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Effect of Agricultural Production Systems on the Potato Metabolome

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Abstract

Potato (*Solanum tuberosum* L.) cv. Santé was grown over two years under both conventional and organic fertiliser and crop protection regimes. The tuber metabolome was analysed using mass-spectrometry (MS) based approaches, principally Liquid Chromatography (LC)-MS and Gas Chromatography (GC)-MS. Data were analysed using Principal Components Analysis (PCA) and ANalysis Of VAriance (ANOVA) to assess any differences between production practices. GC-MS analysis of non-polar metabolites did not detect any statistically significant differences, but GC-MS analysis of polar compounds identified 83 metabolites showing significant differences in the metabolome between the fertiliser treatments. Of the 62 metabolites that were less abundant in tuber samples from organic compared with conventionally fertilised crops, consistent year on year differences were dominated by free amino acids. The effect on free amino acids is associated with the lower nitrogen (N) content of the organically grown potatoes in this instance (50% lower than for conventional production). LC-MS provided indications that levels of certain glycoalkaloids may be lower under the organic fertiliser regime in one growing season. Differences associated with the crop protection measures used were much less consistent, and relatively small, compared with the fertiliser effects found.

Keywords: conventional cultivation, metabolomics, organic cultivation, potato, QLIF, *Solanum tuberosum*

1 **1 Introduction**

2 The links between nutrition and health are well known, and there is an increasing demand for foods with enhanced
3 nutritional qualities, both in the developed and developing worlds (Nishida et al., 2004). Factors that drive variation in
4 crop composition are therefore important nutritionally, but will also impact on other aspects of quality demanded by the
5 fresh and processed market sectors. Variations in crop composition can be driven by genetic background, variety choice
6 and environmental variables, but also by crop management practices, and interactions between all of these factors add
7 further complexity (Davies et al., 2010).

8 Concerns over food security and the need for the development of low input, more sustainable agricultural practices,
9 have increased in recent years. This has been driven by the realisation that natural resources are finite, and that the costs
10 of food have increased substantially due to increasing costs for fuel and fertilisers (FAO, 2009, 2010, 2011; Foresight,
11 2011). Whilst the development of sustainable production systems is not only about organic production *per se*,
12 significant shifts in production practices and climate will be likely to impact on crop performance and composition, and
13 hence on the food chain and diet.

14 The development of broad spectrum, non-targeted, analytical metabolite profiling approaches has offered up the
15 possibility to assess more fully the extent of changes in metabolite pools resulting from differences in plant genetic
16 background, growing environment and production practices. Indeed, profiling approaches have significant potential in
17 plant breeding when integrated with other high-throughput genomic and gene expression technologies (Ferne and
18 Schauer, 2009).

19 With regard to genetic background, Skogerson et al. (2010) demonstrated using metabolite profiling that the small
20 molecule metabolite pool in maize kernels is highly dependent on genotypic variation, and that levels of certain
21 metabolite classes may have an inverse genotypic relationship to each other. Similarly, Röhlig and Engel (2010)
22 observed that differences in kernel composition were mainly due to genetic and environmental influences, but also
23 observed that different input systems used (conventional vs. organic) led to minor differentiations. The importance of
24 environmental factors (i.e. growing locations and seasons) was re-affirmed in a more recent maize study (Frank et al.,
25 2012).

26 With specific regard to the general composition of conventional vs. organically produced crops, a study
27 commissioned by the UK Food Standards Agency (Dangour et al., 2009) assessed 137 published crop studies and
28 concluded that some significant differences can occur in nitrogen (N; higher in conventional crops), magnesium and zinc
29 (higher in organic crops), phytochemicals such as phenolic compounds and flavonoids (higher in organic crops), and

sugars (higher in organic crops). Thus, some compositional differences can be expected under different production practices.

There are several studies comparing metabolite levels in conventional vs. organically produced potato using targeted analysis methods but, as far as we are aware, no such studies using metabolite profiling. Hajšlová et al. (2005) indicated that higher vitamin C (see also Woese et al., 1997) and chlorogenic acid tuber contents most consistently differentiated organically from conventionally produced potatoes. Elevated concentrations of glycoalkaloids were also observed in some potato varieties grown in organic farming systems (see also Wszelaki et al., 2005). Amrein et al. (2003) reported no differences between organic and conventional production with regard to the tuber concentrations of free sugars and asparagine (acrylamide precursors). However, interpretation of data may be confounded depending on whether the values for metabolite concentrations are expressed on a dry or fresh weight basis, as it has been previously reported that organically grown crops may have higher dry matter content vs. conventional (Järvan and Edesi, 2009; Lombardo et al., 2011).

In the European integrated research project SAFEFOODS (<http://www.safefoods.nl>), one of the main objectives was to develop, using modern profiling techniques, comparative safety assessment methods for foods (in this case potato), produced by different breeding approaches and production practices and under different environmental conditions. The work presented in this manuscript on metabolomics complements previously published proteomic (Lehesranta et al., 2007) and transcriptomic (van Dijk et al., 2009, 2012) studies that also used the field-derived potato material from the European project QualityLowInputFood (QLIF; www.qlif.org) discussed here.

2 Material and Methods

2.1 Field Experiment Design

The experiment presented here was carried out within the Nafferton Factorial Systems Comparison (NFSC) trial at the University of Newcastle's Nafferton Experimental Farm, Northumberland, UK (54:59:09 N; 1: 43:56 W). Experiments were established in 2001 in a field with a uniform sandy loam soil (alluvial deposit) of the Stagnogley type, with a mean organic matter content of 3.3%. Each experiment had four blocks and was laid out as a split-split plot design with rotation design as the main plot treatment, crop protection (pesticide) as the sub-plot treatment and fertility management (fertiliser) as the sub-sub-plot treatment (see Cooper et al., 2011, for a detailed description of the experimental design). The treatment plots were 6 x 24 m in size.

The potato samples used for the majority of metabolomic analyses were from crops grown in the NFSC trial in 2005 (year 1) and were established after winter wheat. Potato seed tubers of the variety Santé were planted in ridges (distance between rows: 90 cm, distance between seed within the row: 35 cm) using a semi-automatic two-row potato planter (Reekie, Forfar, Scotland, UK). At the end of the growing season, crops were defoliated and tubers left in the ground for three weeks to allow skin maturation and then harvested using a single row potato harvester (Ransomes, Ipswich, UK). The potato crops for all treatments were planted on 2 May 2005, and harvested on 13 September 2005.

