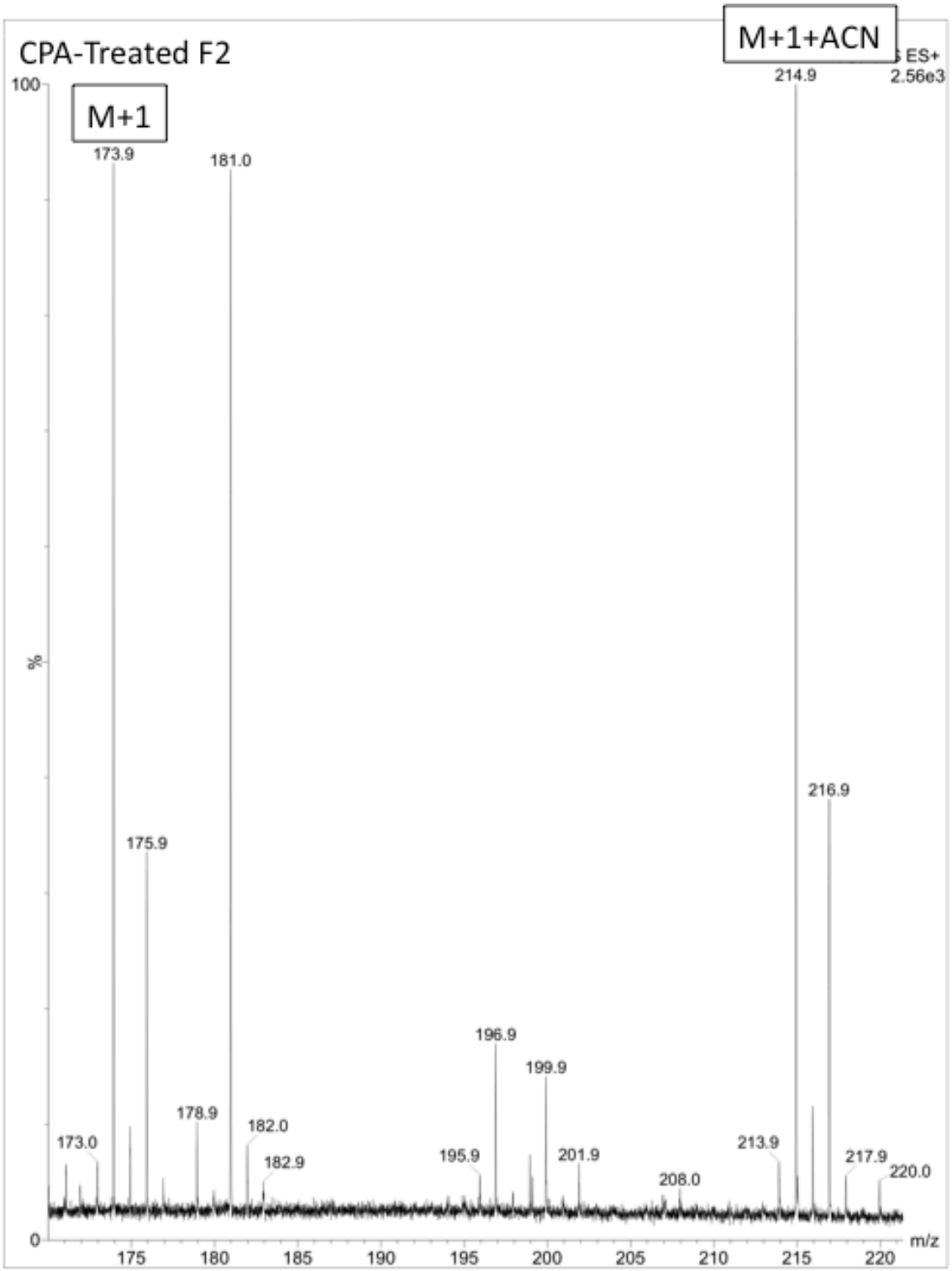
SI Method 4. Detection and quantitation of SA using LC/MS/MS (2 pages)

SI Method 5. HPLC fractionation and detection of 2-HOCPA in CPA-treated leaf extracts (2 pages)

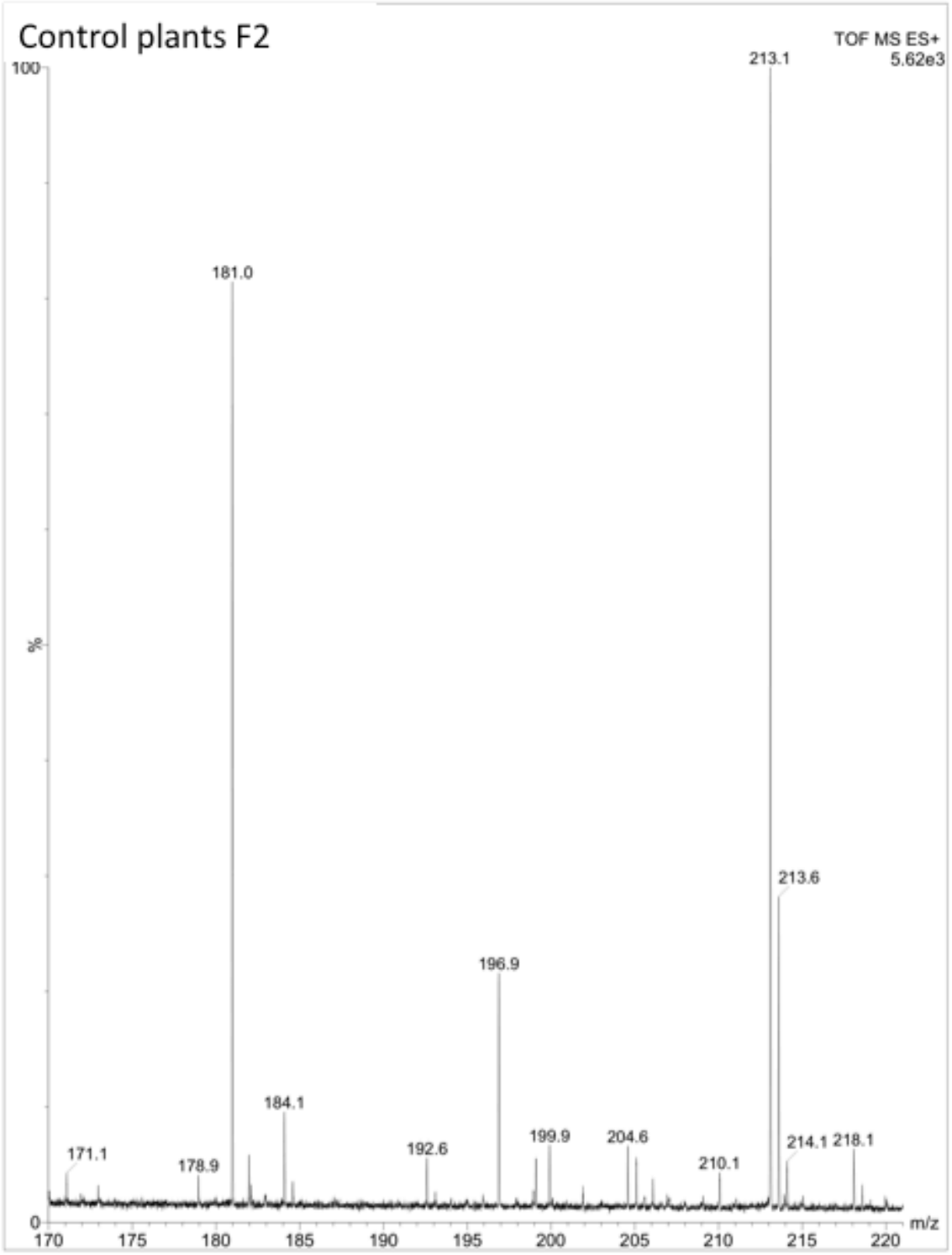
SI Figure 1. MS profile (t_R= 22 min) of 2-HOCPA standard and HPLC fraction F2 from CPA-treated and control plants. $[M+1]^+$= 173.9 and $[M+1+acetonitrile]^+$= 214.9 with ^{35}Cl. Note the characteristic ^{35}Cl: ^{37}Cl ratio of 3:1 for both ions. See Method S5 for experimental procedure.



MS profile of synthetic 2-HOCPA standard.

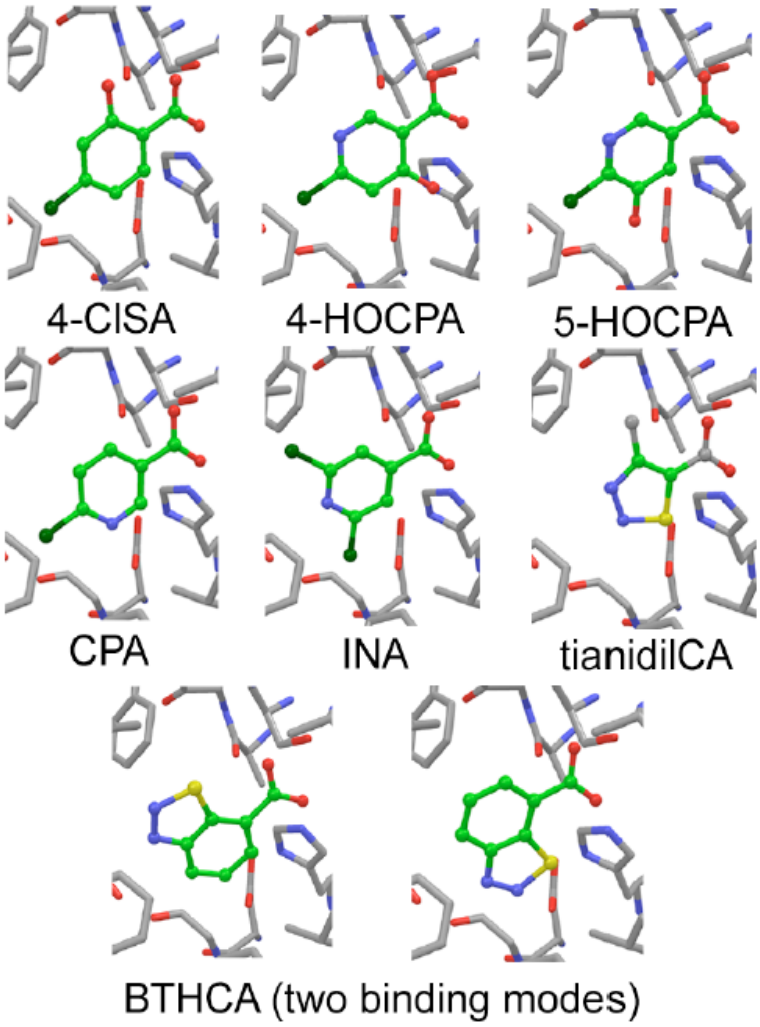


MS profile of HPLC fraction F2 from CPA-treated *Arabidopsis* leaf extracts.



MS profile of HPLC fraction F2 from control *Arabidopsis* leaf extracts.

SI Figure 2. Binding of 2-HOCPA and related compounds in the active site of SABP2.



