The impact of conventional and organic agricultural approaches on blackcurrant polyphenol content and diversity

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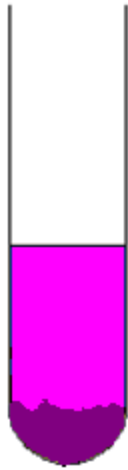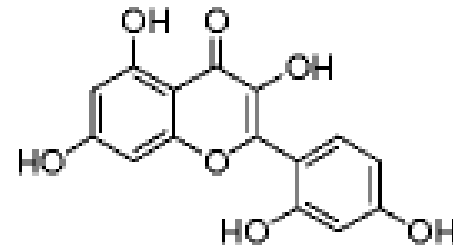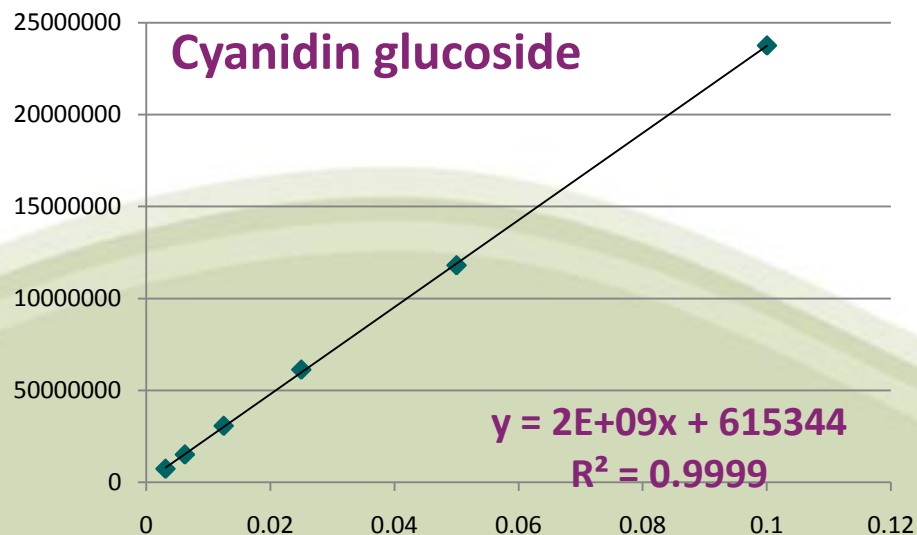# Extraction Protocol

3 ml 60:40 water/acetonitrile 1% acetic acid 0.1 mg/ml morin (internal standard)
100 mg freeze dried (FD) powder

Shaken for 60 minutes at 30 °C

Cyanidin glucoside

$y = 2E+09x + 615344$
$R^2 = 0.9999$

Morin: Internal Standard

Sample analysis – LC-MS

■ Targeted – Anthocyanins (positive mode) & Flavanols (negative mode)

Delphinidin glucoside
Delphinidin rutinoside
Cyanidin glucoside
Cyanidin rutinoside
Petunidin rutinoside
Petunidin glucoside
Pelargonidin glucoside
Peonidin galactoside
Pelargonidin rutinoside
Peonidin rutinoside
Cyanidin arabinoside
Melvidin rutinoside
Delphinidin coumaroylglucoside

Myricetin-3-rutinoside
Myricetin-6-glucoside
Myricetin-3-(6"malonyl)glucoside
Aureusidin glucoside
Quercetin-3-rutinoside
Quercetin-3-(6"-malonyl)glucoside
Kaempferol-3-rutinoside
Isorhamnetin-3-rutinoside
Kampferol-3-glucoside
Kaempferol-3-galactoside
Myricetin-glucuronide
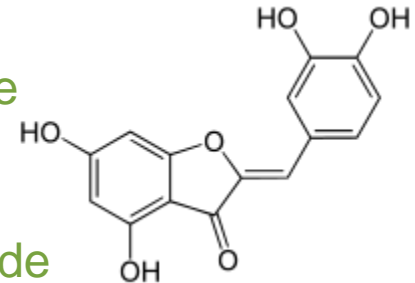
■ Untargeted – Totally unbiased approach

Experiment 1 - Organic vs Conventional

Experimental Design

- Blackcurrant plants

- Two treatments sprayed and unsprayed

- Fruit harvest dates representative of "ripe" – six harvesting events

PCA (1 vs 2) Targeted analysis Coloured by harvest date Organic vs Conventional

Positive & negative MS data combined

Previously demonstrated by harvest time experiment

R2X[1] = 0.373865   R2X[2] = 0.194467   Ellipse: Hotelling T2 (0.95)

SIMCA-P+ 12.0.1 - 2012-09-05 09:06:56 (UTC+0)