Potato samples were also taken from the NFSC trial grown in 2006 (year 2). This has a similar design to that above, but the plants had two different pre-crops: winter barley and spring beans.

2.2 Fertilisation Treatments

The two fertilisation treatments were (a) composted cattle manure, equivalent to 170 kg N/ha (standard fertilisation regime used for commercial organic potato crops) or (b) ammonium nitrate (Nitram), equivalent to 180 kg N/ha and superphosphate and KCl as a compound (N:P:K = 0:20:30; fertiliser equivalent to 134 kg P/ha and 200 kg K/ha), which is the standard fertilisation regime for commercial conventional crops.

2.3 Crop Protection (Pesticide) and Defoliation

Two crop protection (pesticide) regimes typically used in organic and conventional production systems in the North East of England were compared. The organic crop protection regime consisted of two passes with inter-row cultivators/ridgers through potato rows (for weed control and to ensure tubers were well covered with soil to prevent greening), five foliar sprays of copper oxychloride (product: Headland Copper) at a total rate of 6 kg Cu/ha (for control of late blight caused by *Phytophthora infestans*), and mechanical removal of foliage by flailing to minimise the risk of tuber blight infection and encourage skin set in potato tubers. No soil treatments were used. The conventional crop protection consisted of a pre-planting soil application of aldicarb granules (67 kg/ha) (for soil pest control including nematodes), one spray of linuron (3.5 L/ha) (for weed control), four foliar sprays of fluazinam (2.85 L/ha; for late blight control) and one foliar spray of the herbicide diquat (4 L/ha) for desiccation/removal of foliage to minimise tuber blight risk and encourage skin set in potato tubers.

2.4 Yield and Disease Assessment

Potato yields were assessed by harvesting the four middle rows of each plot. Fresh weights were determined by weighing tubers harvested in each plot immediately after harvesting. Dry weights were determined by drying a subsample of harvested tubers at 80°C for two days using a drying oven (Genlab Ltd, Widnes, UK). Plots were examined for visible symptoms of foliar blight at two weekly intervals and other foliar diseases, but in 2005 no symptoms of foliar blight and/or other diseases could be detected.

2.5 Tuber Sampling Strategy

After harvest (post-senescence; Section 2.1), tubers were transported from University of Newcastle to the James Hutton Institute, where the tubers were stored at ambient (*ca.* 10°C) for two weeks in the dark to facilitate skin set. Thereafter, for each treatment and replicate, one bulk sample, comprising the same number of similarly-sized tubers (4-6 tubers) to a combined fresh weight of *ca.* 800 g, was prepared. For each set of tubers, and to minimise the impact of metabolite gradients within these large tubers, two opposite eighths were removed from each tuber, combined, chopped into 1 cm cubes and frozen in liquid N₂ prior to freeze-drying for one week. The freeze-dried samples were milled using a Retsch mill (Tecator Udy) with a 1 mm sieve. Freeze-dried potato powders were stored in re-sealable bags at -80°C (in the dark) until required for analysis. Storage in this way has no impact on metabolite balance (as revealed by metabolomics) for at least 12 months (unpublished data).

2.6 Metabolomics Analysis

For the 2005 trial freeze-dried potato tuber powder was extracted, analysed by LC-MS and GC-MS (polar and non-polar), raw data analysed and quality checked as previously described (Shepherd et al., 2010). For the 2006 trial only polar metabolites (GC-MS) were analysed.

For the 2005 trial, LC-MS involved the analysis of one technical replicate, and GC-MS involved the analysis of two technical replicates per sample. In 2006 only one technical replicate was extracted and analysed per sample with GC-MS (given positive experiences gained in GC-MS analytical reproducibility). For all technologies and years, the values obtained are expressed on the natural scale in units of response ratio to internal standard (LC-MS – reserpine; GC-MS polar – ribitol; GC-MS non-polar – methyl nonadecanoate).

2.7 Metabolite Identification

In accordance with the guidelines of the metabolomics standards initiative (MSI; Sumner et al., 2007), all but four

metabolites (allantoin, trihydroxypentanoic acid, galactinol [GC-MS polar] and solasonine [LC-MS]) were identified on the basis of two independent data i.e. comparison of retention time and mass spectrum to an authentic standard analysed under identical conditions to samples. Annotations of putative compounds for both technologies are described in Table SI.

In LC-MS, unknown compounds were annotated using a U-prefix, followed with a combination of both the prominent mass ion and retention time. For example, an unknown metabolite with a mass of 210.060 and a retention time of 3.00 minutes would be named U0210060_0300P (where P denotes positive mode). In GC-MS polar analysis unknown compounds were annotated using a U-prefix, followed by the relative retention index (RR_i). Some unknown compounds had the RR_i prefixed with USA (unknown sugar acid) or UP (unknown polysaccharide), where the compound class was known (Table SI).

In Table SI, RR_i values quoted in the Golm Metabolome Database (GMD; <http://gmd.mpimp-golm.mpg.de>) are for analytes that have been separated using an instrumental method designated as VAR5, in which a series of n-alkanes (C_{10} , C_{12} , C_{15} , C_{18} , C_{19} , C_{20} , C_{28} , C_{32} , C_{36}) were used for calculation of RR_i values. The VAR5 method uses a 5% phenyl-95% dimethylpolysiloxane GC column (Restek Rtx-5), similar (but not identical) to the DB-5MS column (phenyl arylene, equivalent to (5%-phenyl)-methylpolysiloxane)) used in our laboratories. These differences in the column chemistries, and the use of a different range of alkane RR_i markers (C_{11} , C_{13} , C_{16} , C_{20} , C_{24} , C_{30} , C_{34} , C_{38}) in our analyses results in the Golm RR_i values being mostly slightly lower (range -26 to +7 units) than those found with the DB-5MS column used in this study.

2.8 Statistical Analyses

Two statistical analyses were carried out for each technology. In the first, PCA was used to summarise broad scale variation among the samples using all the metabolites. For metabolomics data, PCA can be carried out using either the sample variance-covariance matrix or the sample correlation matrix: the former focuses on the most abundant metabolites, whereas the latter standardises each metabolite by subtracting the mean and dividing by the standard deviation. Both approaches were investigated here to see which was the most informative. PCA was carried out on five sets of data: the LC-MS data from 2005, the GC-MS polar data from 2005, the GC-MS non-polar data from 2005, the GC-MS polar data from 2006 and the GC-MS polar data from 2005 and 2006 together.