SABP2 binding modes of chlorosalicylic acid, chlorohydroxypyridinylcarboxylic acids and thiadiazolylcarboxylic acids. The corresponding models for SA, CPA and 2-HOCPA are shown in Fig. 6. Potential ligands were docked to the SABP2 crystal structure 1Y7I (1) after addition of hydrogen atoms and removal of nonprotein moieties using Glide as implemented in Maestro (2, 3). The docking includes a spatial fit of the ligand to the receptor grid, followed by minimization and scoring of hits based on a discretized ChemScore function (4,5). Ligands were flexibly docked using standard precision and the top hits were examined.

References:

1. Forouhar F, et al. (2005) Structual and biochemical studies identify tobacco SABP2 as a methyl salicylate esterase and implicate it in plant innate immunity. *Proc Natl Acad Sci USA* 102:1773-1778.
2. Glide 5.5, Maestro 9.0 (2009) Schrödinger, LLC, New York, NY.
3. Friesner RA, et al. (2004) Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J Med Chem* 47:1739-1749.
4. Eldridge MD, Murray CW, Auton TR, Paolini GV, Mee RP (1997) Empirical scoring functions: I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. *J Comput-Aided Mol Des* 11:425-445.
5. Baxter CA, Murray CW, Clark DE, Westhead DR, Eldridge MD (1998) Flexible docking using Tabu search and an empirical estimate of binding affinity. *Proteins* 33:367-382.

SI Table 1. SA, CLO or IMI altered expression of 94 of 107 genes defined as SA-dependent by Wang et al. (2008) MPMI, 21, 1408-20. These genes are induced by Psm ES4326 in a PAD4-, NPR1-, and SID2- dependent manner.

Locus	Description	SA vs. Control	p-value	CLO vs. Control	p-value	IMI vs. Control	p-value
AT1G01560	ATMPK11 (Arabidopsis thaliana MAP kinase 11); MAP kinase/ kinase	5.2	0.53	5.6	0.25	19.5	0.13
AT1G02360	chitinase, putative	5.9	0.17	2.6	0.39	10.4	0.07
AT1G03290	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT4G02880.1)					2.6	0.16
AT1G03660	similar to ankyrin repeat family protein [Arabidopsis thaliana]					5.2	0.27
AT1G03850	glutaredoxin family protein	5.1	0.22	3.6	0.46	25.4	0.08
AT1G07000	ATEXO70B2 (exocyst subunit EXO70 family protein B2); protein binding	3.0	0.61	3.4	0.42	8.9	0.22
AT1G08050	zinc finger (C3HC4-type RING finger) family protein	5.3	0.53	5.1	0.21	19.3	0.07
AT1G11310	MLO2 (MILDEW RESISTANCE LOCUS O 2); calmodulin binding	2.2	0.45			2.1	0.14
AT1G13470	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G13520.1)	7.5	0.42	5.3	0.29	6.8	0.24
AT1G14870 /// AT1G14880		38.4	0.20	25.6	0.18	138.2	0.07
AT1G15790	similar to protein binding / transcription cofactor [Arabidopsis thaliana]	2.2	0.51	2.9	0.23	4.3	0.13
AT1G17600	disease resistance protein (TIR-NBS-LRR class), putative	2.6	0.57			2.6	0.17
AT1G17615	disease resistance protein (TIR-NBS class), putative					3.3	0.41
AT1G35230	AGP5 (ARABINOGALACTAN-PROTEIN 5)	23.9	0.31	34.8	0.05	37.8	0.09
AT1G47890	disease resistance family protein	3.7	0.46	2.0	0.29	6.3	0.07
AT1G51860	leucine-rich repeat protein kinase, putative	7.6	0.35	6.5	0.04	24.6	0.01
AT1G65610	ATGH9A2/KOR2 (ARABIDOPSIS THALIANA GLYCOSYL HYDROLASE 9A2)			2.2	0.45	5.7	0.35
AT1G67520 /// A	CES101 (CALLUS EXPRESSION OF RBCS 101); carbohydrate binding / kinase	3.1	0.49	2.3	0.43	3.2	0.24
AT1G67800	copine-related	2.8	0.56	3.4	0.21	5.9	0.12
AT1G68710	haloacid dehalogenase-like hydrolase family protein					2.3	0.34
AT1G73805	calmodulin binding calmodulin binding	7.5	0.31	6.8	0.20	12.2	0.13
AT2G01650	PUX2 (PLANT UBX DOMAIN-CONTAINING PROTEIN 2)					2.7	0.14
AT2G04430	ATNUDT5 (Arabidopsis thaliana Nudix hydrolase homolog 5); hydrolase			2.7	0.22	3.7	0.26
AT2G04450	ATNUDT6 (Arabidopsis thaliana Nudix hydrolase homolog 6); ADP-ribose diphosphatase	2.7	0.50	2.5	0.46	6.6	0.09
AT2G05940	protein kinase, putative	2.8	0.11	5.7	0.12	9.8	0.08
AT2G14610	PR1 (PATHOGENESIS-RELATED GENE 1)	162.9	0.02	51.1	0.08	534.1	0.02
AT2G14620	xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase					61.4	0.00
AT2G17730	zinc finger (C3HC4-type RING finger) family protein					3.7	0.01
AT2G19190	FRK1 (FLG22-INDUCED RECEPTOR-LIKE KINASE 1); kinase	13.5	0.25	7.8	0.04	17.0	0.24
AT2G19710	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT4G29440.1)					2.5	0.32
AT2G22500	mitochondrial substrate carrier family protein	7.3	0.28	5.8	0.12	6.3	0.13
AT2G26400	ARD/ATARD3 (ACIREDUCTONE DIOXYGENASE)	5.3	0.16	3.8	0.47	18.8	0.20
AT2G27660	DC1 domain-containing protein	8.2	0.36	7.9	0.15	21.4	0.07
AT2G29070	ubiquitin fusion degradation UFD1 family protein	2.3	0.27			3.8	0.05
AT2G30250	WRKY25 (WRKY DNA-binding protein 25); transcription factor	3.2	0.48	3.2	0.35	6.2	0.20
AT2G30550	lipase class 3 family protein	3.8	0.34	2.4	0.17	18.8	0.02

Locus	Description	SA vs. Control	p-value	CLO vs. Control	p-value	IMI vs. Control	p-value
AT2G31020 /// AT2G31030						7.6	0.06
AT2G39210	nodulin family protein	3.6	0.41	4.4	0.17	7.5	0.11
AT2G45760						5.1	0.06
AT2G47130	short-chain dehydrogenase/reductase (SDR) family protein	6.0	0.50	5.7	0.31	15.5	0.17
AT3G02840	immediate-early fungal elicitor family protein	6.5	0.41	5.3	0.01	20.8	0.00
AT3G07600	heavy-metal-associated domain-containing protein	7.3	0.39	2.2	0.32	2.4	0.04
AT3G09010	protein kinase family protein	3.6	0.54	3.7	0.36	9.7	0.18
AT3G09490	chloroplast lumen common family protein	2.5	0.61	2.1	0.59	3.6	0.39
AT3G09960	calcineurin-like phosphoesterase family protein					2.7	0.37
AT3G12230 /// AT3G12240						2.2	0.10
AT3G17420	GPK1 (Glyoxysomal protein kinase 1); kinase			2.0	0.23	3.4	0.14
AT3G12910	transcription factor	5.6	0.43				
AT3G19010	oxidoreductase, 2OG-Fe(II) oxygenase family protein	2.5	0.45			2.5	0.18
AT3G19580	AZF2 (ARABIDOPSIS ZINC-FINGER PROTEIN 2)	5.6	0.10	3.5	0.21	13.0	0.05
AT3G21780	UGT71B6 (UDP-GLUCOSYL TRANSFERASE 71B6); UDP-glycosyltransferase	2.5	0.34			11.1	0.00
AT3G22910	calcium-transporting ATPase, plasma membrane-type, putative / Ca(2+)-ATPase	2.1	0.35	5.5	0.23	44.5	0.01
AT3G28510	AAA-type ATPase family protein	27.2	0.23	2.8	0.25	59.3	0.00
AT3G29250	oxidoreductase	6.6	0.40			38.6	0.01
AT3G44400	disease resistance protein (TIR-NBS-LRR class), putative			2.5	0.41	2.6	0.40
AT3G44400	disease resistance protein (TIR-NBS-LRR class), putative					2.3	0.23
AT3G46280	protein kinase-related	5.9	0.20			11.4	0.03
AT3G47050	glycosyl hydrolase family 3 protein	5.1	0.26			14.0	0.00
AT3G48650	pseudogene, At14a-related protein, similar to At14a	9.1	0.44	14.6	0.26	21.3	0.22
AT3G51330	aspartyl protease family protein	7.3	0.28	5.7	0.28	24.9	0.05
AT3G51440	strictosidine synthase family protein	4.8	0.35	3.0	0.36	15.1	0.10
AT3G53150	UGT73D1 (UDP-GLUCOSYL TRANSFERASE 73D1); UDP-glycosyltransferase	7.4	0.34	3.1	0.40	18.2	0.22
AT3G56400	WRKY70 (WRKY DNA-binding protein 70); transcription factor	2.7	0.24			3.4	0.17
AT3G57480	zinc finger (C2H2 type, AN1-like) family protein					2.4	0.22
AT4G03450	ankyrin repeat family protein	5.8	0.40	10.1	0.11	16.8	0.15
AT4G11850	PLDGAMMA1 (maternal effect embryo arrest 54); phospholipase D	2.5	0.58			2.5	0.21
AT4G11890	protein kinase family protein	7.3	0.54	10.4	0.21	18.3	0.15
AT4G13900 /// AT4G13920		5.4	0.35	4.1	0.20	16.9	0.08
AT4G14610	pseudogene, disease resistance protein (CC-NBS-LRR class), putative	2.4	0.56	2.1	0.53	3.8	0.30
AT4G18430	AtRABA1e (Arabidopsis Rab GTPase homolog A1e); GTP binding	6.8	0.39			6.1	0.17
AT4G20110	vacuolar sorting receptor, putative	5.1	0.27	3.7	0.26	16.3	0.08
AT4G23610 /// A	50S ribosomal protein-related similar to unknown protein [Arabidopsis thaliana]	7.2	0.45	5.9	0.23	7.1	0.23
AT4G39830	L-ascorbate oxidase, putative	6.5	0.42	8.4	0.11	2.5	0.26
AT4G30230	unknown protein					3.1	0.42