Loadings scatter plot score 1v2 Organic vs Conventional
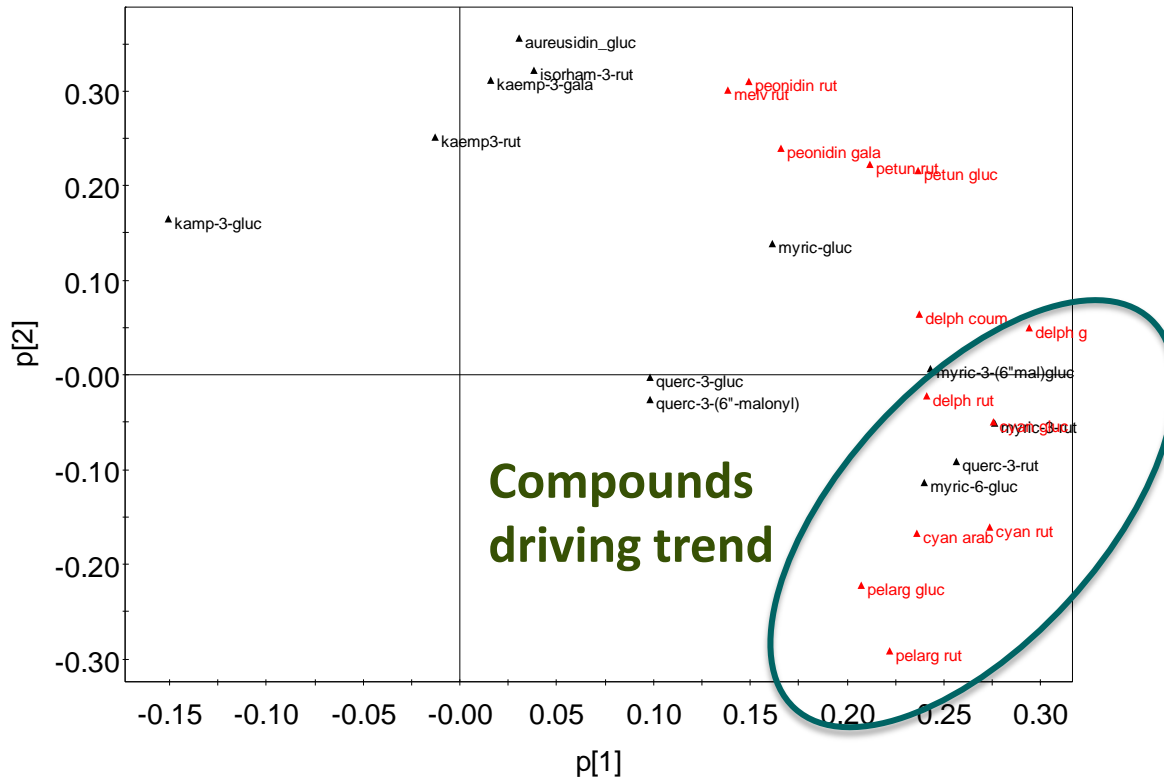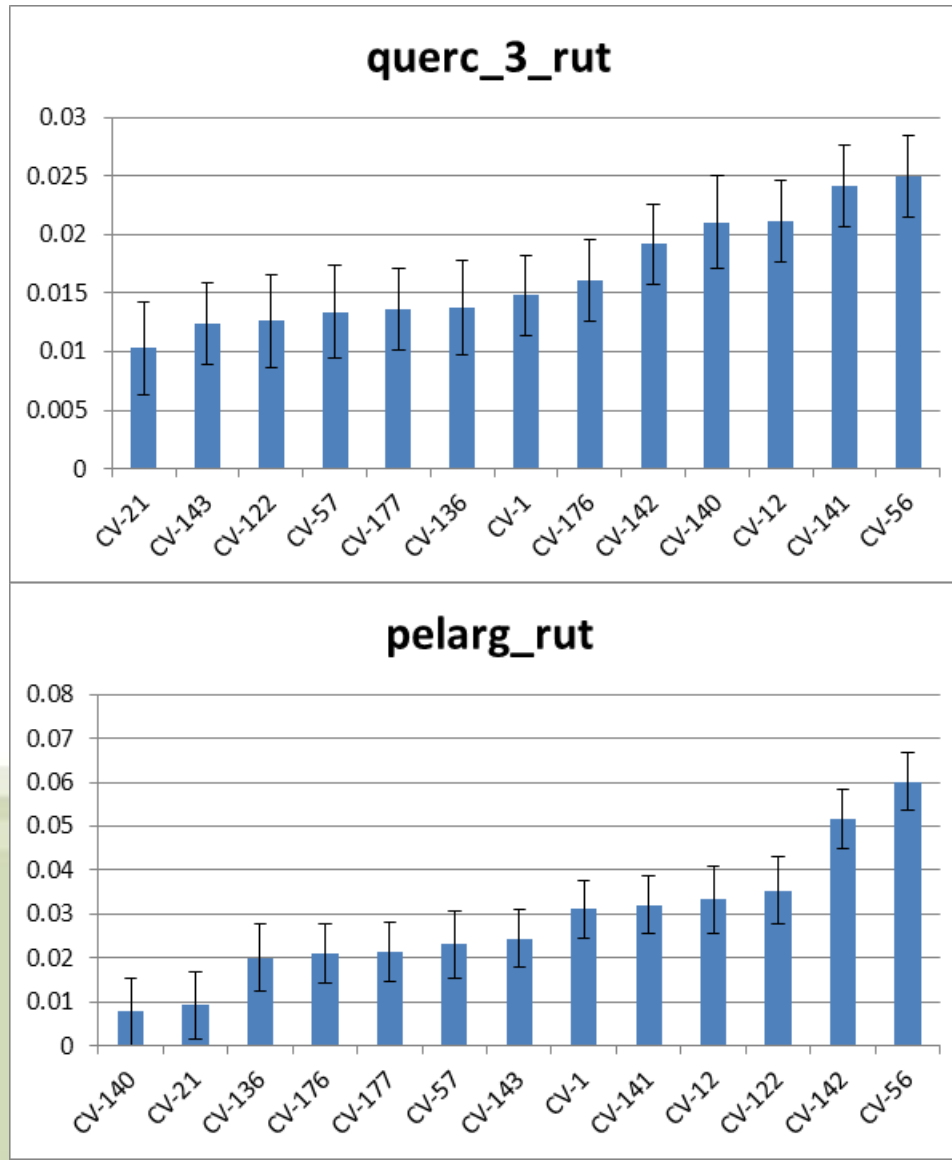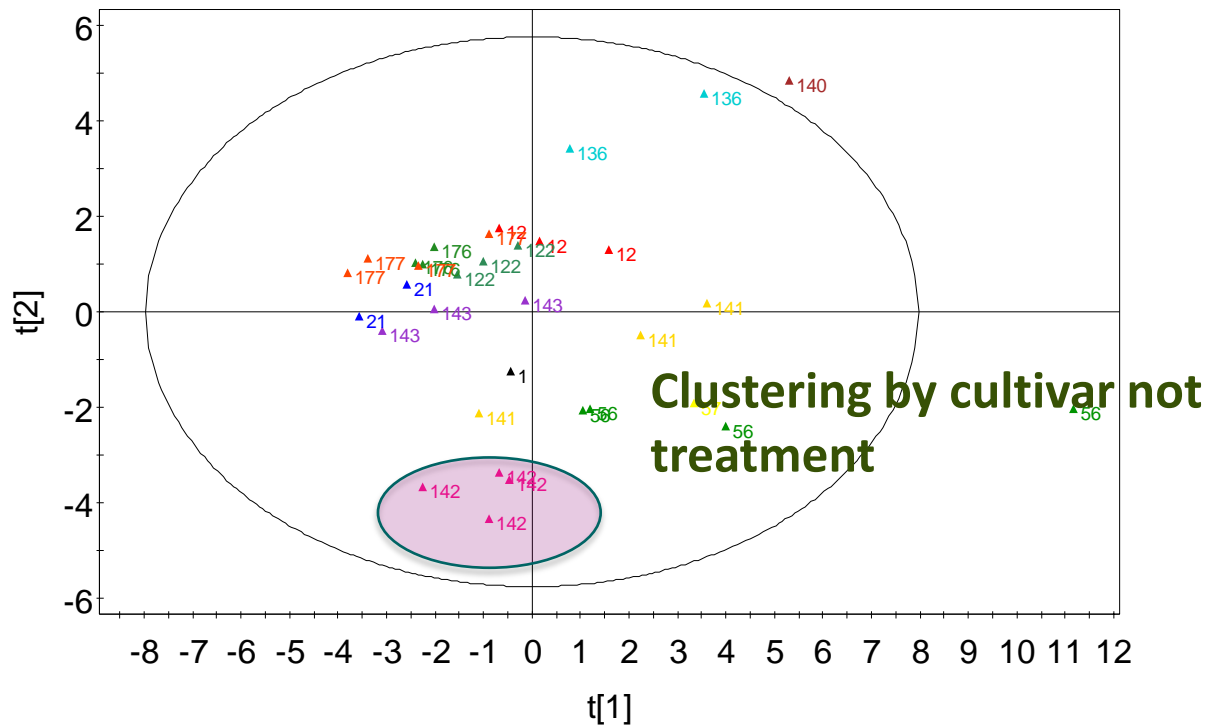

R2X[1] = 0.373865 R2X[2] = 0.1... SIMCA-P+ 12.0.1 - 2012-09-05 09:07:45 (UTC+0)

Targeted analysis Organic vs Conventional mg/100mg DFM

PCA (1 vs 2) Targeted analysis Coloured by cultivar Organic vs Conventional

R2X[1] = 0.373865   R2X[2] = 0.194467
Ellipse: Hotelling T2 (0.95)

SIMCA-P+ 12.0.1 - 2012-08-23 12:36:09 (UTC+0)