The second approach involved an ANOVA of each individual metabolite to identify those with significant differences between the fertiliser treatments, the pesticide treatments or interactions between these. These were carried

out using $\log_e$ -transformed data to normalise the distribution of the residuals. When the metabolomics analysis used more than one sub-sample from each field subplot (i.e. technical replicates) these were included as a separate stratum in the ANOVA. For the data from 2006, differences among the pre-crops and interactions between the pre-crops and the other treatments were also included in the analysis. Metabolites were selected according to their p -values from the ANOVA F-test, taking into account the estimated False Discovery Rate (FDR). If the Benjamini-Hochberg (BH) FDR procedure (Benjamini and Hochberg, 1995) did not reject the null hypothesis of no effect of a treatment at significance level $\alpha = 0.05$ for any metabolites from a technology, this is reported as no significant effects. Otherwise the metabolites with $p < 0.05$ are reported, along with an estimate of the FDR. This was obtained by modelling the p -values as a mixture of a uniform and a gamma distribution (Allison et al., 2002), as implemented in the GenStat procedure FDRMIXTURE.

Finally, a mixed model was used for a joint analysis of the GC-MS polar metabolites over 2005 and 2006, again using $\log_e$ -transformed data. For the 2005 data the mean over the two technical replicates of each field sample was used so that the experimental unit was comparable with the data from 2006. The fixed effects in the model were fertiliser, pesticide and pre-crop and their interactions, and the random effects were field plot within block within year. It was of most interest to identify metabolites with a consistently significant effect over the two years i.e. where interactions involving year are not significant.

All statistical analyses were performed using GenStat for Windows, 13th Edition (VSN International Ltd., Hemel Hempstead, UK).

3 Results and Discussion

3.1 PCA: Separation of Organic and Conventionally Grown Potatoes

3.1.1 LC-MS Analysis (2005 trial)

In the samples analysed in this study, a total of 70 metabolite peaks were detected. In a PCA based on the sample correlation matrix, the first principal component (PC1) explained 38.8% and the second principal component (PC2) explained 18.6% of the total variation. These separated the conventional from the organic fertiliser treatments (Figure 1a).

INSERT FIGURE 1a-1d here.

3.1.2 GC-MS Analysis

For tubers analysed from the 2005 trial experiment, a total of 143 metabolites were detected (91 polar and 52 non-polar). For the 2006 trial, 74 polar metabolites were detected. A total of 93 different polar metabolites were detected when data for both years were combined.

For the polar compounds in 2005, PC1, PC2 and PC3 of the sample correlation matrix accounted for 29.0%, 15.7% and 8.5% of the total variation, respectively. PC1 separated the conventional from the organic fertiliser treatments (Figure Ib), while PC3 partially separated the conventional from the organic pesticide treatments (Figure Ic). The first five principal components of the non-polar data, which explained 59.7%, 11.0%, 8.9%, 4.5% and 2.8% of the total variation respectively, showed no separation of any conventional or organic treatment.

The GC-MS polar data from 2006 showed similar patterns to 2005 for PC1, which explained 33.7% of the variation, and separated the conventional from the organic fertiliser treatment. PC4, which explained 8.1% of the variation, showed some separation of the pesticide treatments in 2006. Both are illustrated in Figure Id.

3.1.3 Over Years Analysis

A combined PCA of the 2005 and 2006 GC-MS polar data using the sample correlation matrix of the 70 metabolites found in both years showed that the first two principal components (PC1 and PC2) clearly distinguished between crops grown in the 2005 and 2006 seasons. In each year it was possible to distinguish between the fertiliser treatments but the differences were not as pronounced as between growing seasons (Figure II). PC1 accounted for 51.4% of the total variation, while PC2 accounted for 11.5%.

PC3, PC4 and PC5 from the combined analysis, which explained 5.6%, 4.1% and 3.7% of the variation, were also inspected but none could distinguish between the organic or conventional pesticide treatments.

INSERT FIGURE II HERE

For potato, metabolomic data on compositional variation in conventional vs. organic systems is basically non-existent, although Hajšlová et al. (2005) used a targeted metabolite analysis approach (nitrate, trace elements, vitamin C, glycoalkaloids, chlorogenic acid) to show that organic and conventionally grown potatoes could be differentiated based on PCA. Yield, dry matter and tuber size were consistently different, but the varieties used and growing locations selected, were also important factors driving the metabolite variation observed. In a metabolomics study with maize, Röhlig and Engel (2010) revealed only minor differences based on conventional vs. organic production systems, and that the major drivers of metabolomic variation were mainly due to genetic differences (cultivars) and environmental influences. Similarly, Zorb (2006) reported only moderate differences in polar metabolites of wheat grain produced

under organic and conventional farming systems. Only eight of the 52 metabolites analysed showed a statistically significant difference with the largest difference being 50%.

With regard to other "omics" technologies, proteomic (Lehesranta et al., 2007) and transcriptomic (van Dijk et al., 2012) analyses of potato tubers from the same experiments used for the metabolomics analysis in this manuscript have already been published. Proteomics (2-DE [dimensional electrophoresis]), and subsequent protein identification by High Performance Liquid Chromatography–Electrospray (HPLC-ESI-) Tandem-MS, was also clearly able to differentiate tubers grown under organic and conventional fertiliser regimes, with differences shown in 160 of the 1100 tuber proteins separated by 2-DE. Seventeen were present at higher levels in tubers grown with a conventional fertilisation regime, while the remaining 143 were more abundant in tubers grown with the organic fertilisation regime. Analysis of gene expression using a potato microarray (van Dijk et al., 2012) revealed separation of the conventional and organic treatment groups with respect to both fertiliser and crop protection practices. The separations were revealed in combinations of principal components rather than individual components.

There is general consensus in the literature that “omics” approaches can differentiate between genotypes, growing seasons and environments, and that these tend to be more extensive qualitatively and quantitatively than those reported between conventional and organically produced crops (Davies 2010; Davies et al., 2010; Röhligh et al., 2009; Barros et al., 2010).

3.2 ANOVA: Identity of Metabolites Driving Changes between Organic and Conventional Samples

Different ANOVA models were used to represent the different designs used for the various technologies and years. Initially, a split-plot ANOVA model was used with residual terms for the main plot and subplot. However these were of similar sizes, indicating a low correlation between subplots, and the residual terms were merged to increase the degrees of freedom for testing the significance of the treatment terms i.e. the experiment was analysed as a randomised block design.