Locus	Description	SA vs. Control	p-value	CLO vs. Control	p-value	IMI vs. Control	p-value
AT4G39830	L-ascorbate oxidase, putative					14.4	0.08
AT5G02490	heat shock cognate 70 kDa protein 2 (HSC70-2) (HSP70-2)			2.3	0.64	13.6	0.15
AT5G09290	3'(2'),5'-bisphosphate nucleotidase, putative	5.5	0.31	3.2	0.13	15.4	0.03
AT5G11210	ATGLR2.5 (Arabidopsis thaliana glutamate receptor 2.5)	2.6	0.43			10.1	0.04
AT5G18270	ANAC087; transcription factor			2.1	0.51	18.4	0.04
AT5G18780	F-box family protein	2.3	0.57	2.2	0.47	5.4	0.21
AT5G19880	peroxidase, putative			2.1	0.42	3.4	0.42
AT5G24210	lipase class 3 family protein	2.7	0.49	3.7	0.33	5.1	0.31
AT5G25820	exostosin family protein	4.1	0.37	2.6	0.29	8.0	0.00
AT5G37740	C2 domain-containing protein	2.7	0.39	2.4	0.37	4.4	0.17
AT5G45000	transmembrane receptor	3.9	0.41	2.8	0.19	10.3	0.02
AT5G45110	NPR3 (NPR1-LIKE PROTEIN 3); protein binding	2.2	0.52	2.2	0.48	3.2	0.34
AT5G46230	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G09310.1)	3.6	0.51	5.6	0.26	12.7	0.15
AT5G46520	ATP binding / nucleoside-triphosphatase/ nucleotide binding / protein binding	2.2	0.51			2.1	0.31
AT5G46950 /// AT5G46960						3.0	0.39
AT5G48410	ATGLR1.3 (Arabidopsis thaliana glutamate receptor 1.3)			2.2	0.36	16.1	0.05
AT5G49570	ATPNG1 (ARABIDOPSIS THALIANA PEPTIDE-N-GLYCANASE 1); catalytic					2.2	0.13
AT5G51630	disease resistance protein (TIR-NBS-LRR class), putative					2.4	0.27
AT5G52810	ornithine cyclodeaminase/mu-crystallin family protein	2.8	0.47	2.8	0.20	6.1	0.08
AT5G60280	lectin protein kinase family protein	3.4	0.46			9.1	0.06
AT5G63990	3'(2'),5'-bisphosphate nucleotidase, putative					5.2	0.00

SI Table 2. *Arabidopsis* genes in functional processes impacted by treatment with CLO, IMI and SA. MapMan analysis (Thimm et al., 2004, Plant J, 37. 914-939) was performed using genes with ≥ 2-fold altered expression in treatment versus control samples and q-value ≤ 0.5 to identify statistically enriched processes. MapMan bins with p-value ≤ 0.05 are shown.

		SA vs control		CLO vs control		IMI vs control	
Bin	Functional process	elements	p-value	elements	p-value	elements	p-value
1	PS	24	0.00			86	0.00
1.1	PS.lightreaction	22	0.00			71	0.00
1.1.1	PS.lightreaction.photosystem II	8	0.04			33	0.00
1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits					27	0.00
1.1.2	PS.lightreaction.photosystem I					13	0.02
1.1.2.2	PS.lightreaction.photosystem I.PSI polypeptide subunits					11	0.05
1.1.30	PS.lightreaction.state transition					2	0.03
1.1.5	PS.lightreaction.other electron carrier (ox/red)	3	0.04			5	0.02
1.1.6	PS.lightreaction.NADH DH						
1.3	PS.calvin cyle						
2	major CHO metabolism						
2.1	major CHO metabolism.synthesis					18	0.01
2.1.2	major CHO metabolism.synthesis.starch					15	0.00
2.1.2.2	major CHO metabolism.synthesis.starch.starch synthase						
2.1.2.3	major CHO metabolism.synthesis.starch.starch branching					2	0.04
2.1.2.4	major CHO metabolism.synthesis.starch.debranching					4	0.01
2.2.1	major CHO metabolism.degradation.sucrose						
2.2.1.1	major CHO metabolism.degradation.sucrose.fructokinase						
2.2.1.3	major CHO metabolism.degradation.sucrose.invertases					4	0.01
2.2.1.3.1	major CHO metabolism.degradation.sucrose.invertases.neutral						
2.2.2	major CHO metabolism.degradation.starch						
2.2.2.2	major CHO metabolism.degradation.starch.starch phosphorylase						
2.2.2.3	major CHO metabolism.degradation.starch.glucan water dikinase						
3	minor CHO metabolism						
3.2.3	minor CHO metabolism.trehalose.potential TPS/TPP						
3.4.3	minor CHO metabolism.myo-inositol.InsP Synthases					3	0.02
3.4.4	minor CHO metabolism.myo-inositol.myo inositol oxygenases					2	0.02
4	glycolysis					24	0.01
4.13	glycolysis.PK					3	0.02
4.9	glycolysis.glyceraldehyde 3-phosphate dehydrogenase					7	0.01
5	fermentation						
6	gluconeogenese/ glyoxylate cycle						
7	OPP						
8	TCA / org. transformation						
8.1	TCA / org. transformation.TCA						
8.1.7	TCA / org. transformation.TCA.succinate dehydrogenase						
8.3	TCA / org. transformation.carbonic anhydrases					4	0.05
9	mitochondrial electron transport / ATP synthesis			12	0.03	29	0.00
9.4	mitochondrial electron transport / ATP synthesis.alternative oxidase					2	0.04

		SA vs control		CLO vs control		IMI vs control	
Bin	Functional process	elements	p-value	elements	p-value	elements	p-value
9.5	mitochondrial electron transport / ATP synthesis.cytochrome c reductase					3	0.05
9.7	mitochondrial electron transport / ATP synthesis.cytochrome c oxidase					6	0.04
10	cell wall	91	0.00	68	0.00	160	0.00
10.1	cell wall.precursor synthesis					19	0.05
10.1.9	cell wall.precursor synthesis.MUR4					3	0.01
10.2	cell wall.cellulose synthesis	10	0.03	8	0.04	24	0.01
10.5	cell wall.cell wall proteins	20	0.00	15	0.02	28	0.01
10.5.1	cell wall.cell wall proteins.AGPs	16	0.00			21	0.01
10.5.3	cell wall.cell wall proteins.LRR			2	0.04		
10.6	cell wall.degradation	21	0.00	14	0.00	34	0.00
10.6.2	cell wall.degradation.mannan-xylose-arabinose-fucose						
10.6.3	cell wall.degradation.pectate lyases and polygalacturonases	16	0.00	12	0.00	26	0.00
10.7	cell wall.modification	19	0.00	17	0.04	29	0.00
10.8	cell wall.pectin*esterases					22	0.00
10.8.2	cell wall.pectin*esterases.acetyl esterase					7	0.02
11	lipid metabolism	50	0.00	31	0.00	130	0.00
11.1	lipid metabolism.FA synthesis and FA elongation	16	0.00			44	0.00
11.1.1	lipid metabolism.FA synthesis and FA elongation.Acetyl CoA Carboxylation					5	0.05
11.1.10	lipid metabolism.FA synthesis and FA elongation.beta ketoacyl CoA synthase					6	0.01
11.2	lipid metabolism.FA desaturation	4	0.00	3	0.00	7	0.05
11.2.1	lipid metabolism.FA desaturation.desaturase	3	0.01	2	0.02		
11.3	lipid metabolism.Phospholipid synthesis			4	0.04		
11.6	lipid metabolism.lipid transfer proteins etc					8	0.00
11.9	lipid metabolism.lipid degradation					33	0.05
11.9.2.1	lipid metabolism.lipid degradation.lipases.triacylglycerol lipase					9	0.04
11.9.4.2	lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH					6	0.01
12	N-metabolism						
13	amino acid metabolism						
13.2	amino acid metabolism.degradation					31	0.00
13.1.3.6	amino acid metabolism.synthesis.aspartate family.misc					3	0.05
13.1.3.6.1	amino acid metabolism.synthesis.aspartate family.misc.homoserine					3	0.05
13.1.3.6.1.1	amino acid metabolism.synthesis.aspartate family.misc.homoserine.aspartate kinase						
13.1.4	amino acid metabolism.synthesis.branched chain group						
13.1.4.1	amino acid metabolism.synthesis.branched chain group.common						
13.1.4.1.4	amino acid metabolism.synthesis.branched chain group.common.branched-chain amino acid aminotransferase						
13.1.6	amino acid metabolism.synthesis.aromatic aa					15	0.01
13.1.6.5	amino acid metabolism.synthesis.aromatic aa.tryptophan					9	0.05
13.2.2	amino acid metabolism.degradation.glutamate family					3	0.01
13.2.2.2	amino acid metabolism.degradation.glutamate family.proline					2	0.02
13.2.4	amino acid metabolism.degradation.branched chain group					10	0.00
13.2.4.1	amino acid metabolism.degradation.branched-chain group.shared					6	0.00

		SA vs control		CLO vs control		IMI vs control	
Bin	Functional process	elements	p-value	elements	p-value	elements	p-value
13.2.4.4	amino acid metabolism.degradation.branched-chain group.leucine						
13.2.6.2	amino acid metabolism.degradation.aromatic aa.tyrosine					2	0.04
14	S-assimilation						
15	metal handling						
16	secondary metabolism						
16.1.5	secondary metabolism.isoprenoids.terpenoids					4	0.02
16.5	secondary metabolism.sulfur-containing						
16.7	secondary metabolism.wax	5	0.04	3	0.02	7	0.00
16.8	secondary metabolism.flavonoids	18	0.01				
16.8.1	secondary metabolism.flavonoids.anthocyanins	7	0.01				
16.8.2	secondary metabolism.flavonoids.chalcones					3	0.03
17	hormone metabolism					175	0.30
17.1.2	hormone metabolism.abscisic acid.signal transduction						
17.2.1	hormone metabolism.auxin.synthesis-degradation						
17.2.2	hormone metabolism.auxin.signal transduction					4	0.03
17.3.1	hormone metabolism.brassinosteroid.synthesis-degradation	9	0.01	3	0.05		
17.3.1.1	hormone metabolism.brassinosteroid.synthesis-degradation.BRs			2	0.05		
17.3.1.2	hormone metabolism.brassinosteroid.synthesis-degradation.sterols	6	0.01				
17.4.1	hormone metabolism.cytokinin.synthesis-degradation					3	0.05
17.5	hormone metabolism.ethylene	15	0.00	19	0.00	33	0.00
17.5.1	hormone metabolism.ethylene.synthesis-degradation					12	0.04
17.5.1.1	hormone metabolism.ethylene.synthesis-degradation.1-aminocyclopropane-1-carboxylate synthase					4	0.01
17.5.2	hormone metabolism.ethylene.signal transduction	11	0.00	12	0.00	15	0.01
17.5.3	hormone metabolism.ethylene.induced-regulated-responsive-activated					6	0.04
17.6	hormone metabolism.gibberelin	14	0.01	9	0.03	15	0.00
17.6.3	hormone metabolism.gibberelin.induced-regulated-responsive-activated	8	0.03			7	0.01
18	Co-factor and vitamine metabolism						
19	tetrapyrrole synthesis						
20	stress	128	0.00	120	0.00	248	0.00
20.1	stress.biotic	87	0.00	72	0.00	119	0.00
20.2.1	stress.abiotic.heat	16	0.04	27	0.00	64	0.00
20.2.2	stress.abiotic.cold			4	0.03	5	0.01
20.2.3	stress.abiotic.drought/salt	9	0.00	7	0.00	24	0.00
20.2.99	stress.abiotic.unspecified					28	0.04
21	redox.regulation						
22	polyamine metabolism						
23	nucleotide metabolism						
23.5	nucleotide metabolism.deoxynucleotide metabolism	3	0.05				
24	Biodegradation of Xenobiotics						
25	C1-metabolism						
26	misc			155	0.00		
26.4	misc.beta 1,3 glucan hydrolases						