Analysis of variance - Organic vs Conventional

- Of the 25 compounds analyses 16 are significantly different between cultivars

compound	Pval	average SED	1	12	21	56	57	122	136	140	141	142	143	176	177
Variate: aureusidin_gluc	<.001	0.001	0.001	0.002	0.001	0.001	0.001	0.003	0.004	0.004	0.002	0.001	0.001	0.002	0.003
Variate: isorham_3_rut	<.001	0.0005	0.0007	0.0028	0.0015	0.0018	0.0008	0.0020	0.0017	0.0028	0.0017	0.0008	0.0011	0.0028	0.0020
Variate: kaemp_3_gala	<.001	0.0003	0.0000	0.0012	0.0004	0.0007	0.0000	0.0009	0.0013	0.0000	0.0008	0.0000	0.0003	0.0007	0.0010
Variate: kamp_3_gluc	<.001	0.0011	0.0019	0.0048	0.0028	0.0024	0.0009	0.0051	0.0049	0.0007	0.0023	0.0050	0.0027	0.0068	0.0069
Variate: myric_3_6_mal_gluc	<.001	0.0017	0.0031	0.0052	0.0030	0.0102	0.0022	0.0041	0.0085	0.0101	0.0110	0.0069	0.0028	0.0037	0.0055
Variate: myric_6_gluc	<.001	0.0072	0.0211	0.0261	0.0203	0.0557	0.0134	0.0301	0.0334	0.0393	0.0468	0.0352	0.0193	0.0182	0.0296
Variate: myric_gluc	<.001	0.0003	0.0003	0.0019	0.0002	0.0026	0.0001	0.0021	0.0009	0.0017	0.0007	0.0001	0.0001	0.0014	0.0009
Variate: pelarg_gluc	<.001	0.0025	0.0106	0.0090	0.0016	0.0175	0.0068	0.0094	0.0069	0.0049	0.0097	0.0131	0.0068	0.0097	0.0112
Variate: pelarg_rut	<.001	0.0101	0.0311	0.0332	0.0093	0.0601	0.0232	0.0353	0.0200	0.0079	0.0321	0.0516	0.0245	0.0210	0.0213
Variate: melv_rut	0.002	0.0091	0.0181	0.0372	0.0216	0.0286	0.0114	0.0387	0.0560	0.0204	0.0312	0.0177	0.0432	0.0191	0.0305
Variate: kaemp3_rut	0.004	0.0006	0.0015	0.0033	0.0014	0.0017	0.0012	0.0014	0.0013	0.0025	0.0019	0.0009	0.0017	0.0028	0.0032
Variate: myric_3_rut	0.006	0.0085	0.0208	0.0304	0.0194	0.0498	0.0188	0.0277	0.0343	0.0360	0.0376	0.0304	0.0159	0.0147	0.0235
Variate: delph_rut	0.014	4.798	9.030	21.080	11.480	22.320	10.950	21.710	22.010	7.010	14.720	18.260	20.350	12.010	11.460
Variate: querc_3_6_malonyl_gluc	0.016	0.0018	0.0041	0.0055	0.0030	0.0063	0.0018	0.0058	0.0061	0.0060	0.0061	0.0088	0.0016	0.0061	0.0045
Variate: petun_rut	0.042	0.0819	0.1593	0.3403	0.1749	0.2685	0.1226	0.3461	0.3856	0.1729	0.2445	0.1760	0.2929	0.1350	0.1913
Variate: cyan_gluc	0.05	0.332	0.657	0.930	0.448	1.708	0.527	0.822	0.842	0.589	0.945	0.952	0.716	1.172	1.043
Variate: cyan_arab	0.051	0.006	0.010	0.025	0.009	0.025	0.011	0.012	0.013	0.006	0.018	0.022	0.016	0.019	0.013
Variate: delph_gluc	0.075	1.174	2.292	3.583	2.175	6.017	2.147	4.132	4.481	2.074	3.148	2.890	3.786	3.471	3.261
Variate: cyan_rut	0.082	2.255	4.966	9.169	4.458	10.808	4.911	7.456	6.936	3.332	7.456	9.491	7.685	7.324	5.874
Variate: querc_3_gluc	0.084	0.0050	0.0100	0.0168	0.0111	0.0225	0.0065	0.0180	0.0203	0.0112	0.0185	0.0217	0.0180	0.0184	0.0168
Variate: querc_3_rut	0.095	0.0052	0.0148	0.0212	0.0103	0.0250	0.0134	0.0126	0.0137	0.0211	0.0242	0.0192	0.0124	0.0160	0.0136
Variate: peonidin_rut	0.278	0.0673	0.1668	0.2875	0.1052	0.1658	0.0704	0.2471	0.2226	0.1581	0.1705	0.1333	0.1951	0.1878	0.1968
Variate: petun_gluc	0.35	0.0175	0.0380	0.0358	0.0287	0.0592	0.0187	0.0484	0.0645	0.0446	0.0416	0.0205	0.0413	0.0284	0.0373
Variate: delph_coum	0.514	0.132	0.288	0.511	0.253	0.482	0.302	0.273	0.372	0.209	0.380	0.296	0.301	0.454	0.330
Variate: peonidin_gala	0.644	0.0048	0.0117	0.0115	0.0062	0.0130	0.0042	0.0117	0.0132	0.0147	0.0111	0.0087	0.0084	0.0123	0.0145

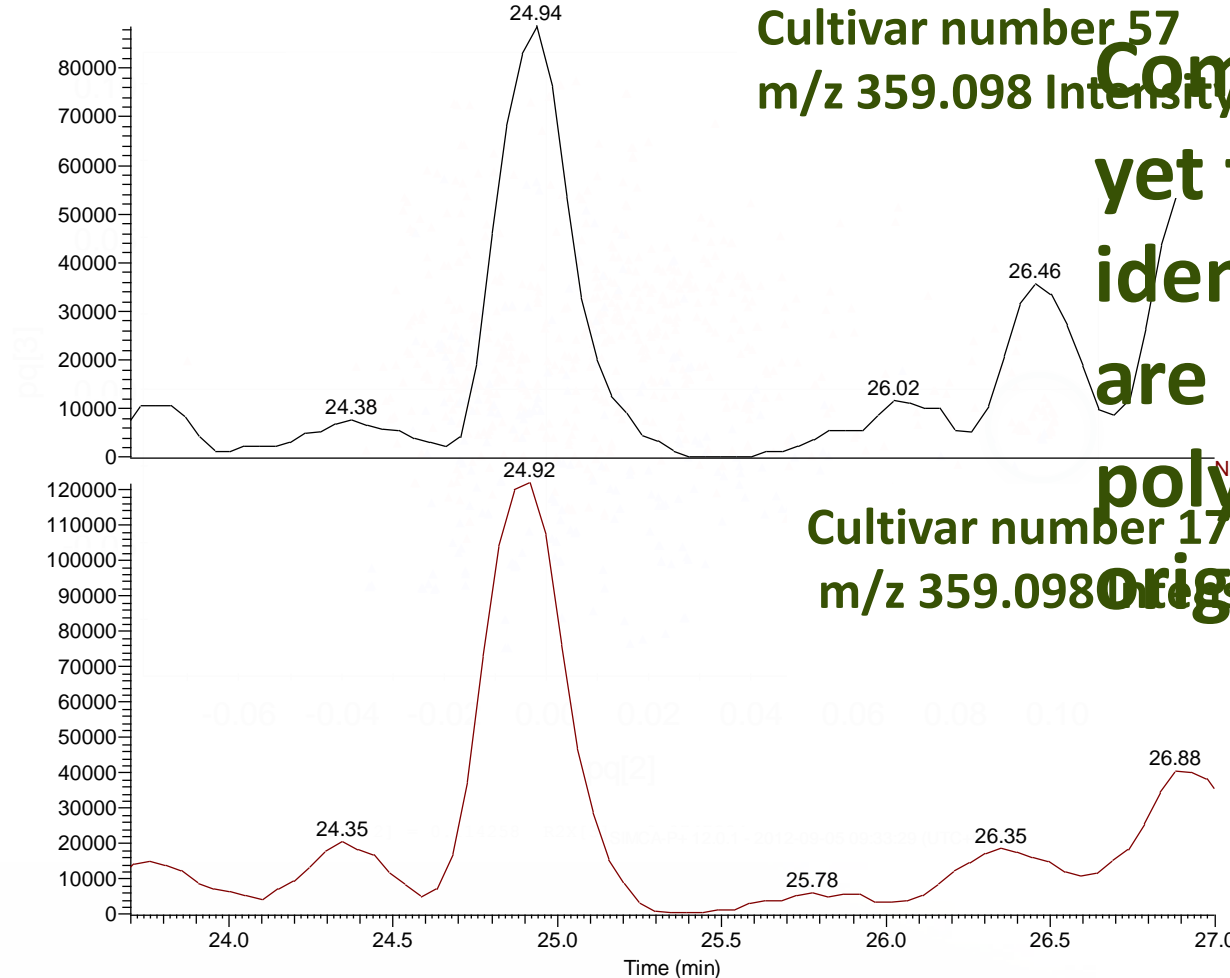# Untargeted analysis PCA 2 vs 3 Organic vs Conventional
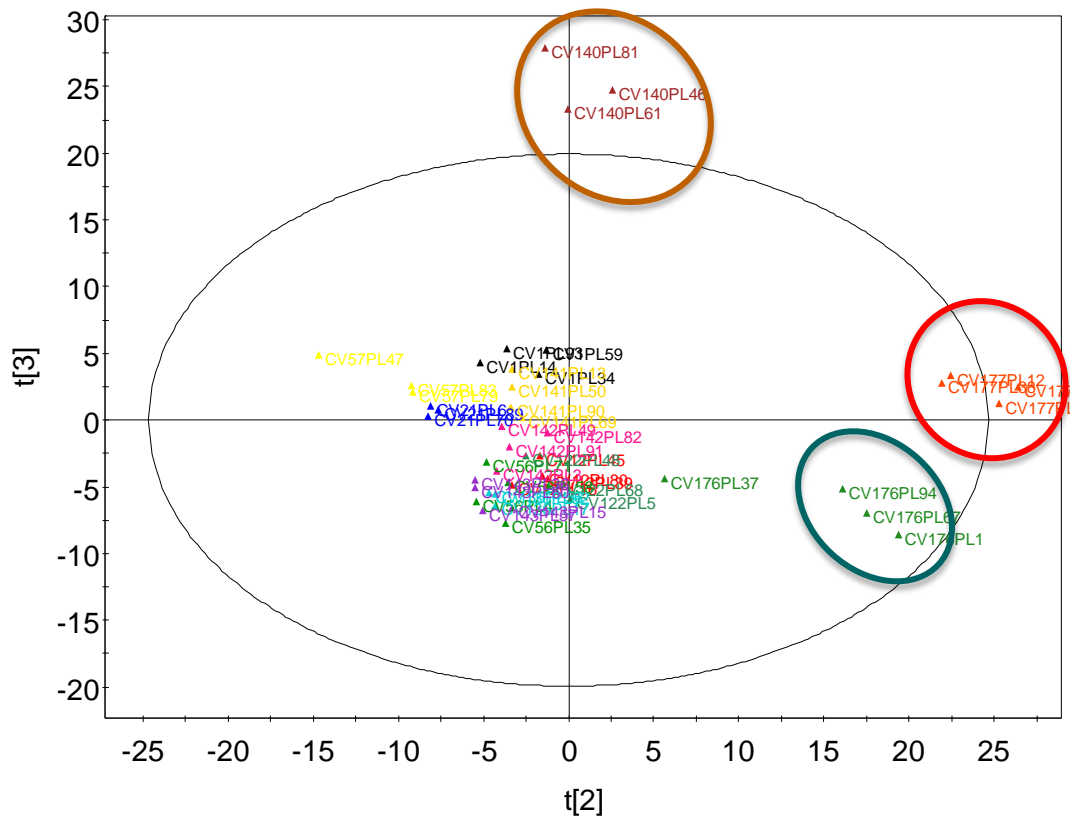
As with targeted analysis we are seeing clustering Untargeted analysis PCA 2 vs 3 due to cultivar

