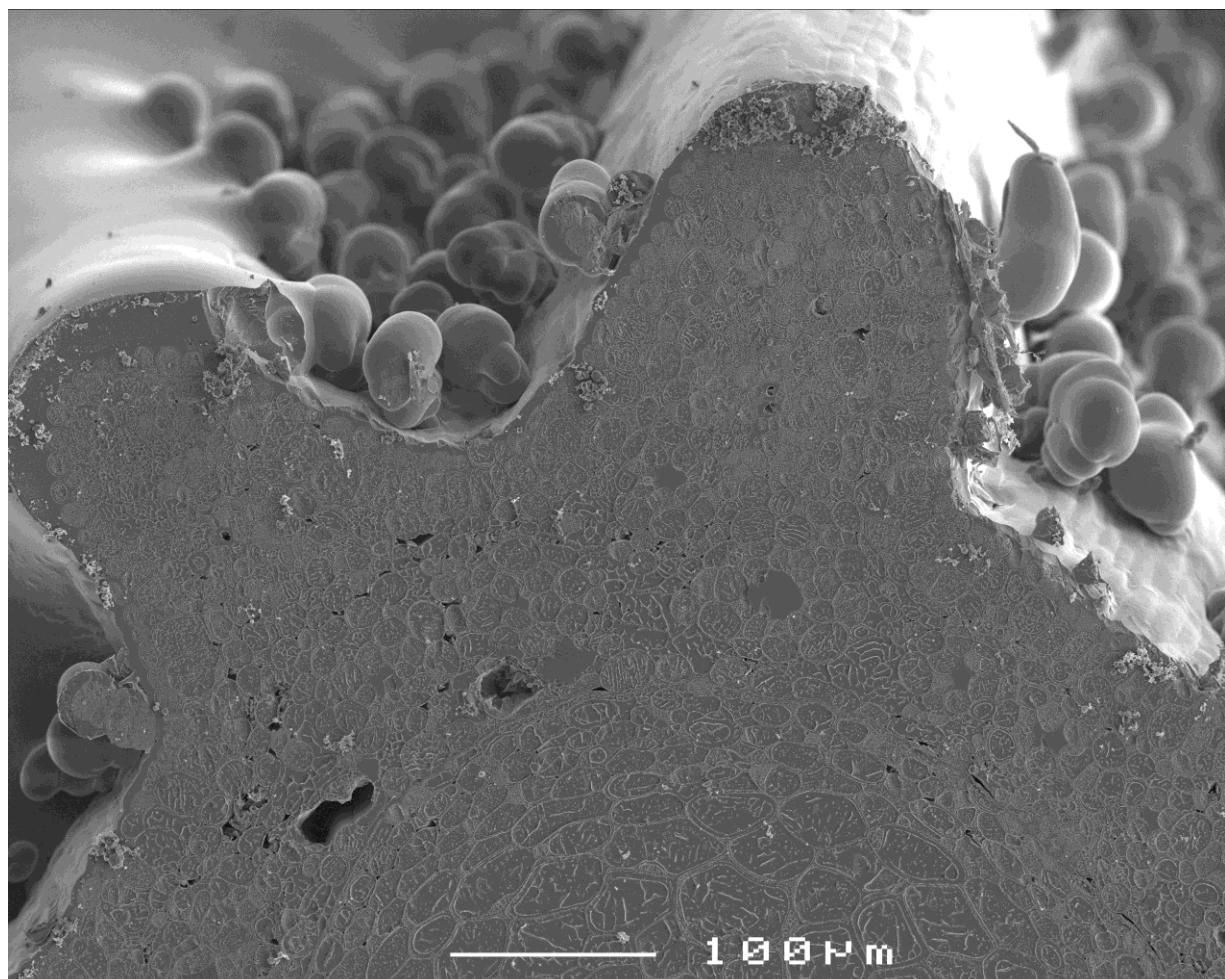


Supplemental Figure 1: Biosynthesis of pyrethrins and sesquiterpene lactones.