		SA vs control		CLO vs control		IMI vs control	
Bin	Functional process	elements	p-value	elements	p-value	elements	p-value
26.6	misc.O- methyl transferases	4	0.05				
26.7	misc.oxidases - copper, flavone etc.						
26.8	misc.nitrilases, *nitrile lyases, berberine bridge enzymes, reticuline oxidases, troponine reductases	12	0.00	13	0.00		
26.9	misc.glutathione S transferases			13	0.00	24	0.00
26.10	misc.cytochrome P450	27	0.05	20	0.00	51	0.00
26.11	misc.alcohol dehydrogenases					5	0.04
26.12	misc.peroxidases	5	0.01	7	0.02		
26.16	misc.myrosinases-lectin-jacalin						
26.23	misc.rhodanese					3	0.04
26.28	misc.GDSL-motif lipase	12	0.00	13	0.00	25	0.00
27	RNA						
27.1	RNA.processing						
27.1.19	RNA.processing.ribonucleases	2	0.03				
27.3.6	RNA.regulation of transcription.bHLH,Basic Helix-Loop-Helix family	12	0.03	11	0.04	32	0.00
27.3.8	RNA.regulation of transcription.C2C2(Zn) DOF zinc finger family	3	0.04				
27.3.11	RNA.regulation of transcription.C2H2 zinc finger family	14	0.05	9	0.01		
27.3.12	RNA.regulation of transcription.C3H zinc finger family	3	0.05	2	0.05		
27.3.22	RNA.regulation of transcription.HB,Homeobox transcription factor family					19	0.01
27.3.30	RNA.regulation of transcription.Trihelix, Triple-Helix transcription factor family	2	0.04				
27.3.32	RNA.regulation of transcription.WRKY domain transcription factor family	22	0.00	19	0.00	26	0.00
27.3.35	RNA.regulation of transcription.bZIP transcription factor family					19	0.02
27.3.40	RNA.regulation of transcription.Aux/IAA family					14	0.01
27.3.64	RNA.regulation of transcription.PHOR1	5	0.05				
27.3.67	RNA.regulation of transcription.putative transcription regulator	11	0.02				
28	DNA	44	0.00	35	0.00	77	0.00
28.1	DNA.synthesis/chromatin structure	33	0.00	26	0.00	52	0.00
28.1.3	DNA.synthesis/chromatin structure.histone	12	0.01	8	0.00	18	0.00
28.2	DNA.repair	6	0.01	6	0.01		
29	protein					672	0.01
29.2	protein.synthesis						
29.2.1	protein.synthesis.mito/plastid ribosomal protein	11	0.03			48	0.00
29.2.1.1	protein.synthesis.mito/plastid ribosomal protein.plastid	10	0.02			36	0.00
29.3.4	protein.targeting.secretory pathway					18	0.01
29.3.4.3	protein.targeting.secretory pathway.vacuole	4	0.04	3	0.04	6	0.01
29.4	protein.postranslational modification					154	0.03
29.4.1.56	protein.postranslational modification.kinase.receptor like cytoplasmatic kinase VI			3	0.03	3	0.04
29.4.1.57	protein.postranslational modification.kinase.receptor like cytoplasmatic kinase VII					13	0.03
29.5	protein.degradation					325	0.00
29.5.1	protein.degradation.subtilases					20	0.00
29.5.4	protein.degradation.aspartate protease	4	0.05			5	0.04
29.5.5	protein.degradation.serine protease					23	0.01
29.5.9	protein.degradation.AAA type	9	0.01			12	0.00

		SA vs control		CLO vs control		IMI vs control	
Bin	Functional process	elements	p-value	elements	p-value	elements	p-value
29.5.11	protein.degradation.ubiquitin	43	0.02			181	0.00
29.5.11.4	protein.degradation.ubiquitin.E3	40	0.01			140	0.00
29.5.11.4.2	protein.degradation.ubiquitin.E3.RING	34	0.02			102	0.00
29.5.11.4.3	protein.degradation.ubiquitin.E3.SCF					36	0.01
29.5.11.4.3.2	protein.degradation.ubiquitin.E3.SCF.FBOX					35	0.02
29.5.11.20	protein.degradation.ubiquitin.proteasom					11	0.05
29.5.11.4.5	protein.degradation.ubiquitin.E3.BTB/POZ Cullin3					2	0.03
29.5.11.4.5.2	protein.degradation.ubiquitin.E3.BTB/POZ Cullin3.BTB/POZ					2	0.03
30	signalling	191	0.00	159	0.01		
30.11	signalling.light	11	0.01			25	0.00
30.2	signalling.receptor kinases	108	0.02				
30.2.2	signalling.receptor kinases.leucine rich repeat II	5	0.04	5	0.04	8	0.01
30.2.3	signalling.receptor kinases.leucine rich repeat III	11	0.00	7	0.00	18	0.00
30.2.4	signalling.receptor kinases.leucine rich repeat IV					3	0.05
30.2.11	signalling.receptor kinases.leucine rich repeat XI						
30.2.13	signalling.receptor kinases.leucine rich repeat XIII					4	0.01
30.2.17	signalling.receptor kinases.DUF 26	20	0.00	17	0.00	20	0.01
30.2.19	signalling.receptor kinases.legume-lectin					5	0.03
30.2.20	signalling.receptor kinases.wheat LRK10 like	5	0.01			5	0.02
30.2.22	signalling.receptor kinases.proline extensin like						
30.2.25	signalling.receptor kinases.wall associated kinase					5	0.01
30.2.99	signalling.receptor kinases.misc	13	0.01				
30.3	signalling.calcium	38	0.00	36	0.00	66	0.00
30.6	signalling.MAP kinases					12	0.04
31	cell	55	0.00	44	0.01	159	0.01
31.1	cell.organisation	35	0.04			95	0.00
31.3	cell.cycle	8	0.00	4	0.02		
31.4	cell. vesicle transport						
32	micro RNA, natural antisense etc						
33	development	67	0.02				
33.99	development.unspecified	59	0.01				
34	transport						
34.1	transport.p- and v-ATPases	2	0.05				
34.19	transport.Major Intrinsic Proteins	8	0.01	6	0.03	12	0.05
34.19.1	transport.Major Intrinsic Proteins.PIP					6	0.02
34.19.2	transport.Major Intrinsic Proteins.TIP	3	0.04	3	0.05	3	0.03
34.19.3	transport.Major Intrinsic Proteins.NIP						
34.99	transport.misc					50	0.01
35	not assigned					1766	0.05
35.1.19	not assigned.no ontology.C2 domain-containing protein	4	0.04			10	0.02
35.1.23	not assigned.no ontology.aconitase C-terminal domain-containing protein					2	0.02
35.2	not assigned.unknown					1076	0.01

		SA vs control		CLO vs control		IMI vs control	
Bin	Functional process	elements	p-value	elements	p-value	elements	p-value
35.3	unknown.disagreeing hits	4	0.01				

SI Table 3. Neonicotinoid-induced enhanced resistance of *Arabidopsis* to powdery mildew. Disease rating scores for each plant summarized in Table 1 and details of scoring system are provided below.

	Number of Plants with Disease Rating							
Treatment	**0**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	***p*-value**
Experiment 1								
Control	0	2	2	13	6	9	4	
SA	0	1	5	11	1	3	0	≤0.02
CLO	0	10	3	14	0	0	0	<0.0001
IMI	0	5	4	16	1	0	0	<0.0001
Experiment 2								
Control	0	1	0	2	3	12	1	
SA	0	0	0	7	1	2	0	≤0.02
CLO	0	7	2	6	0	0	0	<0.0001
IMI	0	2	3	9	3	2	0	<0.0002

Neonicotinoid-induced systemic acquired resistance of *Arabidopsis* to powdery mildew. Four days after treatment with 4 mM SA, CLO or IMI or control, boxes of wild type *Arabidopsis* Col-0 plants were infected with powdery mildew *Golovinomyces orontii* conidia. Visual symptoms of powdery mildew growth were assessed for each plant 10 days post infection using the scoring system of Reuber *et al.* (1): 0, no visible symptoms of infection; 1, isolated spots; 2, ~20% leaf covered by powdery mildew, 3, ~50% coverage; 4, ~100% coverage. Intermediate scores were included to allow finer resolution (e.g., 2.5, 35% coverage). Experiments above were performed one month apart.

Reference:

1. Reuber TL, et al. (1998) Correlation of defense gene induction defects with powdery mildew susceptibility in *Arabidopsis* enhanced disease susceptibility mutants. *The Plant J* 16:473-485.

SI Table 4. Powdery mildew visible disease is compromised in CLO-treated *ics1* mutant vs. wild type *Arabidopsis*.

	Percent of total plants with ≥12.5% mildew coverage		
	Exp 1	Exp 2	*p*-value
Col-0 + 4 mM CLO	0	0	
ics1 + 4 mM CLO	32	36	≤0.001

CLO-induced enhanced resistance of *Arabidopsis* to powdery mildew is compromised in the *ics1* mutant. Four days after soil treatment with 4 mM chemical or control, boxes of *Arabidopsis* Col-0 plants were infected with powdery mildew conidia. Powdery mildew growth and reproduction was assessed at 10+ days post infection using a modified standard scoring system (1) to describe the visible percent coverage on fully expanded leaves of similar age per plant. A score of 1.5 represents ~12.5% mildew coverage. Experiments 1 (n≥19) and 2 (n=11) were performed two weeks apart. *p*-value shown is valid for each experiment.

Reference:

1. Reuber TL, et al. (1998) Correlation of defense gene induction defects with powdery mildew susceptibility in *Arabidopsis* enhanced disease susceptibility mutants. *The Plant J* 16:473-485.

SI Table 5. Replicate experiments measuring SA accumulation in response to neonicotinoids.

(1) **SA accumulation induced by soil treatment with 4 mM neonicotinoids versus control at 4 days post treatment.** Data are mean±SD (n=3). p-values<0.001 for treatment versus control are indicated with * for Total SA (Free SA + SA glucoside). Below we show initial experiments performed using GC/MS and subsequent experiments performed using LC/MS/MS (see Method S4). Experiment 1 LC/MS/MS is shown in Figure 3D. All experiments are performed with *Arabidopsis thaliana* Col-0 (wild type) plants.

Experiment 1- GC/MS

Treatment	Free SA (μg/g)	Total SA (μg/g)
Control	0.4±0.0	2.8±0.5
4 mM IMI	0.5±0.1	2.4±0.4
4 mM CLO	1.5±0.3	8.2±2.8 *

Experiment 2- GC/MS

Treatment	Free SA (μg/g)	Total SA (μg/g)
Control	0.5±0.0	3.2±0.3
4 mM IMI	0.8±0.2	3.5±0.2
4 mM CLO	2.1±0.4	8.4±2.6 *

Experiment 2 – LC/MS/MS analysis

Treatment	Free SA (μg/g)	Total SA (μg/g)
Control	0.6±0.1	3.0±0.2
4 mM IMI	0.8±0.2	3.1±0.2
4 mM CPA	0.8±0.0	3.3±0.1
4 mM CLO	1.1±0.2	6.0±0.2 *
4 mM CTA	1.1±0.1	5.1±0.2 *

In response to 4 mM SA, free SA was 8.1±0.7 and total SA was 34.0±4.6.