11% variation on score 2
7% Variation on score 3

Loadings scatter plot 2V3 Organic vs Conventional

RT: 23.70 - 27.00   SM: 5B

Cultivar number 57
m/z 359.098 Intensity 85000

Cultivar number 176
m/z 359.098 Intensity 120000

Compounds yet to be identified but are not of polyphenol origin

Var ID (MZ)	Var ID (Time)
331.067	9.211
315.072	10.495
361.077	10.495
315.072	11.756
300.08	12.033
299.077	12.033
153.019	17.278
375.092	17.889
376.096	17.889
705.187	17.906
359.098	18.356
707.135	18.833
461.129	18.886
329.087	19.181
311.113	20.87
355.103	20.87
371.098	22.101
415.088	22.101
417.103	22.344
832.187	22.38
831.182	22.397
863.187	22.833
399.092	22.957
800.195	22.957
323.077	22.967
799.192	22.967
372.101	23.316
371.098	23.388
415.088	23.388
137.024	24.912
589.192	30.332
418.135	23.186
423.09	23.214
434.13	23.613
439.085	23.615
417.103	23.615

Time (min)

Untargeted analysis PCA 3 vs 4 Organic vs Conventional
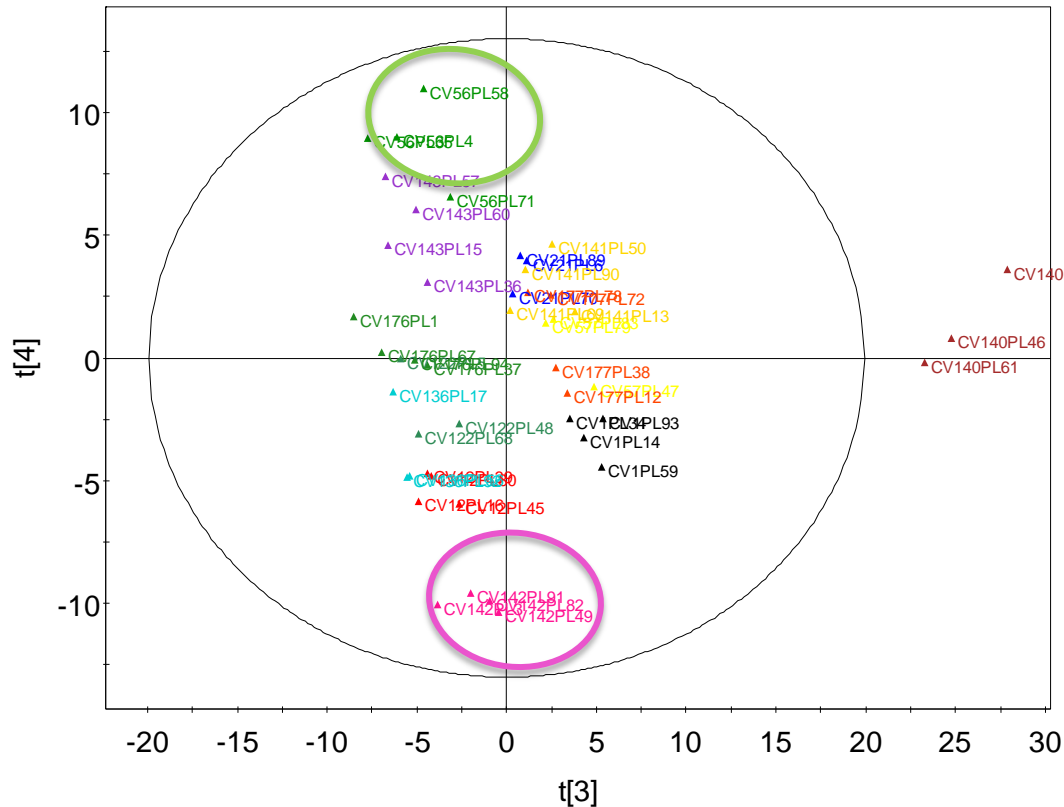
As with PC 2 vs 3 clustering by cultivar is evident

(7.5% variation on score 3)