3.2.1 LC-MS

ANOVA of the metabolites identified by LC-MS indicated no significant differences in their levels due to pesticide treatment, and no significant interactions between pesticide and fertiliser regimes (data not shown). The distribution of *p*-values for each of these resembled a uniform distribution and none were low enough for the null hypothesis of no significant effect to be rejected by the BH procedure at a significance level of $\alpha = 0.05$.

Thirty-four metabolites showed significant differences ($p < 0.05$, corresponding to $FDR < 0.022$) with fertiliser treatment, with the mean values for 26 metabolites lower with the organic fertiliser than with the conventional fertiliser, and eight with higher levels. The means, standard errors of difference (SEDs) and significance levels of the known metabolites are shown in Table I (unknown metabolites are listed in Table SII). Of the 26 metabolites lower in the organic fertiliser, two were glycoalkaloids (α -solanine and the glycoalkaloid derivative solasonine, although α -chaconine was not significantly affected in this study), 14 were amino acids, and the remaining 10 were unknown. Of the eight metabolites higher in the organic fertiliser treatment, one was the antioxidant glutathione and seven were unknown.

Decreased levels of glycoalkaloids in organically grown potatoes agrees with the findings of Hellenas and Branzell (1995) and Abreu et al. (2007), although Hajšlová et al. (2005) reported slightly higher levels of glycoalkaloids in organically grown tubers but with year-to-year and genotypic variation swamping any effects of management practice. Environmental factors associated with organic management have been suggested to induce the biosynthesis of compounds involved in disease resistance, including glycoalkaloids (Friedman, 2006).

The pattern of lower amino acid levels in organic samples was confirmed by GC-MS analysis and is discussed below.

3.2.2 GC-MS - 2005 Experiment

ANOVA of the 91 polar metabolites identified by GC-MS for the 2005 experiment showed no significant interactions between pesticide and fertiliser according to the BH procedure with $\alpha = 0.05$, with the p -values resembling a uniform distribution.

Forty-five metabolites showed significant differences ($p < 0.05$, corresponding to a $FDR < 0.022$) between fertiliser treatments. The means for the known metabolites are listed in Table II and unknowns are listed in Table SIII, with SEDs and the significance levels. All but one metabolite (galactinol) was lower with the organic fertiliser. Of the 44 metabolites lower in the organic fertiliser samples, 20 were amino acids, eight a mixture of organic acids, amides and sugar alcohols, and the remaining 16 were unknown.

For pesticides, the effects were less clear. At a significance level $p < 0.05$, 20 metabolites showed significant differences, but the associated FDR was higher ($FDR < 0.122$). At a significance level $p < 0.01$ ($FDR 0.068$) only four metabolites were significant. Eleven of the 20 metabolites were unknown, and there was little agreement with the significant metabolites in 2006. For pesticide, we focus on the metabolites that were significant in the over-years

analysis, in section 3.2.4 below.

ANOVA of the non-polar metabolites for the 2005 experiment showed no significant effects for fertiliser, pesticide or their interaction according to the BH procedure with $\alpha = 0.05$.

3.2.3 GC-MS - 2006 Experiment

The 2006 trial included a pre-crop treatment (winter wheat or spring beans) as well as the pesticide and fertiliser treatments used in 2005. However, none of the metabolites were affected by the pre-crop used in the rotation, and there were no significant interactions between pesticide and fertiliser, or between pre-crop and pesticide or fertiliser treatments, according to the BH procedure with $\alpha = 0.05$. The same was true for data obtained from proteomic analysis of samples from the same experiment (Lehesranta et al., 2007). Thirty-seven of the 74 metabolites showed significant differences ($p < 0.05$, corresponding to a FDR < 0.025) between fertiliser treatments. The means for the known metabolites are shown in Table II and the means for the unknown metabolites are listed in Table SIII. All but one metabolite (galactinol) had lower means with the organic fertiliser than with the conventional fertiliser. Of the 36 metabolites lower in the organic fertiliser samples, 21 were amino acids, five a mixture of organic acids, amides and sugar alcohols, and the remaining 10 were unknown.

As in 2005, the effects of pesticides were less clear. Nine metabolites showed significant differences ($p < 0.05$, corresponding to a high FDR < 0.32) between pesticide treatments. At a significance level $p < 0.01$ (FDR 0.040) only five metabolites were significant. Only sucrose and glucose were significant for both years with sucrose general higher with organic pesticide treatments and glucose lower. We discuss these further in 3.2.4.

3.2.4 Comparisons of Outcomes over Years and Across Metabolomic Technologies Used

Twelve metabolites (the amino acids methionine, phenylalanine, tryptophan, histidine, asparagine, lysine, isoleucine, leucine, proline, valine, threonine and tyrosine) were detected by both LC-MS and GC-MS (Tables I and II), and in 2005 both technologies showed that lower levels of all of these amino acids accumulated in tubers from the organic compared with conventional fertiliser treatment.

An over-years analysis was used to explore interactions between years and the other treatments. Separate residual terms among the field plots were used for each year, as preliminary plots showed differences in year to year variability. The over-years analysis showed that only two metabolites (proline and threonine) had a significant year by fertiliser interaction at significance level $p < 0.001$, and four further metabolites (histidine, oxoproline, fumaric acid and 2-

1 piperidinecarboxylic acid) had a significant year by fertiliser interaction at significance level $p < 0.05$. As shown in
2 Table II, for the remaining metabolites this interaction was not significant ($p > 0.05$), indicating a consistent effect for
3 the two years analysed.

4 GC-MS was used for two consecutive growing seasons, and the data for fertiliser regime effects for 20 amino acids
5 were consistent for both years. Three other known metabolites, allantoin, inositol and trihydroxypentanoic acid were
6 also lower under organic fertiliser in both years (Table II). In other cases differences between fertiliser treatments were
7 observed in one year only (e.g. L-alanine).

8 As mentioned above, the effect of pesticide treatment on the metabolite levels is less clear, and there was less
9 agreement between the two years. We focus on the metabolites for which the p -value of the pesticide effect in the over-
10 years analysis was < 0.001 , which corresponds to a FDR of 0.002. Table III shows that these metabolites are sugars,
11 where galactose and glucose are lower under organic pesticide, and sucrose is higher. However, the extent of these
12 differences varies with growing season and a larger study would be advisable to confirm these effects.