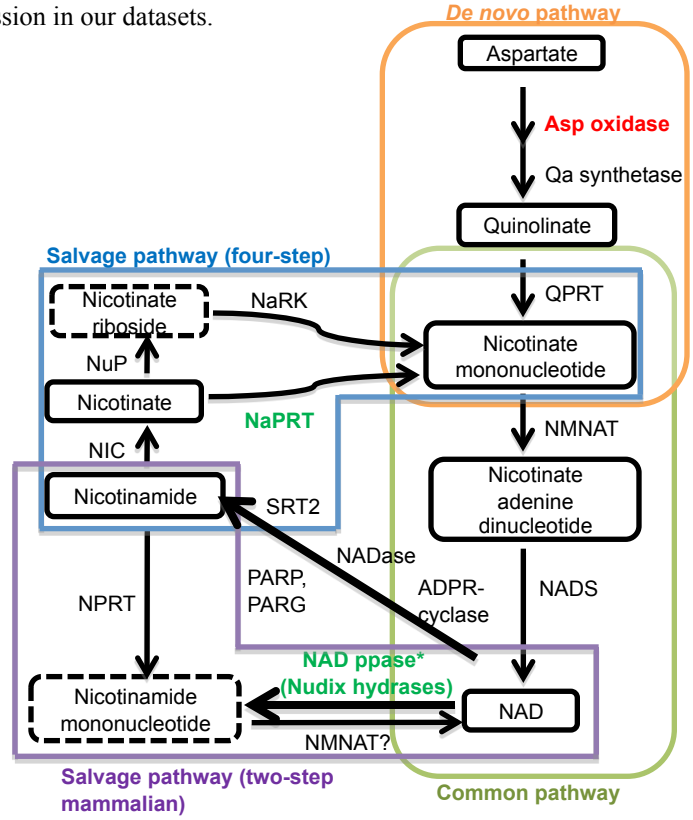
(2) **Total SA accumulation in response to 4 mM CLO treatment in wild type and *ics1* mutant *Arabidopsis* plants at 4 days post treatment.** Data is presented for each of two samples in independent confirmatory experiment. SA extraction and analysis by HPLC with fluorescence detection is as previously described in Nobuta et al. (1).

Sample	Total SA (μg/g)
Col-0 + 4 mM CLO	10.6
Col-0 + 4 mM CLO	8.8
ics1 + 4 mM CL0	0.1
ics1 + 4 mM CLO	0.1

Reference:

1. Nobuta K, et al. (2007) The GH3 acyl adenylase family member PBS3 regulates salicylic acid-dependent defense responses in *Arabidopsis. Plant Physiology* 144:1144-1156.

SI Table 6. *Arabidopsis* genes associated with NAD(P) metabolism with altered expression in response to SA, CLO or IMI. Genes obtained from KEGG, AraCyc and Hashida et al. (1). Schematic diagram is modified from (1). Metabolites in dashed lines have not been detected in *Arabidopsis*. Red=enhanced expression, green=decreased expression in our datasets.



			Ratio (treatment vs. control)		
Gene Name	**Enzyme [EC number]**	**Locus***	**SA**	**CLO**	**IMI**
Asp oxidase	Aspartate oxidase [EC:1.4.3.16]	At5g14760	2.0	2.8	5.9
Qa synthetase	Quinolinate synthase [EC:2.5.1.72]	At5g50210	ND	ND	ND
QPRT	Quinolinate phosphoribosyl transferase [EC:2.4.2.19]	At2g01350	ND	ND	ND
NIC	Nicotinamidase [EC:3.5.1.19]	At5g23230, At5g23220, At3g16190, At2g22570	ND	ND	ND
NuP	Nucleoside phosphorylase [EC:2.4.2.1]	-	ND	ND	ND
NaRK	Nicotinamide riboside kinase 1 [EC:2.7.1.-]	-	ND	ND	ND
NaPRT	Nicotinate phosphoribosyltransferase [EC:2.4.2.11]	At4g36940	ND	ND	0.3
NaPRT	Nicotinate phosphoribosyltransferase [EC:2.4.2.11]	At2g23420	ND	ND	ND
NADS	NAD+ synthase [EC:6.3.5.1]	At1g55090	ND	ND	ND
NMNAT	Nicotinamide-mononucleotide adenylyltransferase [EC:2.7.7.1]	At5g55180	ND	ND	ND
NPRT	Nicotinamide phosphoribosyltransferase [E2.4.2.12]	-	ND	ND	ND
NAD ppase	NAD pyrophosphatase (Nudix hydrolase11) [EC:3.6.1.22]	At5g45940**	ND	ND	0.2
PARP1	Poly(ADP-ribose)polymerase 1 [EC:2.4.2.30]	At4g02390	ND	ND	ND
PARP2	Poly(ADP-ribose)polymerase 2	At2g31320	ND	ND	ND
PARG1	Poly(ADP-ribose)glycohydrolase 1 [EC:3.2.1.143]	At2g31865	NA	NA	NA
PARG2	Poly(ADP-ribose)glycohydrolase 2	At2g31870	ND	ND	ND
SRT2	Silent information regulator 2 protein	At5g09230	ND	ND	ND
ADPR cyclase	ADP-ribose cyclase	-	ND	ND	ND
NADase	NAD glycohydrolase (NAD nucleosidase)	-	ND	ND	ND
NADK1	NAD kinase 1 [EC:2.7.1.23]	At3g21070	ND	ND	ND
NADK2	NAD kinase 2	At1g21640	ND	ND	ND
NADK3	NAD kinase 3	At1g78590	NA	NA	NA
	5'-nucleotidase [EC:3.1.3.5]	At1g72880, At4g14930	NA, ND	NA, ND	NA, ND

*Established loci associated with NADP metabolism. **Only nudix hydrolase with experimental evidence of enzyme activity in *Arabidopsis thaliana*. Genes with ≥2-fold change in expression and q-value ≤0.5. Red=enhanced expression, green=decreased expression. ND=below threshold; NA=not on array.

Reference:

1. Hashida et al. (2009) The role of NAD biosynthesis in plant development and stress responses. *Annals of Botany* 103:819-824.

SI Method 1. RNA isolation, microarray hybridization and analysis.

Total RNA was extracted from 100 mg of each frozen leaf sample using the Qiagen RNeasy Plant RNA Mini kit (Qiagen, Valencia, CA). RNA was quantified using the Nanodrop 1000 (Thermo Scientific, Wilmington, DE) and quality assessed by determining the A260/A280 ratio. Target labeling, microarray hybridizations to Affymetrix ATH1 arrays and array scanning were performed by the Functional Genomics Laboratory (UC Berkeley). Expression values (log_2) for two independent biological replicates per treatment were extracted using robust multiarray analysis with perfect match correction and quantile normalization as in (1). Genes with ≥2 fold change in treated versus control samples were computed using a false discovery rate (q-value) ≤ 5% with Partek Genomics suite (Partek). TAIR9 gene functional descriptions (2) were employed. MAPMAN (3) and Biomaps in Virtual Plant (4) were used to identify statistically enriched functional processes.

References:

1. Chandran, D., et al. (2009) Temporal global expression data reveal known and novel salicylate-impacted processes and regulators mediating powdery mildew growth and reproduction on Arabidopsis, *Plant Physiology 149*, 1435-1451.
2. Swarbreck, D., et al. (2008) The Arabidopsis Information Resource (TAIR): gene structure and function annotation, *Nucleic Acids Research 36*, D1009-1014.
3. Thimm, O., et al. (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes, *The Plant J. 37*, 914-939.
4. Katari, M.S., et al. (2009) VirtualPlant: a software platform to support systems biology research. *Plant Physiology* 152: 500-15.

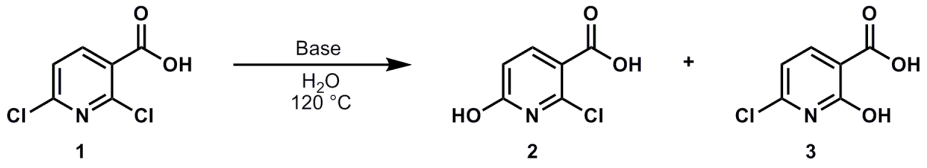
SI Method 2. Induction of *PR1* by reverse transcription PCR.

First strand cDNA synthesis was performed on DNase-treated total RNA samples using Superscript III Reverse Transcriptase (Invitrogen). PCR reactions were performed using *PR1* or *UBQ5* primers with amplified products analyzed by gel electrophoresis. PR1 forward primer, 5'-GAAAACTTAGCCTGGGGTAGC-3', PR1 reverse primer, 5'-TTCATTAGTATGGCTTCTCGTTCA-3'; UBQ5 forward primer, 5'-GAAGACTTACACCAAGCCGAAG-3'; UBQ5 reverse primer, 5'-TTCTGGTAAACGTAGGTGAGTCC-3.

SI Method 3. Synthesis of 6-chloro-2-hydroxypyridinyl-3-carboxylic acid.

2-HOCPA has been previously prepared as a byproduct in the basic hydrolysis of 2,6-dichloro-3-pyridinecarboxylic acid in refluxing sodium hydroxide (5 equivalents) aqueous solution which yields some 2-hydroxy but mostly 6-hydroxy isomer (1). Hydrolysis conditions were studied in order to improve the 6-hydroxy to 2-hydroxy ratio. Unfavorable ratios (given in parenthesis) were observed when using 5 equivalents of sodium hydroxide (4:1 ratio), lithium hydroxide (1:0), or potassium hydroxide (1:0) for 5 h at 120 °C or 2.5 equivalents of magnesium oxide (2:1) for 24 h at 120 °C. The ratio was greatly improved with calcium oxide (1:3 ratio) or barium hydroxide (1:3) for 24 h at 120 °C allowing greater ease of crystallization to obtain pure 2-HOCPA in 17% recovered yield. The improved selectivity of hydrolysis is possibly associated with coordination of the divalent cation to the carboxylate moiety or, less likely, to the heterogeneous nature of the reaction mixture when alkali earth bases are used.

Variation of Base in the Hydrolysis of 2,6-Dichloropyridinyl-3-carboxylic Acid. 2-Hydroxy-6-chloropyridinyl-3-carboxylic acid (compound **3**) has been previously prepared by microbial oxidation of 6-chloropyridinyl-3-carboxylic acid and is also produced during the hydrolysis of 2,6-dichloropyridinyl-3-carboxylic acid in refluxing sodium hydroxide solution (2). Our observation on repeating the hydrolysis of compound **1** with NaOH was a 4:1 ratio of compound **2** to compound **3** in quantitative yield (Scheme A). We greatly improved the synthesis of compound **3** by using a variety of bases to conduct the hydrolysis of compound **1**.