Loadings scatter plot score 3 vs 4 Organic vs Conventional

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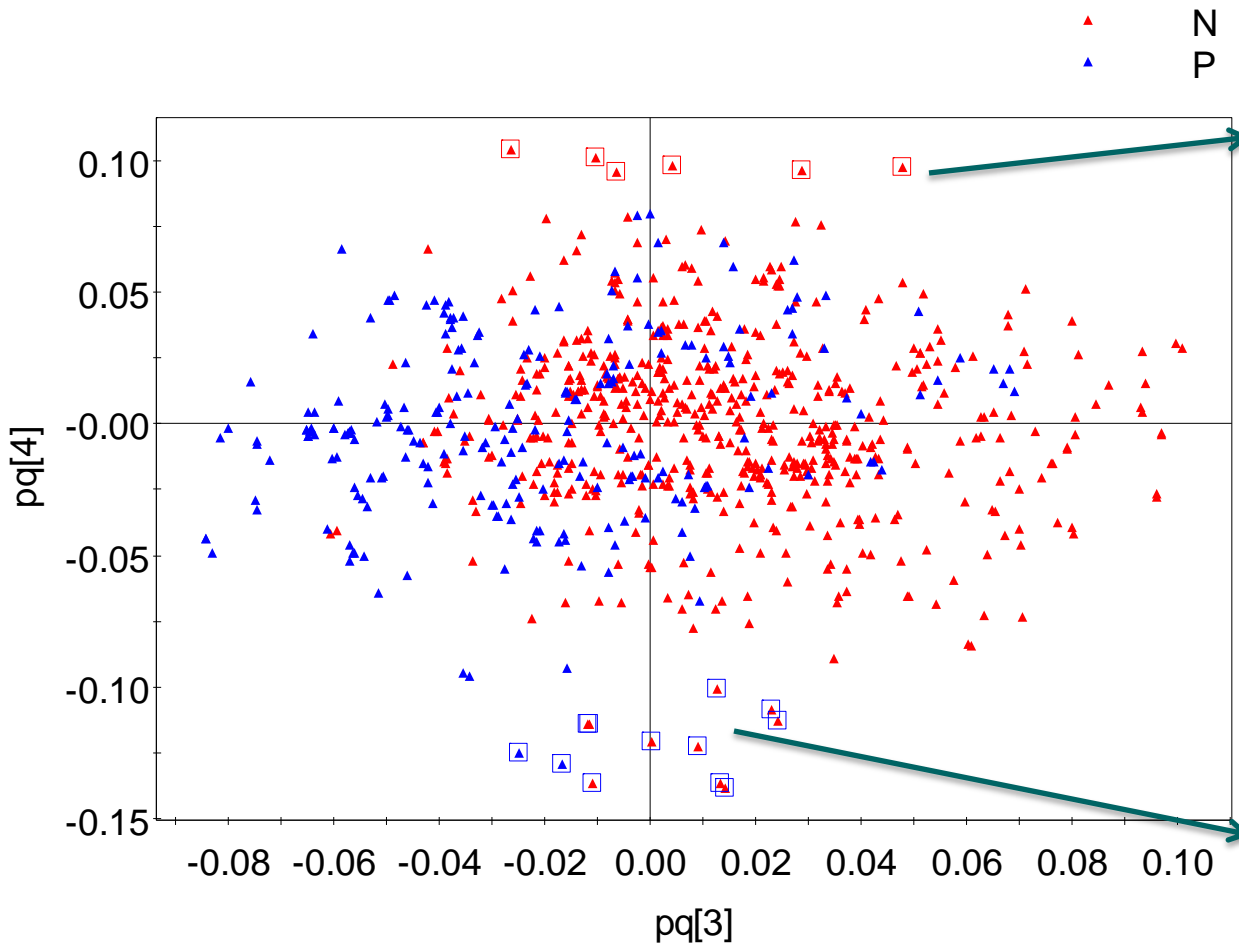
Var ID (MZ)	Var ID (Time)
304.103	9.796
436.124	10.904
436.145	11.115
306.119	12.037
138.031	12.621
543.28	31.402

m/z 304 Delphinidin

Others not known phenolic In blackcurrant

Var ID (MZ)	Var ID (Time)
305.066	12.239
351.072	12.239
337.092	12.332
643.165	12.332
675.192	12.362
338.095	12.362
965.255	12.438
627.155	12.814
628.158	12.814
481.134	25.789
339.108	12.986
147.044	12.986

N
P

pq[4]

pq[3]

R2X[3] = 0.0747334 R2X[4]

Experiment 1
# Comments & Conclusions

- Harvest date has a major effect on polyphenol content

- Samples segregating by cultivar and **<u>NOT</u>** treatment

- Metabolites not from known polyphenol origin causing segregation: are these important?

Experiment 2 – Organic vs Conventional

- Blackcurrant plants

- 2 field sites

- 2 Planting dates

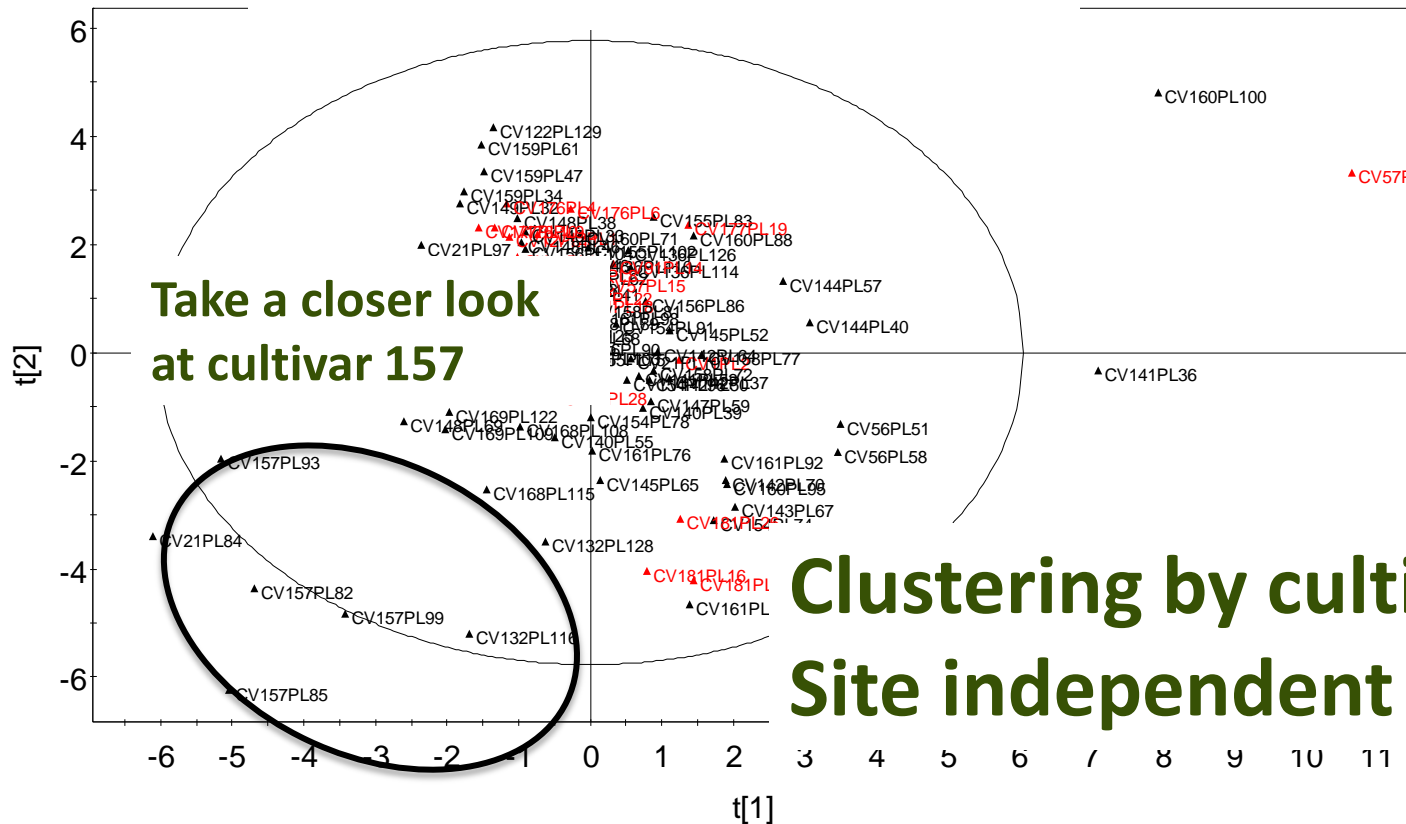# Varietal trial – Targeted Data
PCA plot 1 vs 2; coloured by cultivar
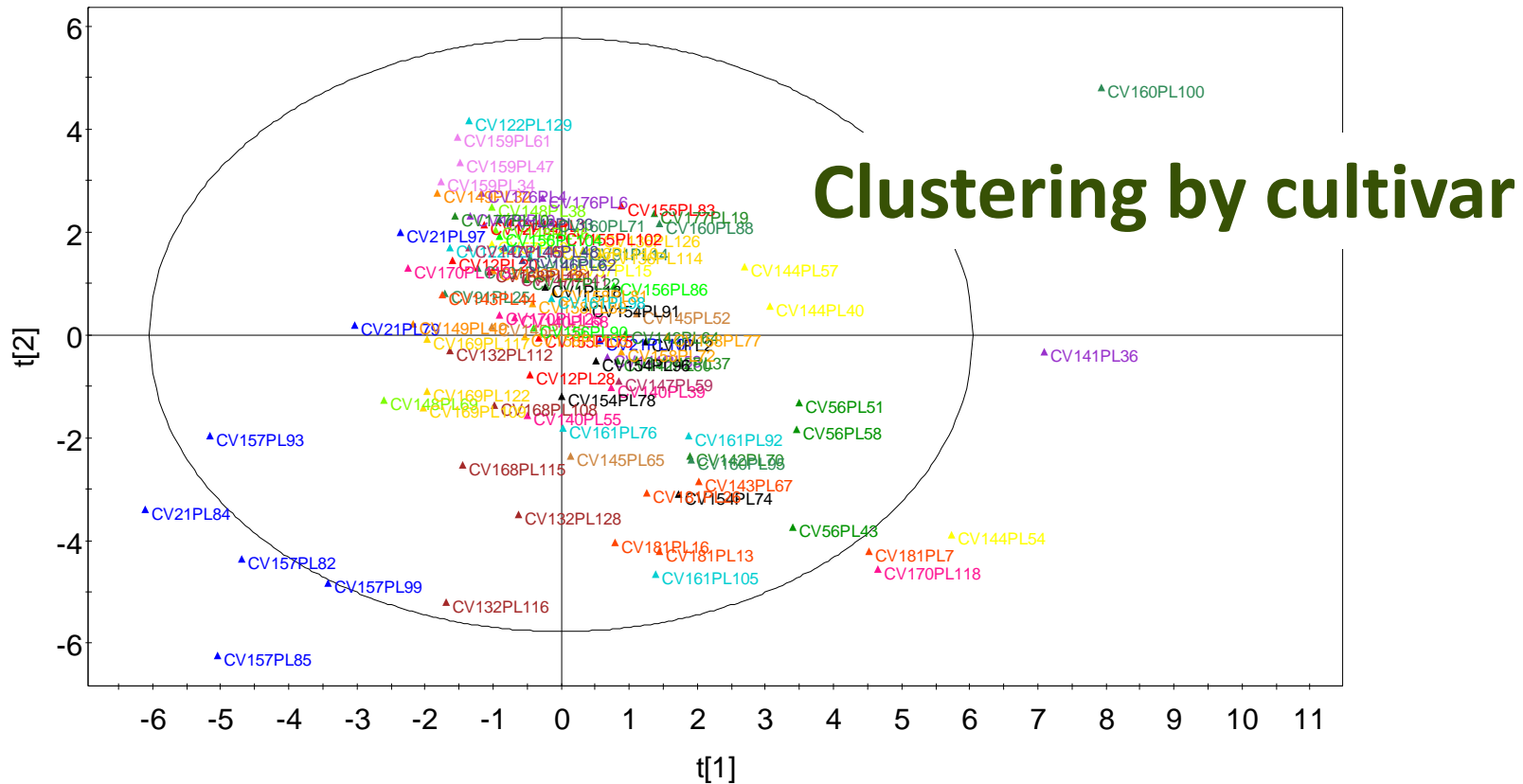
Clustering by cultivar