13 The dominance of amino acids in driving consistent differences between the metabolite profiles of conventional and
14 organic fertiliser regimes is perhaps not surprising as, for this field trial, Lehesranta et al. (2007) reported that total N
15 levels in the tubers were significantly higher with the conventional fertilisation regime (by *ca.* 50%), despite the fact that
16 similar amounts of N were applied in the organic and conventional fertilisation regimes. Since free amino acids
17 constitute *ca.* 50% of the total potato N pool, different levels of amino acids can be expected where N availability from
18 inorganic and organic sources differ both temporally and quantitatively during the growing season. Theoretically, all of
19 the N in conventional ammonium nitrate fertiliser is readily available to plant roots, whereas only *ca.* 10-15% of the N is
20 readily available to the current crop from organic manures, with the remainder mineralised throughout the current and
21 subsequent seasons (Lehesranta et al., 2007, and references therein).

22 In maize, Röhlig and Engel (2010) found that only three metabolites (malic acid, *myo*-inositol, and phosphate) were
23 consistently different between organic and conventionally produced maize. This is a very low number considering the
24 broad range of low-molecular-weight constituents they were able to analyse by with their GC-MS approach. For wheat,
25 Zorb et al. (2006) detected lower levels of alanine and valine using organic fertiliser, but the differences were small.
26 Other differences reported were for glycerate and hydroxyglurate.

27 Proteomic (Lehesranta et al., 2007) and transcriptomic (van Dijk et al., 2012) analyses of potato tubers from the
28 same experiments used for the metabolomics analysis in this manuscript have already been published. Proteins that
29 were more abundant in tubers grown with the organic regime included several involved in protein synthesis, folding and

degradation, such as various heat shock proteins and putative chaperonins. Several were matched against enzymes involved in glycolysis and energy metabolism. Various proteins that are commonly up-regulated in stress responses were also increased in tubers grown with the organic fertilisation regime e.g. Kunitz-type enzyme inhibitor, superoxide dismutases and ascorbate peroxidases. Using transcriptomics, van Dijk et al. (2012) also revealed potato differential expression of Kunitz-type enzyme inhibitor and other genes generally involved in abiotic stress, such as maintenance of redox balance and detoxification mechanisms. One of the most consistent differences found was for the lipoxygenase pathway (higher expression with organic fertiliser). Lipoxygenases are enzymes that catalyse the conversion of (poly)unsaturated fatty acids to hydroperoxide derivatives which can in turn be further metabolised to elicitors of defence mechanisms in different stress. The analysis of non-polar metabolites in our study has not revealed any differences between conventional and organic systems in the relative levels of such (poly)unsaturated fatty acids (α -linoleic and α -linolenic acids). However, the action of lipoxygenase on specific lipid fractions cannot be excluded. van Dijk et al. (2012) also checked their array for expression of 18 potato homologues of genes involved with amino acid transport and metabolism (both synthesis and degradation), and also transport and metabolism of nitrate and ammonia. None of the identified pathways showed differential expression for fertiliser treatment. Thus, gaps remain in our understanding of linkages between transcriptome, proteome and metabolome with regard to the various differences observed. Lu et al. (2005), however, have identified several genes whose expression appears to be indicative of the 'organic status' of the wheat grain in relation to the type of nitrogenous fertiliser. In the treatments with solely organic-N inputs (but not in the inorganic-N treatments, or organic-N treatments supplemented with inorganic-N), N subsequently becomes limiting and in this situation genes involved in N uptake, N metabolism and storage are up-regulated - possibly in an attempt to fulfil the initial yield potential.

Consistent and significant metabolite differences between conventional and organic pesticide regimes were really only observed for three sugars. It would be highly speculative to explain these differences in terms of differential effects on specific physiological and metabolic processes. The chemicals used obviously differed between the treatments, and the organic system involved mechanical removal of foliage rather than the use of a herbicide desiccant. The latter would no doubt result in some differences in carbohydrate transport to tubers prior to harvest.

4 Concluding Remarks

There is a small, but growing, body of literature on the use of omics approaches to differentiate organic and conventionally grown crops. This metabolomics manuscript provides data which supplements and complements the

1 range of publications which already exist for specific metabolites. We have used potato as a model system to assess
2 metabolome variation under organic fertiliser and pesticide treatment regimes, and to complement existing publications
3 on the potato proteome and transcriptome which used materials from the same field trial (Lehesranta et al., 2007; van
4 Dijk et al., 2012). In total, ANOVA identified 71 metabolites (22 unique to LC-MS, 37 unique to GC-MS polar and 12
5 amino acids detected in both) showing significant differences in the metabolome between the fertiliser treatments. All
6 of the 12 amino acids detected by both LC-MS and GC-MS polar analysis were less abundant in organic fertilised
7 samples. Of the 59 other metabolites, 50 were less abundant in organic fertilised samples, but differences were
8 dominated by free amino acids. This can be associated with the much lower N content of the organically grown potatoes
9 in this instance. Differences associated with the crop protection measures used were much less consistent and relatively
10 small compared with the fertiliser effects found.

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Fig. I Selected scores plots from PCA of all metabolites identified for all potato samples in 2005 (a-c) and 2006 (d), with samples denoted by either fertiliser (a-b) or fertiliser and pesticide (c-d) application.

a. LC-MS positive mode: Plot of PC1 (38.8%) against PC2 (18.6%) in 2005.

b. GC-MS polar fraction: Plot of PC1 (29.0%) against PC2 (15.7%) in 2005.

c. GC-MS polar fraction: Plot of PC1 (29.0%) against PC3 (8.5%) in 2005.

d. GC-MS polar fraction: Plot of PC1 (33.7%) against PC4 (8.1%) in 2006.

Where in Ia-Ib filled squares = conventional fertiliser, and open squares = organic fertiliser; and in Ic-IId filled squares = conventional fertiliser and conventional pesticide, filled circles = conventional fertiliser and organic pesticide, open squares = organic fertiliser and conventional pesticide, open circles = organic fertiliser and organic pesticide

Fig 1.a

Fig I.b

Fig I.c

Fig I.d

Fig. II Principal component plot of PC1 (51.4%) against PC2 (11.51%) based on all metabolites identified by GC-MS (polar fraction) for the potato samples from the 2005 and 2006 experiments, with samples denoted by year and fertiliser application.

Table I. LC-MS: Mean levels on the natural and log scale for known metabolites under conventional and organic fertiliser treatment in 2005, ordered by significance from an ANOVA F-test of the log-transformed data.