The biosynthesis of sesquiterpenes generally initiates from the cytosolic precursor molecules IPP and DMAPP by means of a farnesyl diphosphate synthase (*FDS1*) to form FPP. Subsequently FPP is the substrate for many sesquiterpene synthases and modifying enzymes resulting in sesquiterpene lactones A-M (see also Table S1). Pyrethrin ester formation by the GDSL Lipase-like Protein (*GLIP*) is dependent on two dedicated precursor pathways. The irregular monoterpene branch of the plastidic precursor pathway initiates with the dedicated enzyme chrysanthemyl diphosphate synthase (*CDS*). *CDS* uses two DMAPP molecules to form CPP, which after dephosphorylation results in chrysanthemol (COH) and through oxidative steps in chrysanthemic acid (CA). The acid is CoA-activated and modified to form chrysanthemoyl or pyrethroyl CoA through a number of unknown steps. The oxylipin branch of the precursor pathway initiates from linolenic acid and finally leads to the lipid

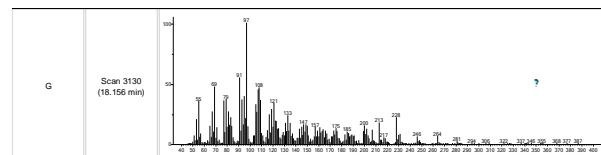
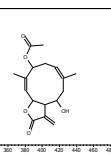
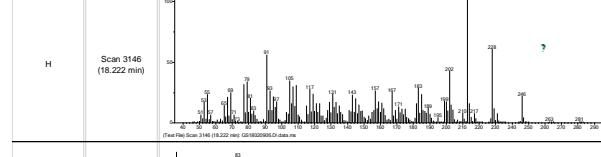
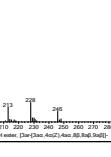
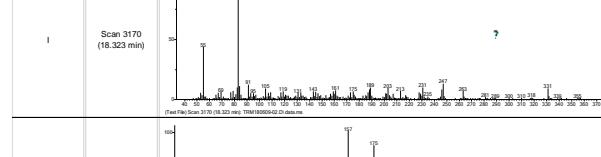
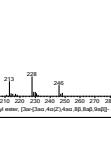
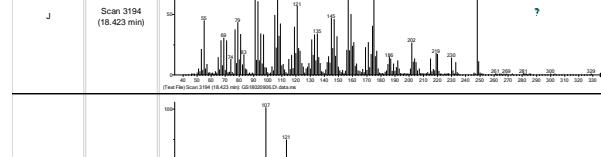
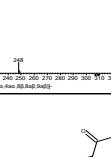
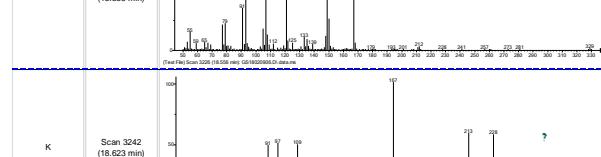
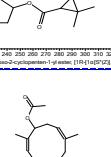
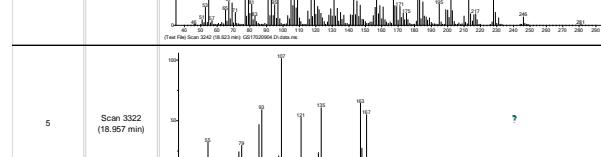
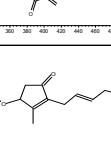
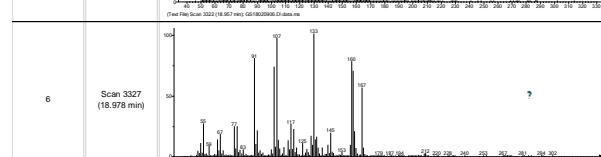
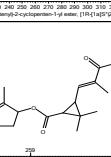
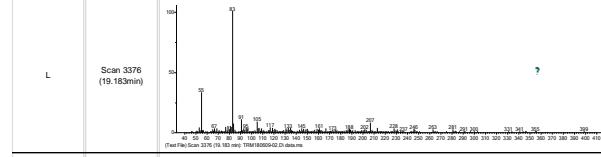
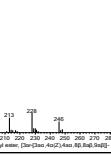
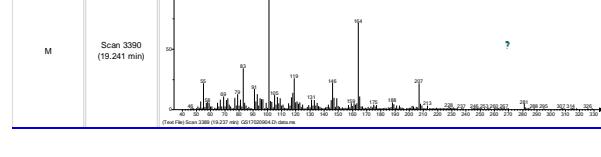
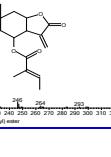
alcohols jasmolone and/or pyrethrolone and/or cinerolone. GLIP then esterifies these two precursors into the six pyrethrin esters. It is not known whether interconversions between the esters occur after esterification or whether these conversions occur already at the alcohol stage or at both.



Supplemental Figure 2: CryoSEM image of a cross section of an achene that was used to draw Figure 6.