R2X[1] = 0.226145   R2X[2] = 0.205704
Ellipse: Hotelling T2 (0.95)

SIMCA-P+ 12.0.1 - 2012-09-05 09:52:01 (UTC+0)

Varietal trial – Targeted Data
PCA plot 1 vs 2: coloured by location

Take a closer look
at cultivar 157

Clustering by cultivar
Site independent

R2X[1] = 0.226145   R2X[2] = 0.205704
Ellipse: Hotelling T2 (0.95)

SIMCA-P+ 12.0.1 - 2012-09-05 09:56:54 (UTC+0)

Varietal trial – Loadings scatter score1 vs 2 Coloured pos/neg
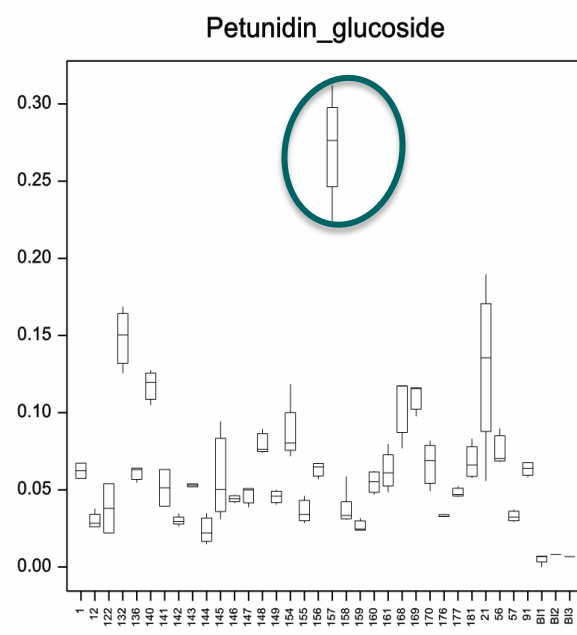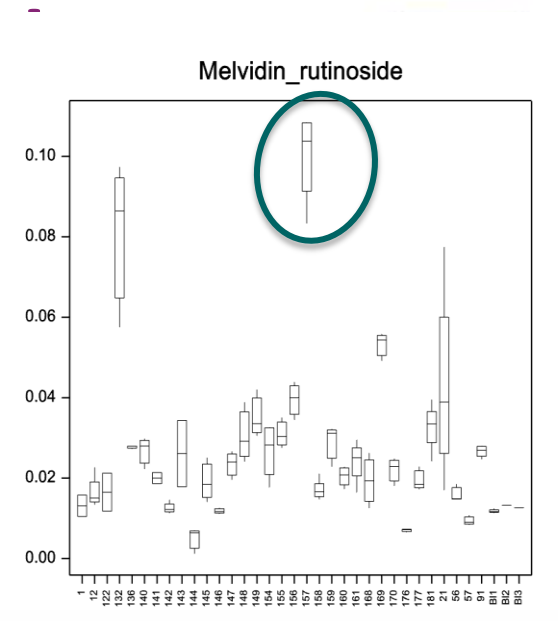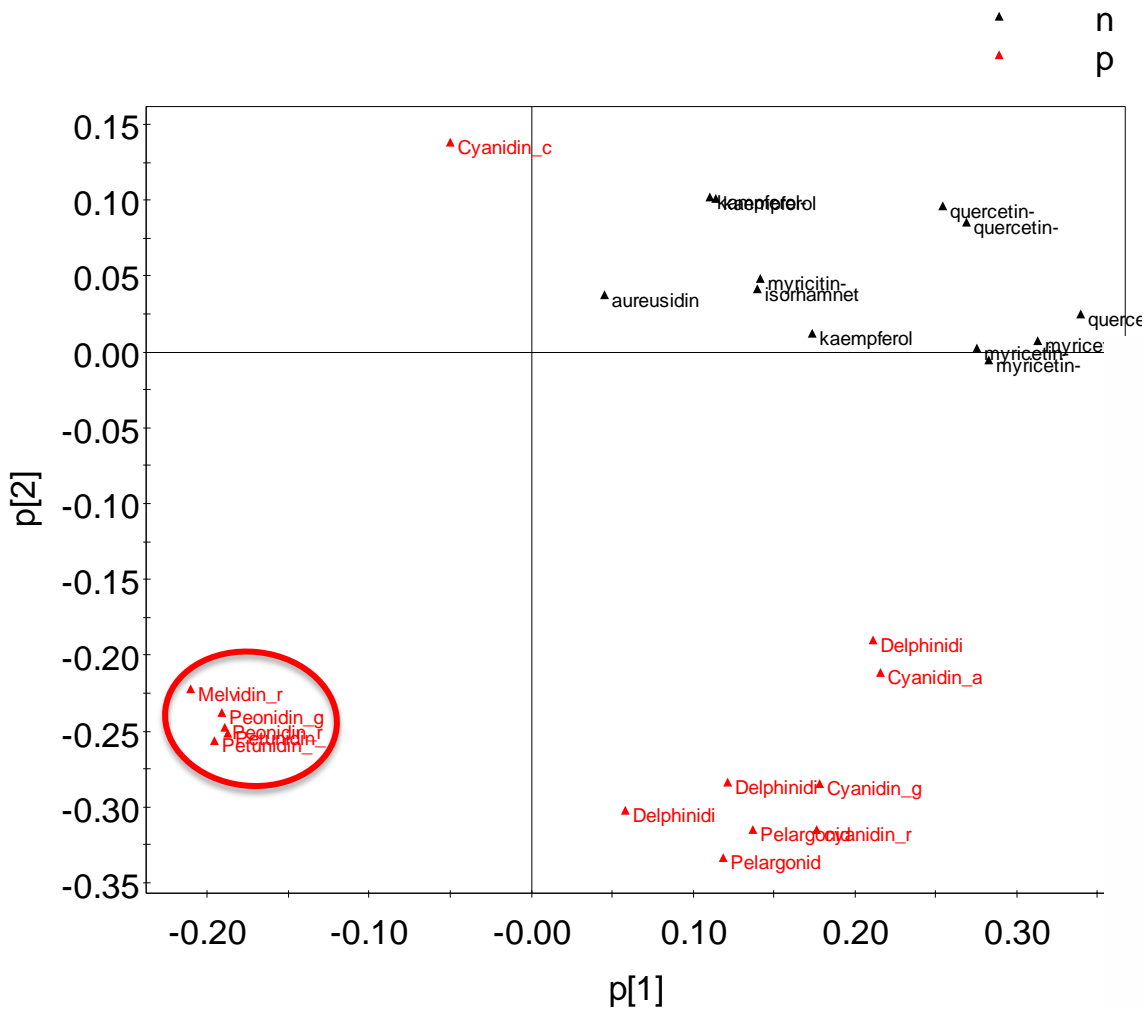
Varietal Trial - Untargeted Analysis PCA 3 vs 4 Coloured by cultivar