1 2 3

Scheme A: Solvolysis of 2,6-Dichloropyridinyl-3-carboxylic acid

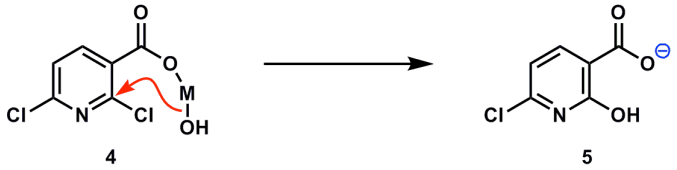
Interestingly, as shown in Table A, alkali metal bases (e.g. LiOH, NaOH, KOH) favored formation of 6-hydroxy isomer (compound **2)** upon hydrolysis of compound **1** whereas alkali earth bases (CaO, $Ba(OH)_2$) tended to favor formation of 2-hydroxy isomer (compound **3)**. A notable exception to this trend was MgO, which favored formation of compound **2**. In most cases, nearly quantitative conversion of chloronicotinate to hydroxynicotinate occurred within the given time. However, when MgO or $Ba(OH)_2$ was used as the base, the reaction did not proceed to completion even after 24 h. An important difference is that solvolyses conducted with alkali metal bases proceeded as homogeneous reaction solutions, whereas alkali earth bases are only sparingly soluble in water and therefore result in heterogeneous mixtures.

base	ratio **2:3**
alkali metal base, 5 equiv., 120 °C, 2 h	
LiOH	1:0
NaOH	4:1
KOH	1:0
alkali earth base, 2.5 equiv., 120 °C, 24 h	
MgO	2:1
CaO	1:3
$Ba(OH)_2$	1:3

Table A. Effect of Base for the Solvolysis of compound **1** on the product ratio

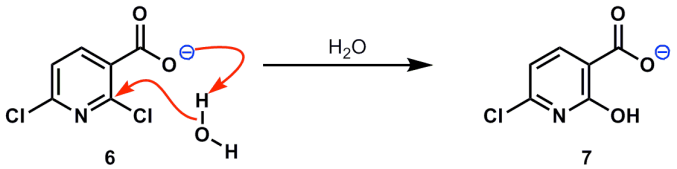
Although solvolysis reactions with CaO typically resulted in complete conversion of compound **1** to **2** and **3**, isolation of compound **3** by crystallization from the crude mixture was challenging. Even after optimizing the purification protocol, only 17% of **3** could be obtained pure.

Mechanistic Considerations. The reversal in selectivity in the solvolysis of compound **1** employing a base with a divalent counterion (e.g. $Ca(OH)_2$ or $Ba(OH)_2$), might involve an intramolecular delivery of hydroxide (Scheme B) to yield compound **5** and ultimately **3** after workup. With monovalent cations (such as Na^+ or K^+) the nicotinate would undergo preferential substitution at C-6 because Coulombic repulsion of the carboxylate and the hydroxide nucleophile disfavors substitution at C-2.



Scheme B: Intramolecular Delivery of Hydroxide

As another possible effect of the counterion, the bases that favored the 2-substituted product (e.g. CaO, $Ba(OH)_2$) had significantly lower dissociation constants (3) than those that favored the 6-substituted product (e.g. LiOH, NaOH, KOH). This suggested that the reversal in selectivity could simply be a hydroxide concentration effect.



Scheme C: Pathways of Base-Promoted Hydrolysis of Nicotinate **4.19**

In order to explore the possibility that hydroxide concentration was responsible for the selectivity of the reaction, dichloropyridinyl-3-carboxylic acid **1** was subjected to hydrolysis conditions for 2 h with exactly one equivalent of sodium hydroxide (in order to generate carboxylate **6**). Under these conditions, hydrolysis was not observed, and only starting material

was recovered. This observation disfavors a mechanism where water is acting as the nucleophile (Scheme C) in the presence of the carboxylate.

Yet another mechanistic possibility is that the reversal of reactivity could be caused by the heterogeneous nature of the reaction mixture when CaO is used, i.e., at the surface of the undissolved CaO (or $Ca(OH)_2$) a manifold of reactivity different from solution-phase reactions could operate. This particular scenario cannot be discounted.

General Methods for Conditions in Table A. A mixture of 2,6-dichloropyridinyl-3-carboxylic acid (60 mg, 0.313 mmol) and the designated base (specified equivalents) in water (2 ml) was placed into a 4 ml vial with a Teflon® lined cap and heated to reflux for the indicated amount of time, then cooled and acidified with with 12 N hydrochloric acid (142 µl, 1.703 mmol). When LiOH, NaOH, or KOH was used, the reaction mixture was cooled for 30 min in an ice bath and the resulting solid was collected by filtration and washed with water. When MgO, CaO, or $Ba(OH)_2$ was used, the acidified mixture was diluted with water (4 ml), then extracted with ethyl acetate (3 x 3 ml). The combined extract was dried over magnesium sulfate and concentrated. In all cases, nearly complete mass recovery was possible, taking into account a small loss on glassware. Yields were not calculated, but the ratio of products was determined by integration of characteristic resonances in the ^1H NMR.

Synthetic Methods.

2-Chloro-6-hydroxypyridinyl-3-carboxylic acid (compound 2). A mixture of 2,6-dichloropyridinyl-3-carboxylic acid (60 mg, 0.31 mmol) and aqueous potassium hydroxide (2.0 M, 780 µl, 1.6 mmol) was placed into a 4 ml vial with a Teflon®-lined cap. The reaction mixture was heated at 120 °C for two hours and then cooled to room temperature. The reaction solution was acidified with hydrochloric acid (12 M, 140 µl, 1.7 mmol) causing a precipitate to

form. The mixture was cooled for 30 min in an ice bath and the resulting solid was filtered and washed with water to yield 2-chloro-6-hydroxypyridinyl-3-carboxylic acid (45 mg, 0.26 mmol, 83 % yield) as an off-white solid. **^{1}H NMR** (600 MHz, DMSO-d_6)) δ ppm 12.75 (bs, 2 H), 8.10 (d, *J*=8.3 Hz, 1 H), 6.65 (d, *J*=8.2 Hz, 1 H); **^{13}C NMR** (150 MHz, DMSO-d_6) δ ppm 164.6, 146.8, 143.6, 116.8, 109.3, 65.0; **IR** (KBr pellet) ν_{max} 3434, 2819, 1642, 1410, 1322, 1281, 1248, 1157, 1050 cm^{-1}; **MS** (EI$^+$), m/z 175/173 (M^+), 138 ([M-Cl]$^+$); **HRMS** (EI$^+$) calc'd for $[C_6H_4ClNO_3]^+$: *m/z* 172.9880, found 172.9876.

6-Chloro-2-hydroxypyridinyl-3-carboxylic acid (compound 3). 2,6-Dichloropyridinyl-3-carboxylic acid **1** (300 mg, 1.6 mmol), calcium oxide (290 mg, 5.1 mmol), and water (20 ml) were heated in a 50 ml Schlenk flask for 48 hours at 128 °C and then acidified with 1M hydrochloric acid (20 ml) and extracted with ethyl acetate (4 x 20 ml). The combined ethyl acetate extract was washed with brine (10 ml), dried over magnesium sulfate and concentrated to give a quantitative yield of a mixture of two products determined by ^{1}H NMR to be a 3:1 ratio of compound **3** to **2**. The crude material was taken up in boiling ethanol (about 5 ml) and the solution was decanted away from some remaining insoluble material. The ethanol solution was concentrated, and the remaining solid was dissolved in boiling ethanol (2 ml) and allowed to crystallize over 2 days at rt. The remaining crystals were washed with diethyl ether, and dried in vacuo to give **3** (48 mg, 0.28 mmol, 17 % yield) as a brown solid. **^{1}H NMR** (600 MHz, DMSO-d_6) δ ppm 8.19 (d, *J*=7.9 Hz, 1 H), 7.02 (d, *J*=7.9 Hz, 1 H); **^{13}C NMR** (150 MHz, DMSO-d_6) δ ppm 167.5, 164.2, 149.8, 143.9, 114.3, 110.7; **IR** (KBr pellet) ν_{max} 3090, 2736, 1730, 1642, 1548, 1409, 1323, 1280 cm^{-1}; **MS** (EI$^+$), m/z 175/173 (M^+), 138 ([M-Cl]$^+$); **HRMS** (EI$^+$) calc'd for $[C_6H_4ClNO_3]^+$: m/z 172.9880, found 172.9878. ^{1}H NMR data were consistent with previously reported data (1).