Supplemental Table 1: List of compounds detected by GC-MS

Notation	Retention Time	Mass spectrum Compound	Mass spectrum NIST	Name	Probability	KI
A	Scan 2758 (16.602 min)			Naphthalocarbonyl[2,3-b]furan-2(3H)-one, 4-(acetoxy)decahydro-8-hydroxy-3,8-dimethyl-5-methylene-[3R-(3S,3aR,4aR,8aR,9aR)]-3,4,4a,8a-tetrahydro-2,3-dimethyl-5-acetyl-6-methoxy-1H,1aH-dihydro-2H-furan-2-one	27.40%	2202
1	Scan 2773 (16.665 min)			Cinerin I	59.30%	2212
B	Scan 2810 (16.819 min)			Dihydroxanthin	29.70%	2238
C	Scan 2842 (16.953 min)			2H-Cyclohepta[b]furan-2-one, 6[1-(acetoxyl)-3-oxobutyl]-3a,4,7,8,8a-hexahydro-7-methyl-3-methylene acetate	47.30%	2261
2	Scan 2882 (17.120 min)			Jasminol I	87.90%	2290
3	Scan 2890 (17.153 min)			Pyrethrin I	95.70%	2296
D	Scan 2986 (17.554 min)			Xanthatin, 1'acetoxy-3-methyl-3-demethylene-1,2-dihydro-2H-furan-2-one	29.60%	2367
E	Scan 3052 (17.830 min)			Cyclohexa[b]furan-2(3H)-one, 9-(acetoxy)-3a,4,5,6,9,11a-hexahydro-4-hydroxy-5,10-dimethyl-3-methylene-10H,11H-dihydro-2H-furan-2-one	32.20%	2416
F	Scan 3103 (18.043 min)			Naphthalocarbonyl[2,3-b]furan-2(3H)-one, 4-(acetoxy)decahydro-8-hydroxy-3,8-dimethyl-5-methylene-[3R-(3S,3aR,4aR,8aR,9aR)]-3,4,4a,8a-tetrahydro-2,3-dimethyl-5-acetyl-6-methoxy-1H,1aH-dihydro-2H-furan-2-one	77.50%	2454

G	Scan 3130 (18.156 min)			Cyclohex-2-en-1-ene, 9-(acetoxy)-3a,4,5,8,9,11-hexahydro-4-hydroxy-6,10-dimethyl-3-methylene-2-butenyl acetate	55.10%	2475
H	Scan 3146 (18.222 min)			2-Butenic acid, 2-methyl-, dodecahydro-8-hydroxy-8a-methyl-3,5-bis(methyl-2-oxospiro[2,3-b]furan-4-yl)ester, [3aR-[3a(2R,4aR,8S,8aS,9aS)]]	54.30%	2487
I	Scan 3170 (18.323 min)			2-Butenic acid, 2-methyl-, dodecahydro-8-hydroxy-8a-methyl-3,5-bis(methyl-2-oxospiro[2,3-b]furan-4-yl)ester, [3aR-[3a(2R,4aR,8S,8aS,9aS)]]	67.00%	2506
J	Scan 3194 (18.423 min)			Naphtho[2,3-b]furan-2(3H)-one, (acetoxy)decahydro-8-hydroxy-3,8-dimethyl-5-methylene-, [3R-[3a(4aR,8S,8aS,9aS)]]	53.00%	2525
4	Scan 3226 (18.556 min)			Cinerin II	93.40%	2550
K	Scan 3242 (18.623 min)			Cyclohex-2-en-1-ene, 9-(acetoxy)-3a,4,5,8,9,11-hexahydro-4-hydroxy-6,10-dimethyl-3-methylene-2-butenyl acetate	37.20%	2563
5	Scan 3322 (18.557 min)			Jasmin II	82.80%	2627
6	Scan 3327 (18.578 min)			Pyrethin II	95.30%	2631
L	Scan 3376 (19.183 min)			2-Butenic acid, 2-methyl-, dodecahydro-8-hydroxy-8a-methyl-3,5-bis(methyl-2-oxospiro[2,3-b]furan-4-yl)ester, [3aR-[3a(2R,4aR,8S,8aS,9aS)]]	63.70%	2671
M	Scan 3390 (19.241 min)			2-Methylbut-2-enic acid, 5,8-dihydroxy-5,8-dimethyl-3-methylene-2-oxo-2,3-dihydro-1,3-dihydroxy-2-methoxy-2-oxocyclopentyl-2-oxo-2-cyclopentenyl-1-yl ester	89.20%	2683

Supplemental Table 2: Non-normalized and normalized RT-qPCR results for 4 biosynthetic genes and the GAPDH gene (used for normalization). In yellow the presented data