Indication of clustering by cultivar e.g. 176 & 177
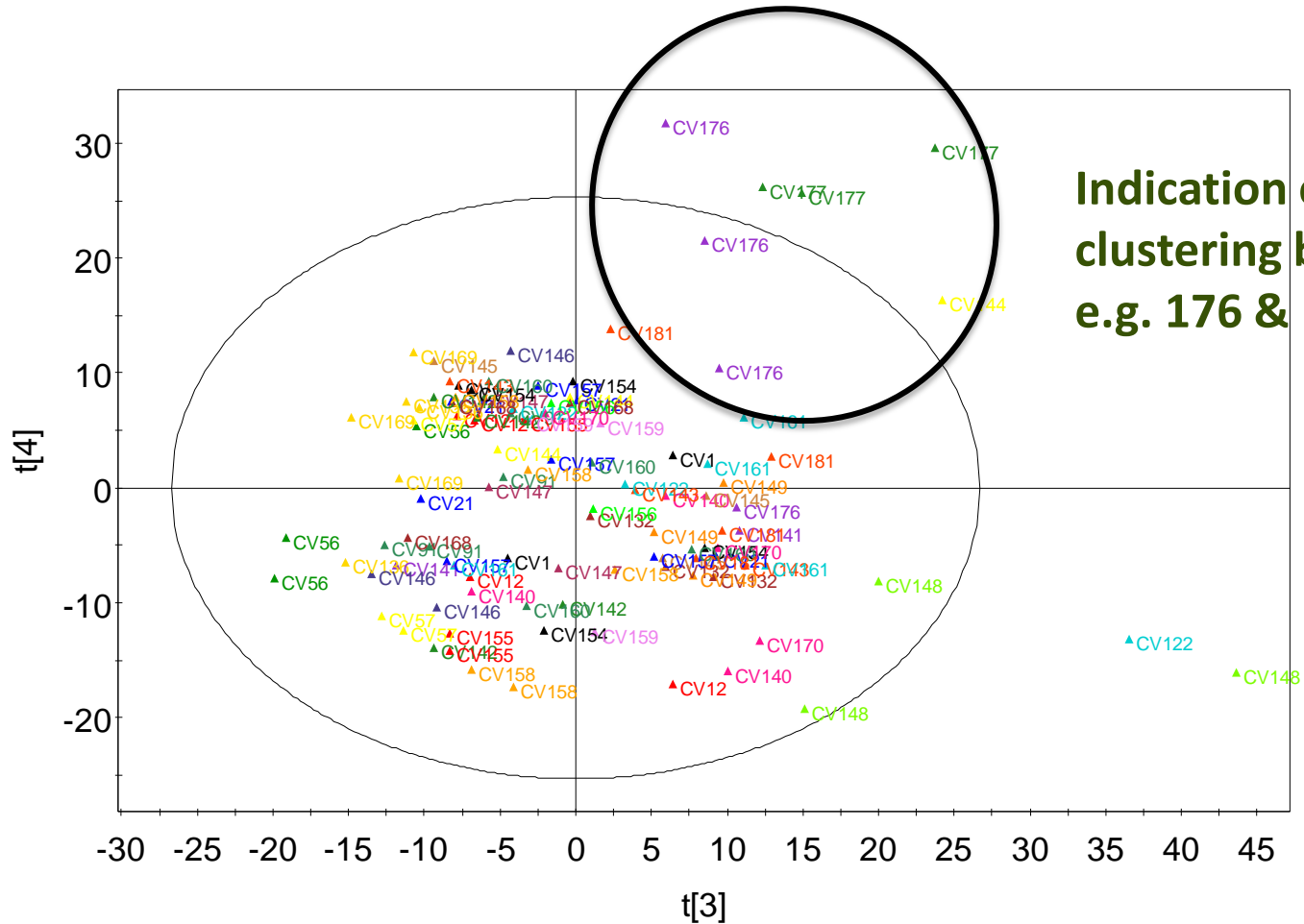
R2X[3] = 0.0653126   R2X[4] = 0.0586434
Ellipse: Hotelling T2 (0.95)

SIMCA-P+ 12.0.1 - 2012-09-05 10:06:11 (UTC+0)

Varietal trial – Loadings scatter plot score 3v4 Coloured to pos neg

Metabolites not polyphenols

Var ID (MZ)	Var ID (RT)
423.086	23.42
439.081	23.85
418.131	23.434
434.125	23.85
337.086	18.413
153.053	18.497
220.029	23.42
353.081	19.52
434.125	22.56
417.099	23.811
485.122	19.414
439.08	22.56
323.071	10.793
480.167	19.414
348.126	20.984
823.183	23.42
228.026	23.85
231.048	23.811
401.104	23.42
424.089	23.42
417.099	22.56
153.053	23.404
855.173	23.85
497.109	23.404
818.228	23.42
348.126	19.534
440.084	23.85
205.54	19.52
420.077	23.42
850.218	23.811
651.184	18.413
436.072	23.85
443.187	21.485
127.038	23.811
284.168	9.233
231.048	22.56
363.137	12.444
833.191	23.811
338.09	18.413
623.153	10.793
479.099	23.42
350.072	18.109
457.236	22.698