Metabolites lower under organic F in 2005	Natural scale		Log scale			
	Conv	Org	Conv	Org	SED	Sig
arginine	0.9941	0.5284	-0.0210	-0.6501	0.0671	***
methionine	0.6505	0.4474	-0.4361	-0.8163	0.0469	***
phenylalanine	7.3480	4.9883	1.9884	1.5988	0.0564	***
isoleucine	4.3933	3.2818	1.4767	1.1855	0.0448	***
leucine	4.3420	3.2261	1.4647	1.1683	0.0458	***
valine	4.1240	3.1790	1.4123	1.1506	0.0404	***
tryptophan	3.0387	2.0488	1.1010	0.7122	0.0667	***
histidine	0.1585	0.0924	-1.8596	-2.3968	0.0861	***
asparagine	1.0071	0.6852	-0.0026	-0.3854	0.0633	***
lysine	0.2980	0.1875	-1.2310	-1.6835	0.0837	***
proline	0.3395	0.2443	-1.1008	-1.4179	0.0660	***
threonine	0.0963	0.0673	-2.3564	-2.7062	0.0801	**
thiamine	0.0084	0.0036	-4.8438	-5.6688	0.2021	**
solasonine	0.0023	0.0017	-6.0417	-6.3370	0.0829	**
tyrosine	2.0432	1.6884	0.7040	0.5131	0.0692	*
α -solanine	1.2784	0.9675	0.1936	-0.0360	0.0980	*
Metabolites higher under organic F in 2005	Conv	Org	Conv	Org	SED	Sig
glutathione	0.0643	0.0759	-2.7597	-2.5993	0.0595	*

Where F = Fertiliser; Conv = Conventional; Org = Organic; SED = Standard Error of Difference; Sig = Significance.

* = significant ($p < 0.05$); ** = significant ($p < 0.01$); *** = significant ($p < 0.001$).

Table II. GC-MS Polar: Levels of known metabolites present in at least one of the two years, with differences between conventional and organic fertiliser treatment.

Metabolites lower under organic F in both years	2005						2006						Combined		
	Natural scale		Log scale				Natural scale		Log scale				Y	F	Y F
	Con v	Org	Con v	Org	SED	Si g	Con v	Org	Con v	Org	SED	Si g	Si g	Si g	Si g
lysine	0.40 69	0.21 27	- 0.90 65	- 1.55 78	0.05 85	** *	0.10 53	0.06 80	- 2.25 50	- 2.71 31	0.09 09	** *	** *	** *	ns
asparagine	2.77 29	1.21 67	1.01 07	0.17 00	0.08 09	** *	1.47 78	0.81 62	0.37 67	- 0.26 44	0.10 44	** *	** *	** *	ns
glutamine	1.30 53	0.44 20	0.22 83	- 0.86 27	0.10 66	** *	0.56 16	0.23 56	- 0.61 75	- 1.62 97	0.18 97	** *	** *	** *	ns
threonine	0.13 76	0.06 30	- 1.99 34	- 2.76 25	0.08 06	** *	0.06 04	0.04 50	- 2.80 08	- 3.10 64	0.07 65	** *	** *	** *	** *
serine	0.26 29	0.12 70	- 1.34 70	- 2.07 16	0.07 73	** *	0.12 02	0.06 70	- 2.11 95	- 2.73 34	0.09 19	** *	** *	** *	ns
phenylalanine	0.82 37	0.46 90	- 0.20 13	- 0.76 28	0.06 58	** *	0.29 52	0.19 12	- 1.23 42	- 1.68 62	0.09 78	** *	** *	** *	ns
valine	1.81 33	1.06 06	0.58 68	0.04 97	0.06 35	** *	0.59 72	0.33 54	- 0.53 57	- 1.13 85	0.10 82	** *	** *	** *	ns
oxoproline	3.88 54	1.32 23	1.32 68	0.22 66	0.13 22	** *	1.16 10	0.74 36	0.09 79	- 0.47 07	0.18 39	** *	** *	** *	*
methionine	0.20 20	0.10 35	- 1.61 10	- 2.26 92	0.07 93	** *	0.06 97	0.04 02	- 2.66 02	- 3.26 29	0.11 64	** *	** *	** *	ns
tryptophan	0.17 15	0.07 25	- 1.79 35	- 2.65 39	0.10 63	** *	0.02 18	0.00 97	- 3.84 23	- 4.60 93	0.14 13	** *	** *	** *	ns
proline	0.26 67	0.15 23	- 1.33 89	- 1.88 98	0.06 96	** *	1.21 19	0.26 11	0.07 00	- 1.90 39	0.32 63	** *	** *	** *	** *
isoleucine	0.55 52	0.32 69	- 0.59 62	- 1.12 70	0.07 33	** *	0.17 91	0.11 60	- 1.73 84	- 2.18 21	0.09 73	** *	** *	** *	ns
histidine	0.08 65	0.03 34	- 2.67 53	- 3.56 80	0.12 61	** *	0.00 69	0.00 47	- 4.88 51	- 5.18 94	0.11 73	*	** *	** *	*
allantoin	0.02 67	0.01 57	- 3.60 36	- 4.12 74	0.07 52	** *	0.01 68	0.01 06	- 4.06 63	- 4.51 55	0.11 68	** *	** *	** *	ns
γ - aminobutyric acid (GABA)	1.83 34	1.13 09	0.58 36	0.11 69	0.07 14	** *	0.72 99	0.46 91	- 0.31 49	- 0.79 13	0.07 84	** *	** *	** *	ns
inositol	0.68 02	0.56 93	- 0.38 67	- 0.56 33	0.02 90	** *	0.63 28	0.48 50	- 0.47 08	- 0.73 79	0.06 55	** *	** *	** *	ns
tyrosine	0.51 19	0.30 95	- 0.68 36	- 1.19 27	0.08 49	** *	0.08 05	0.04 71	- 2.53 36	- 3.05 87	0.08 17	** *	** *	** *	ns
glycine	0.13 09	0.08 07	- 2.04 44	- 2.52 05	0.08 04	** *	0.06 24	0.03 51	- 2.77 01	- 3.36 52	0.09 63	** *	** *	** *	ns

leucine	0.18 89	0.12 82	- 1.67 89	- 2.05 98	0.07 69	** *	0.07 54	0.06 22	- 2.58 45	- 2.78 41	0.07 36	*	** *	** *	ns
β-alanine	0.01 26	0.00 79	- 4.30 98	- 4.77 23	0.09 64	** *	0.00 76	0.00 41	- 4.79 34	- 5.37 13	0.17 11	**	** *	** *	ns
aspartic acid	0.56 57	0.44 62	- 0.57 32	- 0.81 54	0.06 31	**	0.35 80	0.27 78	- 1.03 92	- 1.30 40	0.08 12	**	** *	** *	ns
trihydroxyphen- tanoic acid	0.00 80	0.00 49	- 4.76 17	- 5.14 95	0.12 47	*	0.00 92	0.00 72	- 4.60 81	- 4.83 53	0.07 39	**	ns	** *	ns
glutamic acid	1.10 02	0.96 62	0.09 34	- 0.04 03	0.04 57	*	0.61 34	0.46 54	- 0.49 94	- 0.80 48	0.09 63	**	** *	**	ns