	Ovaries with trichomes	Ovaries without trichomes	Trichomes	Leaves	Seedlings	Ovaries with trichomes	Ovaries without trichomes	Trichomes	Leaves	Seedlings	
Ct values (uncorrected with GAPDH)											
GAPDH	17.14	16.62	17.59	17.00	18.26	GAPDH	0.31	0.35	0.29	0.45	0.37
CDS	19.20	20.74	15.57	20.99	31.00	CDS	0.21	0.20	0.50	0.50	1.01
GLIP	19.76	17.76	24.02	18.88	21.25	GLIP	0.22	0.09	0.35	0.45	0.54
FDS	18.75	19.45	16.75	19.39	20.4	FDS	0.04	0.11	0.16	0.28	0.33
Percentage values relative to the highest expressed gene (not corrected with GAPDH)											
GAPDH	33.7	48.3	24.7	37.1	15.5	GAPDH	4.7	7.7	3.2	7.8	2.6
CDS	8.1	2.8	100.0	2.3	0.0	CDS	0.7	0.2	23.9	0.6	0.0
GLIP	5.5	21.9	0.3	10.1	2.0	GLIP	0.5	0.8	0.0	2.1	0.5
FDS	11.0	6.8	44.1	7.1	3.5	FDS	0.2	0.3	3.0	0.9	0.5
Percentage values relative per gene (not corrected with GAPDH)											
GAPDH	69.7	100.0	51.1	76.8	32.1	GAPDH	9.7	15.9	6.6	16.2	5.4
CDS	8.1	2.8	100.0	2.3	0.0	CDS	0.7	0.2	23.9	0.6	0.0
GLIP	25.0	100.0	1.3	46.0	8.9	GLIP	2.4	3.7	0.2	9.7	2.3
FDS	25.0	15.4	100.0	16.0	8.0	FDS	0.4	0.7	6.8	2.0	1.2
Percentage values relative to the highest expressed gene (corrected with GAPDH)											
GAPDH	24.7	24.7	24.7	24.7	24.7	GAPDH	3.4	3.9	3.2	5.2	4.2
CDS	5.9	1.4	100.0	1.6	0.0	CDS	0.5	0.1	23.9	0.4	0.0
GLIP	4.0	11.2	0.3	6.7	3.1	GLIP	0.4	0.4	0.0	1.4	0.8
FDS	8.1	3.5	44.1	4.7	5.6	FDS	0.1	0.2	3.0	0.6	0.8
Percentage values relative per gene (corrected with GAPDH)											
GAPDH	100.0	100.0	100.0	100.0	100.0	GAPDH	13.8	15.9	12.9	21.1	16.9
CDS	5.9	1.4	100.0	1.6	0.0	CDS	0.5	0.1	23.9	0.4	0.0
GLIP	59.9	167.0	4.3	100.0	46.3	GLIP	5.7	6.2	0.7	21.1	12.1
FDS1	18.3	7.9	100.0	10.7	12.7	FDS	0.3	0.4	6.8	1.3	1.9
Percentage error (STE, uncorrected with GAPDH)											
GAPDH	4.7	7.7	3.2	7.8	2.6	GAPDH	9.7	15.9	6.6	16.2	5.4
CDS	0.7	0.2	23.9	0.6	0.0	CDS	0.7	0.2	23.9	0.6	0.0
GLIP	0.5	0.8	0.0	2.1	0.5	GLIP	2.4	3.7	0.2	9.7	2.3
FDS	0.2	0.3	3.0	0.9	0.5	FDS	0.4	0.7	6.8	2.0	1.2
Percentage error (STE, corrected with GAPDH)											
GAPDH	3.4	3.9	3.2	5.2	4.2	GAPDH	9.7	15.9	6.6	16.2	5.4
CDS	0.5	0.1	23.9	0.4	0.0	CDS	0.7	0.2	23.9	0.6	0.0
GLIP	0.4	0.4	0.0	1.4	0.8	GLIP	2.4	3.7	0.2	9.7	2.3
FDS	0.1	0.2	3.0	0.6	0.8	FDS	0.4	0.7	6.8	2.0	1.2
Percentage error (STE, corrected with GAPDH)											
GAPDH	13.8	15.9	12.9	21.1	16.9	GAPDH	9.7	15.9	6.6	16.2	5.4
CDS	0.5	0.1	23.9	0.4	0.0	CDS	0.7	0.2	23.9	0.6	0.0
GLIP	5.7	6.2	0.7	21.1	12.1	GLIP	2.4	3.7	0.2	9.7	2.3
FDS	0.3	0.4	6.8	1.3	1.9	FDS	0.4	0.7	6.8	2.0	1.2