5. Spectral Data

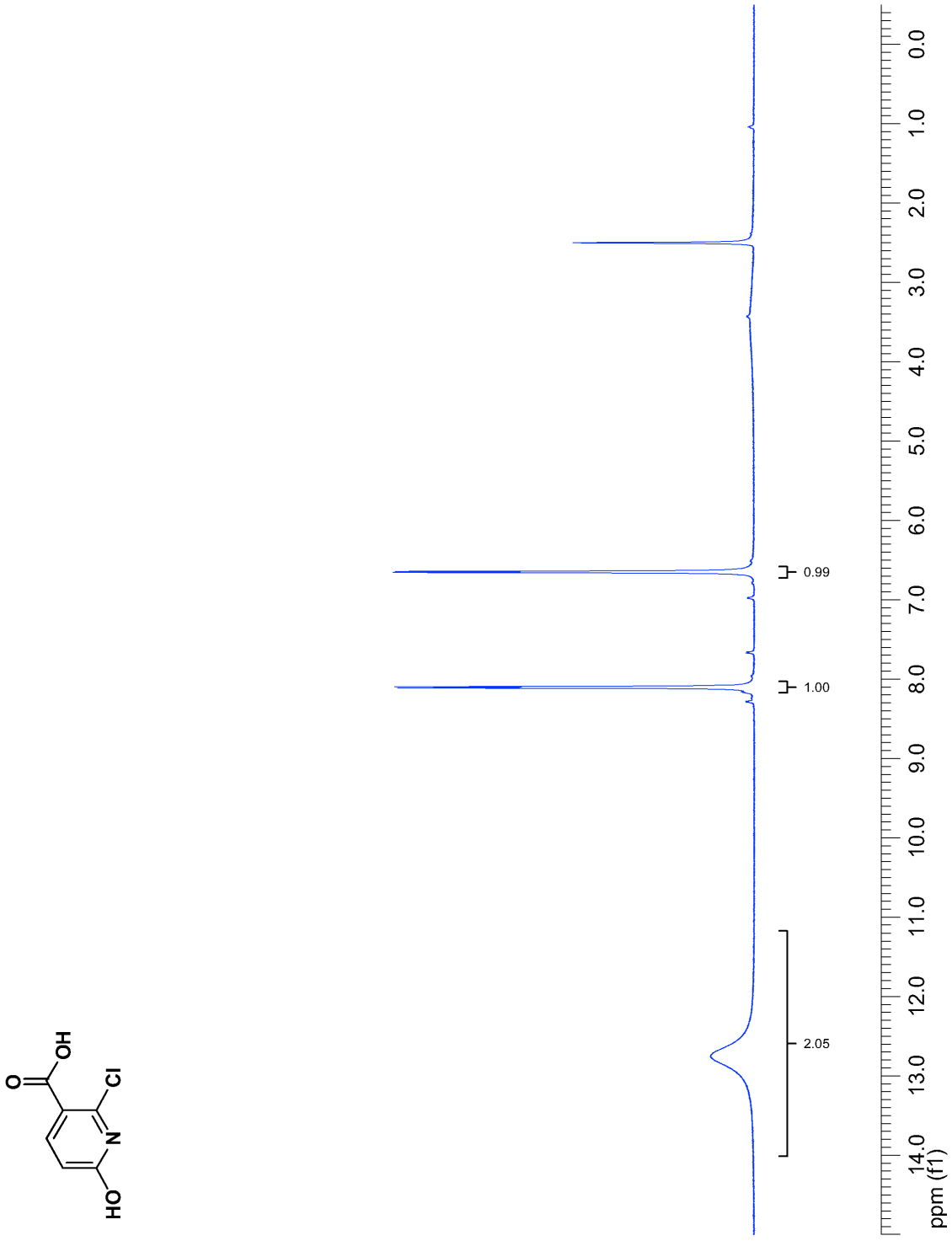


Figure A: ^{1}H NMR (600 MHz, DMSO-d_6) of compound **2.**

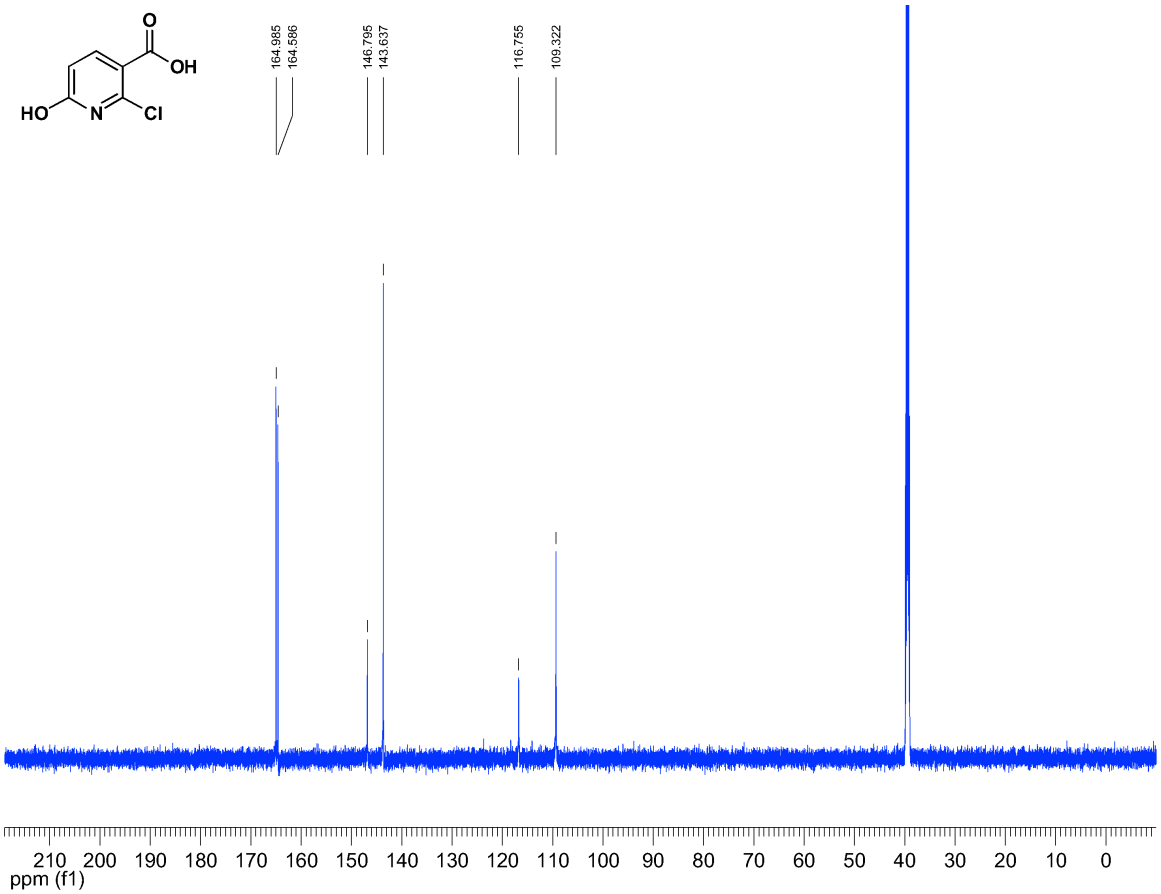


Figure B: ^{13}C NMR (150 MHz, DMSO-d_6) of compound **2.**

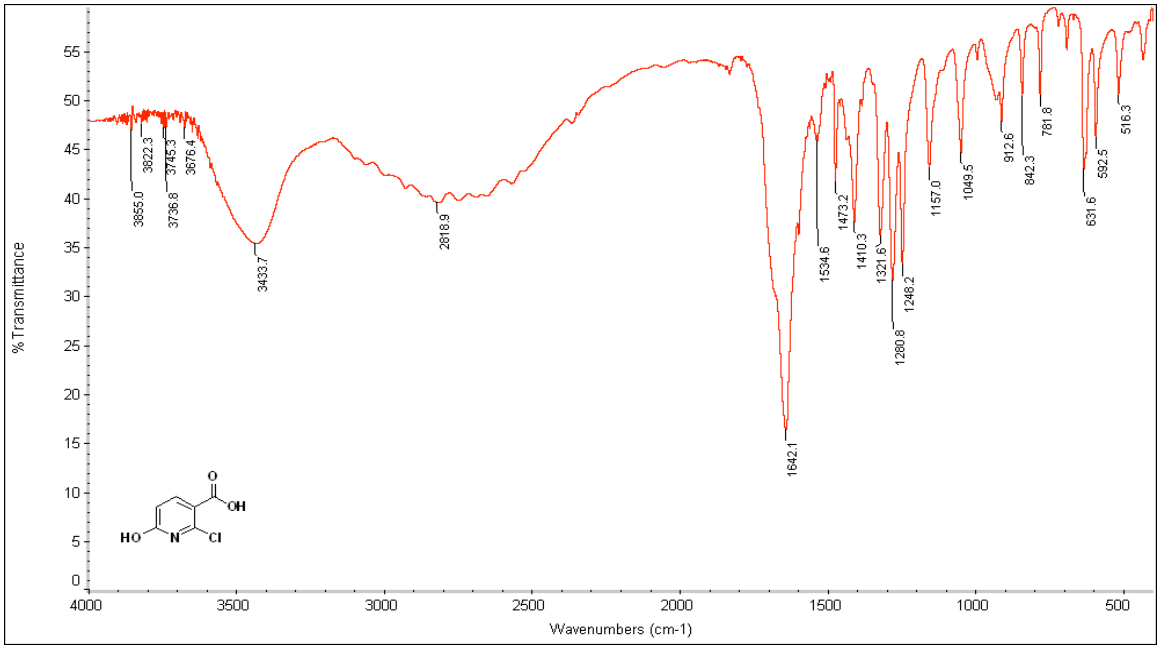


Figure C: IR (KBr pellet) of compound **2.**

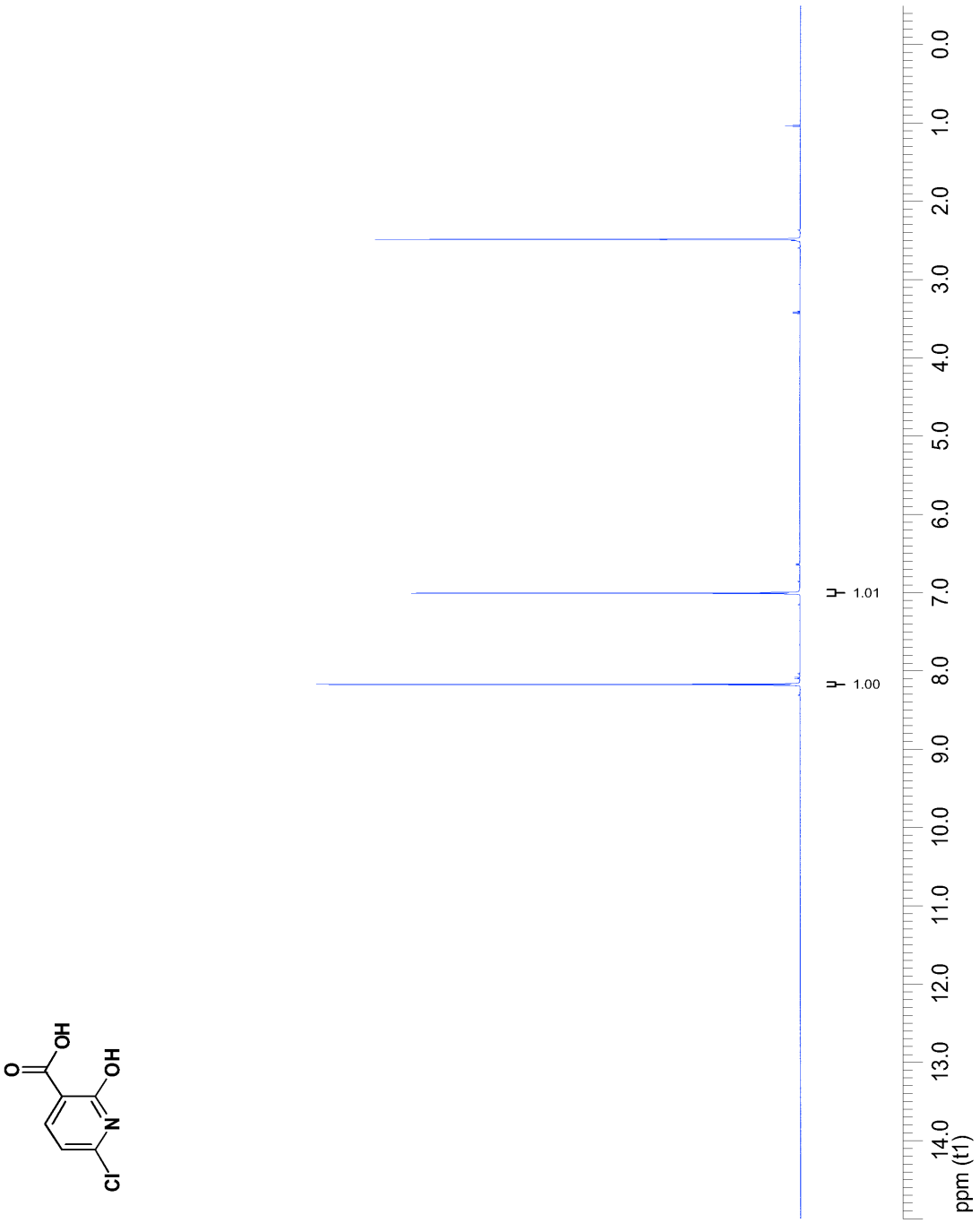


Figure D: ^{1}H NMR (600 MHz, DMSO-d_6) of compound **3.**

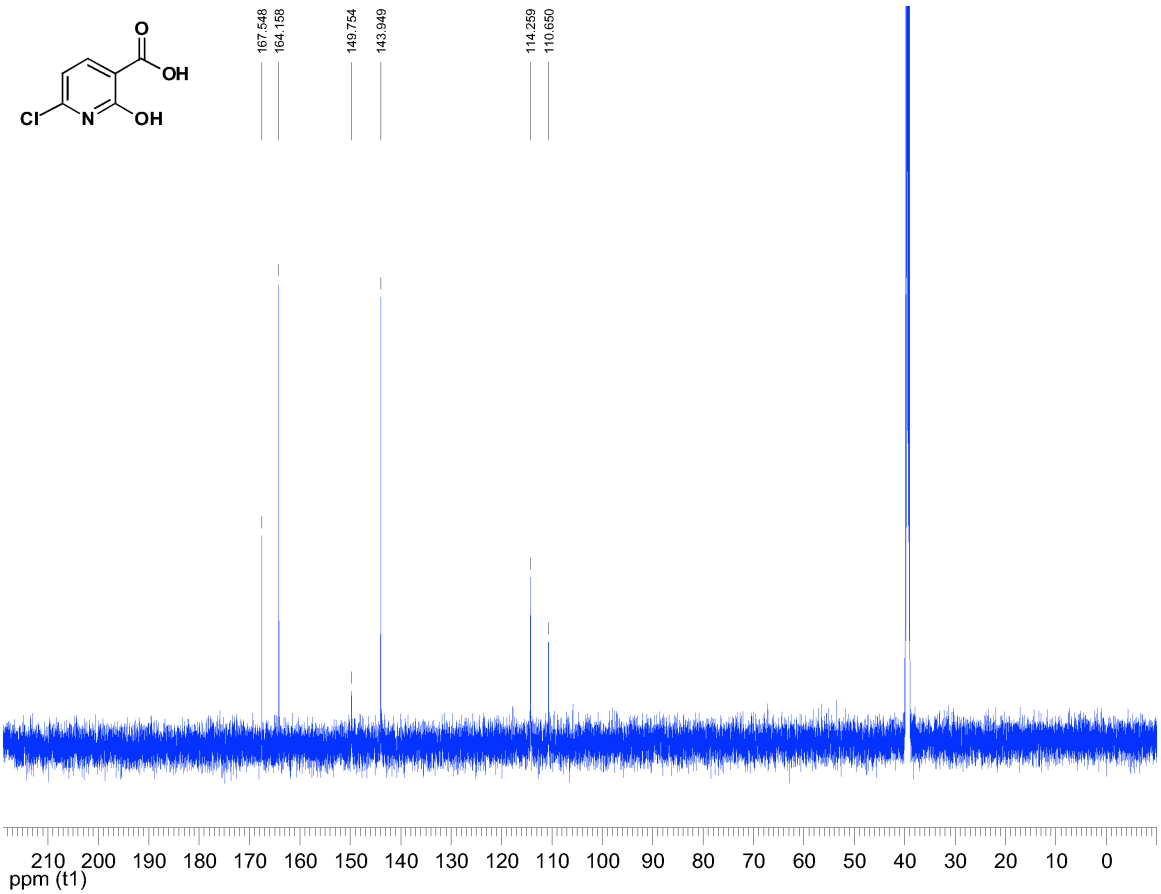


Figure E: ^{13}C NMR (150 MHz, DMSO-d_6) of compound **3.**

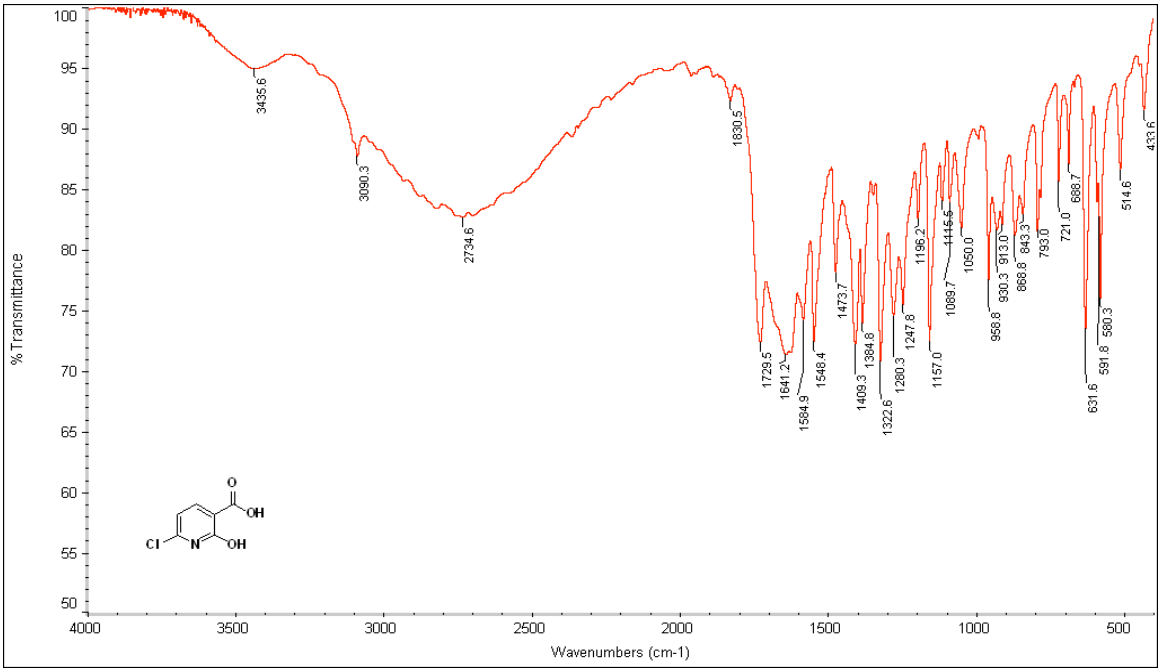


Figure F. IR (KBr pellet) of compound **3**.

References:

1. Tinschert, A., Tschech, A., Heinzmann, K.,; Kiener, A. (2000) Novel regioselective hydroxylations of pyridine carboxylic acids at position C2 and pyrazine carboxylic acids at position C3. *Appl. Microbiol. Biotechnol.* 53: 185-195.

2. F. Hoffman. La Roche & Co. "Nicotinsäurederivate. German Patent 2,153,334, filed November 18, 1971, and issued May 31, 1972.

3. According to *CRC Handbook of Chemistry and Physics. 77th Ed.;* Lide, D.R. Ed.; CRC Press; 1996, the solubility of $Ca(OH)_2$ is 1.6 g/L ($k_{sp} = 9.9 \times 10^{-6}$ M^3), and that of $Ba(OH)_2$ is 49.1 g/L ($k_{sp} = 2.35 \times 10^{-2}$ M^3).

SI Method 4. Detection and quantitation of SA by LC/MS/MS.

SA, both free and total (with glucoside cleaved by β-glucosidase), were first analyzed by LC/MS with t_R and [M+1] for SA of 24.5 min and 139.1. However high background noise and interfering peaks meant that a more reliable and sensitive method was required. Detection and characterization of SA by LC/MS/MS in multiple reaction monitoring (MRM) mode proved more reliable based on a detection limit with a signal-to-noise ratio of 3:1 of 0.9 ng SA per g for spiked control plant.

HPLC analyses were carried out using an Agilent 1100 HPLC system equipped with a diode array detector (set at 237 nm and 307 nm) and a multi-well autosampler. A Luna C_{18} (Phenomenex, Torrance, CA) column (5 μm, 100 Å, 250 mm x 4.6 mm) was used at 30 °C and the injected volume was 10 μl. The elution gradient was carried out with a solvent system consisting of 0.05% HOAc in H_2O (solvent A) and acetonitrile (solvent B) at a constant flow-rate of 500 μl/min. A linear gradient profile with the following proportions (v/v) of solvent B was applied (*t* (min), %B): (0, 15), (10, 50), (20, 100), (30, 25), (40, 15) with 10 min for re-equilibration. The t_R for SA under these conditions was 20.4 min. Quantitation was done by spiking control plants with SA solutions (ranging from 0.5 to 2000 ng ml^{-1}) and extracting as described. A standard curve of spiked SA amounts *vs* ion intensity was used for calculating the amount of induced-SA.

MS/MS experiments were on an Applied Biosystems API 3000 triple quadrupole mass spectrometer (Foster City, CA) using turbo ionspray source in negative ion mode and the following settings: capillary voltage -3500 V, declustering potential -30 V, focusing potential -150 V, entrance potential -10 V, collision energy -20 V (137/93). Full scan data acquisition was performed scanning from *m/z* 100 to 600 using a cycle time of 2 s. In product ion scan