Metabolites lower under organic F in 1 year, ND in other	2005						2006						Combined		
	Natural scale		Log scale				Natural scale		Log scale				Y	F	Y F
	Con v	Org	Con v	Org	SED	Sig	Con v	Org	Con v	Org	SED	Sig	Sig	Sig	Sig
fumaric acid	0.00 51	0.00 28	- 5.12 37	- 5.64 37	0.14 55	**	0.00 04	0.00 00	- 6.75 47	- 6.90 78	0.11 14	ns	** *	**	*
galactose	0.03 39	0.03 71	- 3.38 25	- 3.28 73	0.03 47	*	0.00 81	0.00 68	- 4.76 07	- 4.94 67	0.15 60	ns	** *	*	ns
2,3- dihydroxypro- panoic acid	0.00 48	0.00 37	- 5.17 28	- 5.38 40	0.07 80	*	ND	ND	ND	ND	ND	ns	N A	N A	N A
quinic acid	0.18 93	0.16 96	- 1.66 67	- 1.77 70	0.04 13	*	0.18 71	0.17 62	- 1.68 52	- 1.75 97	0.05 76	ns	ns	*	ns
glycerol	0.03 26	0.02 42	- 3.41 65	- 3.72 31	0.12 21	*	0.00 69	0.00 63	- 4.90 88	- 4.97 00	0.11 33	ns	** *	*	ns
L-alanine	ND	ND	ND	ND	ND	ns	0.11 66	0.05 27	- 2.16 71	- 3.02 99	0.13 31	** *	N A	N A	N A
2- piperidinecarb- oxylic acid	0.03 48	0.03 01	- 3.37 18	- 3.48 47	0.09 71	ns	0.04 12	0.02 66	- 3.29 01	- 3.66 59	0.08 00	** *	ns	** *	*
oxalic acid	ND	ND	ND	ND	ND	ns	0.00 73	0.00 42	- 4.83 46	- 5.39 44	0.13 31	** *	N A	N A	N A

Metabolites higher under organic F in both years	2005						2006						Combined		
	Natural Scale		Log Scale				Natural Scale		Log Scale				Y	F	Y F
	Con v	Org	Con v	Org	SED	Sig	Con v	Org	Con v	Org	SED	Sig	Sig	Sig	Sig
galactinol	0.08 77	0.11 70	- 2.45 39	- 2.19 30	0.05 93	**	0.04 36	0.05 28	- 3.12 33	- 2.94 12	0.06 93	*	** *	** *	ns

Where F = Fertiliser; Conv = Conventional; NA = not available; ND = not detected; ns = not significant; Org = Organic; SED = Standard Error of Difference; Sig = Significance from an ANOVA F-test; Y = Year; YF = Year by Fertiliser interaction.

* = significant ($p < 0.05$); ** = significant ($p < 0.01$); *** = significant ($p < 0.001$). Shaded values denote no significance.

Table III. GC-MS Polar: Levels of known and unknown metabolites present in at least one of the two years, with differences between conventional and organic pesticide treatment.

Metabolites lower under organic P in one or both years	2005						2006						Combined		
	Natural scale		Log scale				Natural scale		Log scale				Y	P	Y P
	Con v	Org	Con v	Org	SED	Sig	Con v	Org	Con v	Org	SED	Sig	Sig	Sig	Sig
galactose	0.03 87	0.03 23	- 3.24 08	- 3.42 90	0.03 47	** *	0.00 83	0.00 66	- 4.74 13	- 4.96 61	0.15 60	ns	** *	** *	ns
glucose	2.09 00	1.63 63	0.71 04	0.46 78	0.07 56	*	0.60 72	0.30 78	- 0.64 16	- 1.29 98	0.12 76	** *	** *	** *	*
Metabolites higher under organic P in one or both years	2005						2006						Combined		
	Natural scale		Log scale				Natural scale		Log scale				Y	P	Y P
	Con v	Org	Con v	Org	SED	Sig	Con v	Org	Con v	Org	SED	Sig	Sig	Sig	Sig
galactinol	0.07 67	0.12 81	- 2.57 11	- 2.07 57	0.05 93	** *	0.04 65	0.04 99	- 3.06 04	- 3.00 41	0.06 93	ns	** *	** *	** *
2- piperidinecarb oxylic acid	0.03 03	0.03 46	- 3.49 47	- 3.36 17	0.09 71	ns	0.02 42	0.04 36	- 3.72 97	- 3.22 63	0.07 99	** *	ns	** *	** *
sucrose	2.87 19	3.52 57	1.02 94	1.24 49	0.05 71	**	2.23 81	2.68 57	0.79 55	0.97 99	0.04 11	** *	*	** *	ns

Where Conv = Conventional; ns= not significant; Org = Organic; P = Pesticide; SED = Standard Error of Difference; Sig = Significance from an ANOVA F-test; Y = Year; YP = Year by Pesticide interaction.

* = significant ($p < 0.05$); ** = significant ($p < 0.01$); *** = significant ($p < 0.001$). Shaded values denote no significance.

Table SI. Annotation convention of GC-MS polar and LC-MS metabolites.