Supplemental Table 3: Content of pyrethrin biosynthesis related compounds in ovaries and trichomes in terms of specific masses and relative to ovaries with less trichomes before and after normalization with the content of STL (measure for trichome quantities). In yellow the presented data

	mass	specific mass peaks			standard error of specific mass peaks		
		O+	O-	T			
Chrysanthemol (COH)	123	13,707	4,398	41,200	6,405	1,126	14,916
Chrysanthemic acid (CA)	123	146,786	107,356	139,779	36,959	4,396	30,307
Cinerin I (C1)	123	19,529,560	22,255,871	662,826	3,114,186	513,384	40,493
Jasmolin I (J1)	123	9,098,617	7,838,073	210,668	1,518,776	237,640	14,255
Pyrethrin I (P1)	123	4,662,062	4,548,377	133,909	159,101	269,554	14,750
Cinerin II (C2)	133	1,692,842	2,108,493	52,890	252,861	20,433	2,895
Pyrethrin II Jasmolin II (P/J2)	133	3,103,531	3,505,555	114,003	136,800	112,014	22,493
Dihydro-β-cyclo-pyrethosin (peak E)	84	5,240,300	1,826,885	9,613,170	705,367	95,580	1,957,667
		relative % mass peaks			STE relative % mass peaks		
		O+	O-	T	O+	O-	T
Chrysanthemol (COH)	123	312	100	936.72	146	26	339.13
Chrysanthemic acid (CA)	123	137	100	130.20	34	4	28.23
Cinerin I (C1)	123	88	100	2.98	14	2	0.18
Jasmolin I (J1)	123	116	100	2.69	19	3	0.18
Pyrethrin I (P1)	123	102	100	2.94	3	6	0.32
Cinerin II (C2)	133	80	100	2.51	12	1	0.14
Pyrethrin II Jasmolin II (P/J2)	133	89	100	3.25	4	3	0.64
Dihydro-β-cyclo-pyrethosin (peak E)	84	287	100	526.21	39	5	107.16
		relative % normalized for STL			STE relative % normalized for STL		
		O+	O-	T	O+	O-	T
Chrysanthemol (COH)	123	109	100	178.01	51	26	64.45
Chrysanthemic acid (CA)	123	48	100	24.74	12	4	5.36
Cinerin I (C1)	123	31	100	0.57	5	2	0.03
Jasmolin I (J1)	123	40	100	0.51	7	3	0.03
Pyrethrin I (P1)	123	36	100	0.56	1	6	0.06
Cinerin II (C2)	133	28	100	0.48	4	1	0.03
Pyrethrin II Jasmolin II (P/J2)	133	31	100	0.62	1	3	0.12
Dihydro-β-cyclo-pyrethosin (peak E)	84	100	100	100.00	13	5	20.36

Supplemental Table 4. Peak areas based on specific masses (blue panels) of chrysanthemic acid, STL peak E and the different pyrethrin esters in husk extracts, seedling washes and seedling extracts relative to intact seeds (set at 100%). Yellow panels are presented data

	Average specific mass				STE average specific mass				
	Seed	Husk	Wash	Seedling	Seed	Husk	Wash	Seedling	
CA	6874350	1682126	35985	284801	667373	138626	6552	16777	
Peak E	884562	79720			31874	22519			
CI	6115784	558022	249916	7757030	894003	92851	27612	183378	
CII	390053	63153	37203	535508	54339	8561	8917	24568	
JI/PI	11241149	2244962	617091	10192002	1218054	208433	49623	1305371	
JII/PII	2103551	142430	214486	3586957	196549	30822	29716	13279	
	Relative content compared to seeds (%)				STE of the relative content compared to seeds (%)				
	Seed	Husk	Wash	Seedling	Seed	Husk	Wash	Seedling	
CA	100.0	24.5	0.5	4.1	9.7	2.0	0.1	0.2	
Peak E	100.0	9.0	0.0	0.0	3.6	2.5	0.0	0.0	
CI	100.0	9.1	4.1	126.8	14.6	1.5	0.5	3.0	
CII	100.0	16.2	9.5	137.3	13.9	2.2	2.3	6.3	
JI/PI	100.0	20.0	5.5	90.7	10.8	1.9	0.4	11.6	
JII/PII	100.0	6.8	10.2	170.5	9.3	1.5	1.4	0.6	