experiments, MS/MS product ions were produced by collision-activated dissociation of selected precursor ions in the collision cell of the triple quadrupole mass spectrometer and mass analyzed using the second analyzer of the instrument. In negative mode, the SA spectrum gave the deprotonated molecule $[M - H]^-$. Quantitation was performed by injection of samples in MRM mode. The combination of the parent mass and unique fragment ions was used to monitor selectively SA in crude plant extracts. MRM acquisition was done by monitoring the 137/93 transitions for SA.

SI Method 5. HPLC fractionation and detection of 2-HOCPA in CPA-treated leaf extracts.

Sample Preparation

Arabidopsis leaves (~500 mg fresh weight) were harvested at 4 days post soil treatment with 4 mM CPA or water (control), frozen for storage and then extracted as for SA [method described in Nobuta et al. (1)]. HPLC fractionation utilized a Shimadzu SCL-10A system with a Shimadzu RF-10A scanning fluorescence detector and Shimadzu SPD-M10A photodiode array detector. Samples were separated on a 5 μm, 15-cm x 4.6-mm inner diameter Supelcosil ABZ+PLUS column (Supelco) preceded by a 7.5 cm x 4.6 mm guard column maintained at 27°C. Prior to loading the 50 μl sample, the column was equilibrated with 25 μM formate, 5% acetonitrile (ACN), pH 2.8 at a flow rate of 1 ml/min. The concentration of ACN was increased linearly to 10.7% over 15 min, followed by isocratic flow for 5 min, a linear increase from 10.7% to 36.4% ACN over 23 min, a linear increase from 36.4% to 62% ACN over 2 min, isocratic flow for 5 min, a linear decrease from 62% to 5% ACN over 5 min, and isocratic flow at 5% ACN for 3 min. Synthesized 2-HOCPA was used as a standard (t_R~28.7 min) for HPLC analysis and 1 ml fractions (F1-F4) from 26-30 min were collected for the CPA-treated and control samples.

Sample Analysis

LC/MS analysis for detection of 2-HOCPA was performed on the HPLC-fractionated (F1-F4) extracts from CPA-treated and control leaves using methods previously described for analysis of Cl-pyr neonicotinoids and their derivatives (2). LC used the Agilent 1100 series instrument and MS the Waters LCT premier XE micromass instrument. The column was Luna 5μ C18 100Å 250 x 4.60 mm and the gradient was ACN with 0.1% formic acid and water with a flow rate of 0.3 ml/min and a column temperature of 30°C. A linear gradient of 5% ACN to 80%

ACN for 25 min was followed by isocratic 5% ACN at 30 min and 35 min. For MS the capillary voltage was 2000 and core voltage was 68 V, the desolvation temperature 250°C, the source temperature 100°C and the gas flow 40 L/h for the core and 700 L/h for desolvation. The sample was dissolved in methanol (50 μL) and a 20 μl aliquot injected for analysis. The synthetic 2-HOCPA standard (m/z=173.9) eluted at t_R=22 min under the conditions employed.

References:

1. Nobuta, K. et al. (2007) The GH3 Acyl Adenylase Family Member PBS3 Regulates Salicylic Acid-Dependent Defense Responses in Arabidopsis. *Plant Physiology* 144:1144-1156.

2. Ford, K.A. and J.E. Casida (2008) Comparative metabolism and pharmacokinetics of seven neonicotinoid insecticides in spinach. *J Agric Food Chem* 56:10168-10175.