Technology	Metabolite	Metadata Relative to Metabolite Identification
GC-MS (polar)	allantoin	Identification of this component, present in the form of the tetra- and penta-TMS derivatives (RR _i 1886, 1906), is based on comparison with entries for these metabolites in the NIST2005, PAL600K (Palisade Corporation, USA) and GMD mass spectral databases. Predicted RR _i for these in the latter are 1876 and 1897.
	trihydroxypentanoic acid	This component with RR _i 1649 is tentatively identified as 2,4,5-trihydroxypentanoic acid, alternatively named as 2,4,5-trihydroxyvaleric acid or 3-deoxy-2,4,5-trihydroxypentonic acid (tetra TMS derivative) by comparison with entries in the NIST2005 and PAL600K mass spectral databases. The mass spectrum of this metabolite also matches that of the unknown metabolite NA165004 (predicted RR _i 1638) listed in the GMD, also tentatively identified as 3-deoxy-2,4,5-trihydroxypentonic acid (tetra TMS derivative).
	galactinol	This component with RR _i 2973 is tentatively identified as galactinol (TMS) ₉ , a conjugate of galactose and myo-inositol. The mass spectrum of this metabolite matches that of galactinol (9TMS), RR _i 2966, in the GMD. Other hexose-sugar alcohol conjugates of similar structure (maltitol, 2811; lactitol, 2741, cellobitol, 2750) have lower RR _i values in the GMD, and should elute earlier.
	UP2993	This component with RR _i 2993 is tentatively identified as a polysaccharide (TMS) _n due to the presence of ions (e.g. <i>m/z</i> 204, 217, 361) characteristic of TMS derivatives of polysaccharides, and its elution in a region of the chromatogram where other polysaccharides elute. The mass spectrum of this component matches that of an unnamed analyte in the GMD - M000000_A300001-101-1_MST_2,987.50_PRED_VAR5_ALK_NA, RR _i 2987.
	USA1768	This component with RR _i 1768 is tentatively identified as a sugar acid (TMS) ₅ , and closely matches entries for ribonic acid (TMS) ₅ in the NIST2005 and PAL600K mass spectral databases. The mass spectrum of this metabolite also matches those of the penta-TMS derivatives of ribonic acid (RR _i 1752), xylonic acid (RR _i 1750) and lyxonic acid (RR _i 1762) listed in the GMD.
LC-MS	solasonine	This component is putatively identified as solasonine, and has an accurate mass measurement within 5 ppb of the theoretical accurate mass of solasonine. The MS/MS (MS ₂) fragmentation pattern was identical to the predicted theoretical pattern and eluted in the region of the chromatogram for glycoalkaloids.

Where GMD = Golm Metabolome Database; ppb = parts per billion; RR_i = relative retention index; UP = Unknown Polysaccharide; USA = Unknown Sugar Acid.

Table SIII. GC-MS Polar: Levels of unknown metabolites, and those with no significant effects, present in at least one of the two years, with differences between conventional and organic fertiliser treatment.

Metabolites lower under organic F in both years	2005						2006						Combined		
	Natural scale		Log scale				Natural scale		Log scale				Y	F	YF
	Conv	Org	Conv	Org	SED	Sig	Conv	Org	Conv	Org	SED	Sig	Sig	Sig	Sig
U1586	0.0303	0.0058	-3.6161	-5.1207	0.1996	***	0.0151	0.0074	-4.1716	-4.9701	0.2293	***	ns	***	*
U1703	0.0130	0.0036	-4.3482	-5.5153	0.1840	***	0.0038	0.0023	-5.4000	-5.8721	0.1673	*	***	***	*
U1585	0.0904	0.0453	-2.4312	-3.0563	0.1028	***	0.0395	0.0265	-3.1688	-3.6693	0.1865	*	**	***	ns
U1791	0.0033	0.0018	-5.5763	-5.9409	0.1463	*	0.0392	0.0173	-3.2601	-4.1495	0.1786	***	***	***	*
U1598	0.0452	0.0224	-3.1597	-3.8750	0.1667	**	0.0436	0.0223	-3.1892	-3.8557	0.1705	**	ns	***	ns
U1567	0.0190	0.0105	-3.7861	-4.2072	0.0884	**	0.0087	0.0057	-4.3346	-4.6327	0.1124	*	**	***	ns
U1509	0.0143	0.0074	-4.0379	-4.4586	0.1259	**	0.0351	0.0188	-3.3771	-4.0048	0.2785	*	**	***	ns
UP2993	0.0431	0.0341	-3.1404	-3.3793	0.0763	*	0.0350	0.0282	-3.3559	-3.5560	0.0884	*	***	***	ns
U1948	0.0053	0.0034	-4.6174	-4.8183	0.0847	*	0.0024	0.0011	-4.9368	-5.1149	0.0837	*	**	**	ns
Metabolites lower under organic F in 1 year	2005						2006						Combined		
	Natural scale		Log scale				Natural scale		Log scale				Y	F	YF
	Conv	Org	Conv	Org	SED	Sig	Conv	Org	Conv	Org	SED	Sig	Sig	Sig	Sig
U1570	0.0047	0.0022	-5.2293	-5.8163	0.1466	**	ND	ND	ND	ND	ND	ns	NA	NA	NA
U1593	0.0067	0.0028	-4.9930	-5.6979	0.1903	**	ND	ND	ND	ND	ND	ns	NA	NA	NA
U1871	0.0203	0.0149	-3.8716	-4.1698	0.0848	**	0.0073	0.0054	-4.8552	-5.1608	0.1558	ns	***	***	ns
U1809	0.0234	0.0137	-3.8559	-4.5544	0.2019	*	0.0127	0.0118	-4.5757	-4.8519	0.2676	ns	ns	*	ns
U1751	0.0051	0.0039	-5.1194	-5.3366	0.0683	**	0.0055	0.0055	-5.0783	-5.0711	0.0975	ns	ns	*	ns
U2502	0.0007	0.0002	-6.4643	-6.7390	0.1149	*	ND	ND	ND	ND	ND	ns	NA	NA	NA
USA1768	0.0085	0.0074	-4.7220	-4.8024	0.0990	ns	0.0076	0.0059	-4.7710	-5.0117	0.0702	**	ns	0.001	ns
U2125	0.0058	0.0041	-5.0895	-5.5214	0.1779	*	ND	ND	ND	ND	ND	ns	NA	NA	NA

Where Conv = Conventional; F = Fertiliser; NA = not available; ND = not detected; ns= not significant; Org = Organic; SED = Standard Error of Difference; Sig = Significance from an ANOVA F-test; U = Unknown; UP = Unknown Polysaccharide; USA = Unknown Sugar Acid; Y = Year; YF = Year by Fertiliser interaction.

* = significant ($p < 0.05$); ** = significant ($p < 0.01$); *** = significant ($p < 0.001$). Shaded values denote no significance.