R2X[3] = 0.0653126 R2X[4] = 0.0596126

SIMCA-P+ 12.0.1 - 2012-09-05 10:15:16 (UTC+0)
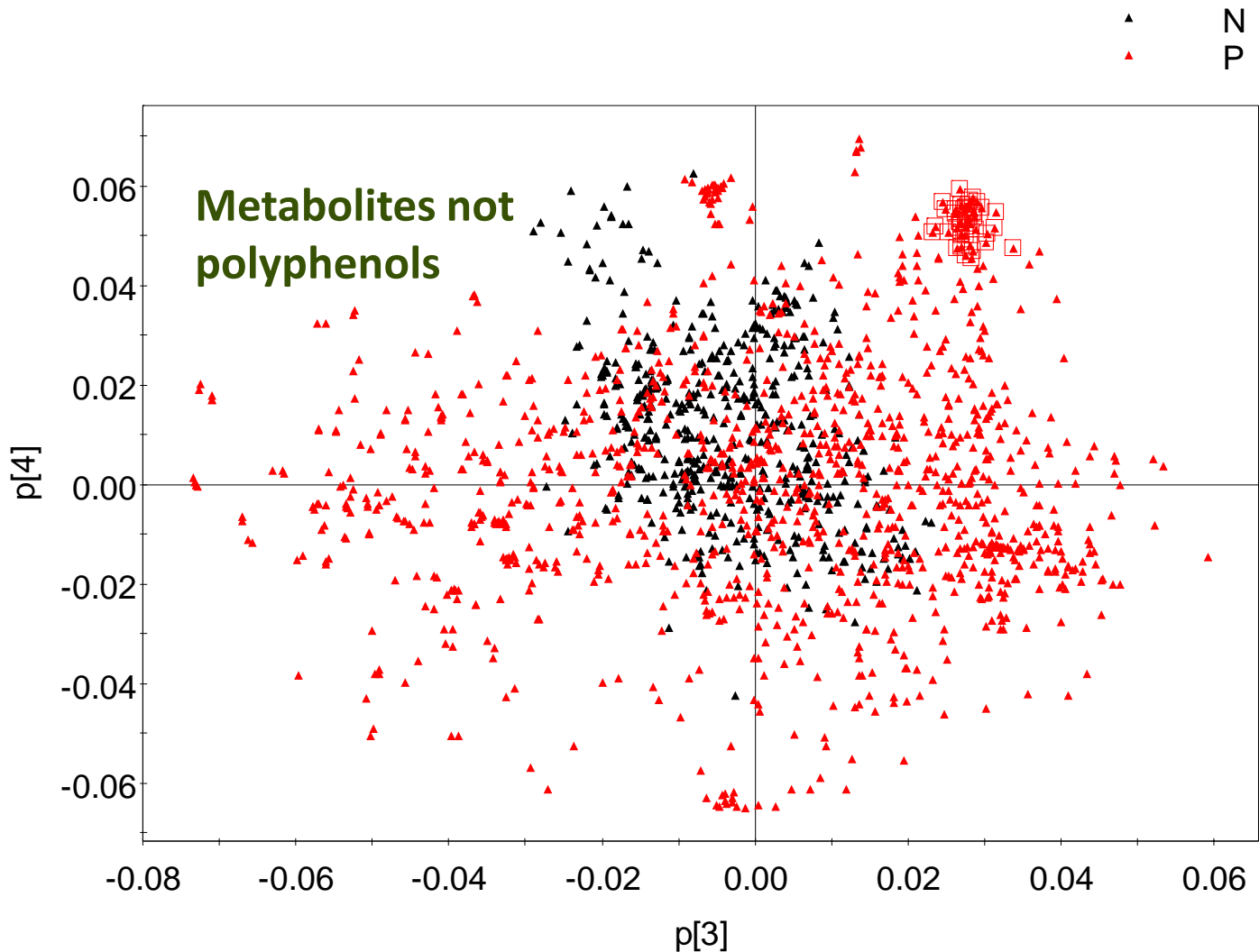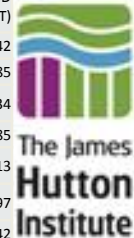
Organic and Traditional confirmation cross comparison cultivar 176

Traditional		Organic	
MZ	**RT**	**MZ**	**RT**
423.086	23.42	423.09	23.214
417.099	22.56	417.103	22.344
434.125	23.85	434.13	23.613
439.081	23.85	439.085	23.615

Cyanidin_arabinoside_TRAD

Pelargonidin_glucoside_TRAD
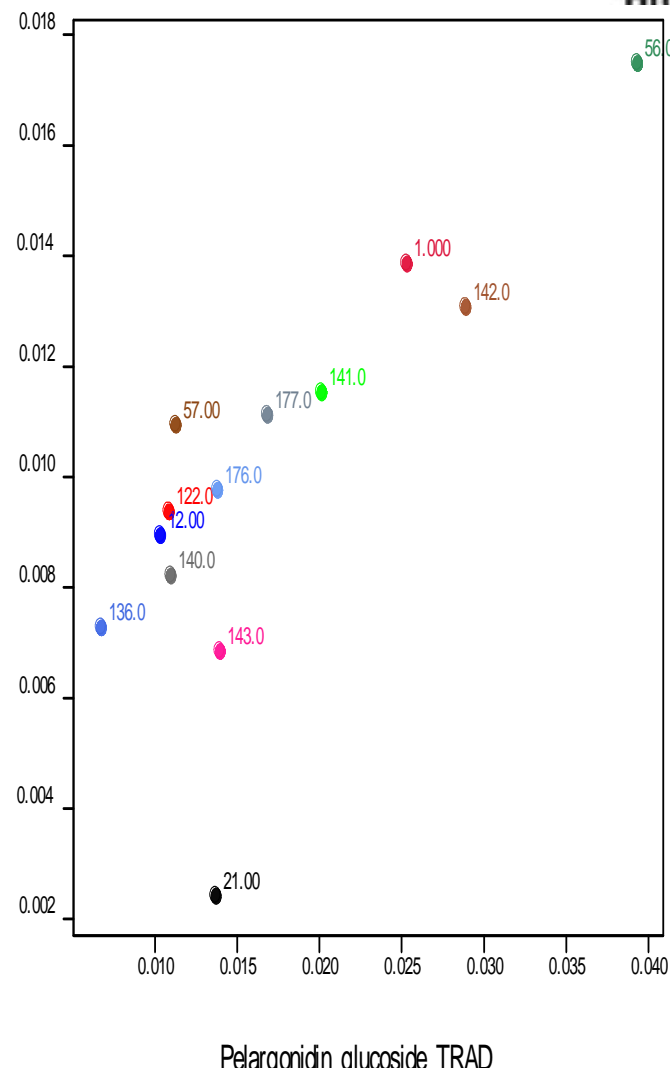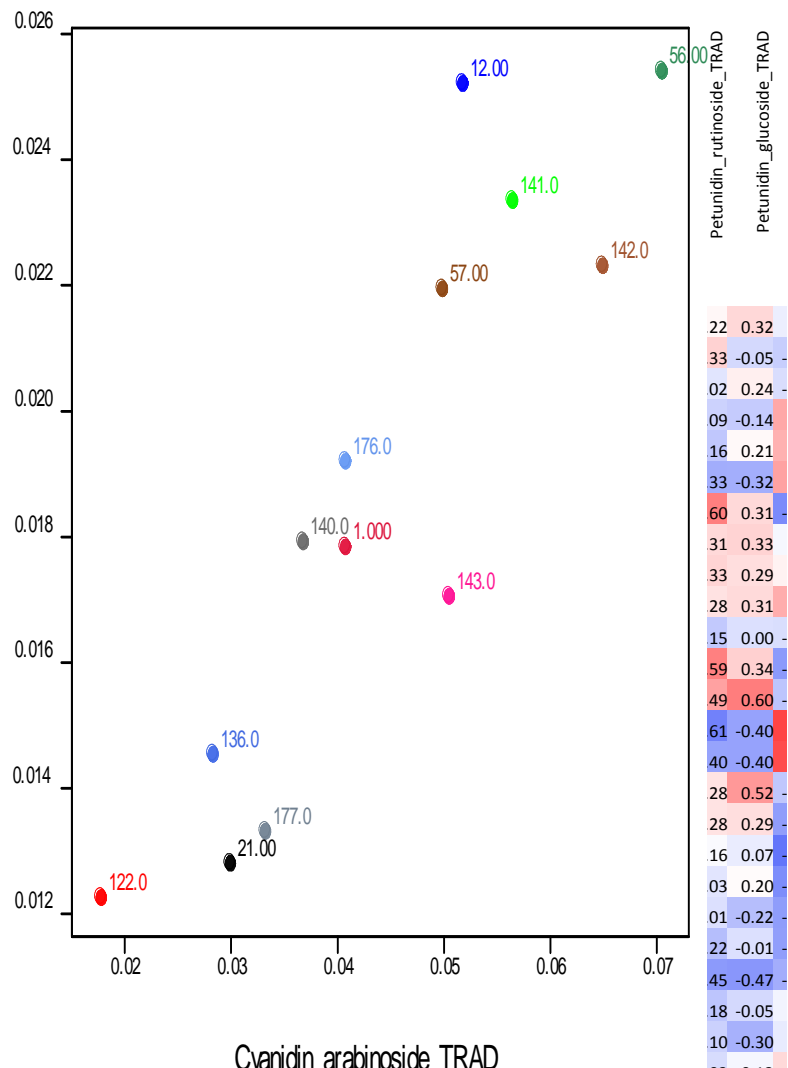
Experiment 2
Comments & Conclusions

- Samples clustered by cultivar and not origin

- Good correlation between cultivars grown for the organic and traditional experiments