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Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans

Short title: Glyphosate accumulates in GM soy

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ABSTRACT

This article describes the nutrient and elemental composition, including residues of herbicides and pesticides, of 31 soybean batches from Iowa, USA. The soy samples were grouped into three different categories: i) genetically modified, glyphosate-tolerant soy (GM-soy); ii) unmodified soy cultivated using a conventional "chemical" cultivation regime; and iii) unmodified soy cultivated using an organic cultivation regime. Organic soybeans showed the healthiest nutritional profile with more sugars, such as glucose, fructose, sucrose and maltose, significantly more total protein, zinc and less fibre than both conventional and GM-soy. Organic soybeans also contained less total saturated fat and total omega-6 fatty acids than both conventional and GM-soy. GM-soy contained high residues of glyphosate and AMPA (mean 3.3 and 5.7 mg/kg, respectively). Conventional and organic soybean batches contained none of these agrochemicals. Using 35 different nutritional and elemental variables to characterise each soy sample, we were able to discriminate GM, conventional and organic soybeans without exception, demonstrating "substantial non-equivalence" in compositional characteristics for 'ready-to-market' soybeans.

Keywords: Agricultural practice, GMO, herbicide residues, pesticides, nutrition, substantial equivalence

1. Introduction

Food and food quality is crucial. Given its significance for human and animal health, we investigate whether plant products from a defined geographical region, produced under different agricultural practices are substantially equivalent or not, in terms of quality indicators like nutritional content, elemental characteristics and herbicide/pesticide residues.

By comparing herbicide tolerant ("Roundup Ready") GM soybeans directly from farmers' fields, with extended references to both conventional, i.e. non-GM soybeans cultivated under a conventional "chemical" cultivation regime (pre-plant herbicides and pesticides used), and organic, i.e. non-GM soybeans cultivated under a "no chemical" cultivation regime (no herbicides or pesticides used), a test of real-life samples 'ready-to-market' can be performed.

Globally, glyphosate-tolerant GM soy is the number one GM crop plant. The herbicide glyphosate is the most widely used herbicide globally, with a production of 620 000 tons in 2008. The world soybean production in 2011 was 251.5 million Metric tons, with the United States (33%), Brazil (29%), Argentina (19%), China (5%) and India (4%) as the main producing countries.

In 2011-2012, soybeans were planted on about 30 million hectares in the USA, with Roundup Ready GM soy contributing 93-94 % of the production. Also in the other leading producing countries, this same GM soy dominates the market accounting for 83 and 100 % of production, respectively in Brazil and Argentina. Globally, Roundup Ready

GM soybeans contributed to 75 % of production in 2011.

The first-generation glyphosate-tolerant GM-soy plant (event 40-3-2), produced and patented by Monsanto Company, has been genetically modified to tolerate exposure to glyphosate-based herbicides during the entire growth season. For herbicide-tolerant GM plants, herbicide co-technology is an integral part of the production system and will always be used by the farmer. However, in early studies of the composition of Roundup-Ready GM soy, the researchers did not spray the tested plants with the recommended herbicide (Millstone, Brunner, & Mayer, 1999). This shortcoming was quickly corrected, and also sprayed GM soybeans were claimed to be substantially equivalent to non-GM soybeans (Harrigan et al., 2007). Still, and surprisingly, even in these studies, the residues of herbicides were not measured.

The concept of 'substantial equivalence' (i.e. close nutritional and elemental similarity between a genetically modified (GM) crop and a non-GM traditional counterpart) has been used to claim that GM crops are substantially equivalent to, and therefore as safe and nutritious as, currently consumed plant-derived foods (Aumaitre, 2002). However, we argue that compositional studies that have overlooked (not measured) pesticide residues contain serious shortcomings. Chemical residues, if present, are important because i) they are clearly a part of a plants composition, and ii) they may add toxic properties to the final plant product either by itself or by affecting the plant metabolism. This is particularly relevant for herbicide-tolerant varieties.

For the predominantly used GM soy on the market, the 40-3-2 event, herbicide tolerance was achieved by insertion of a transgene construct into the plant genome which constitutively expresses the *Agrobacterium* strain CP4 analogue of the plant enzyme

EPSPS (5-enolpyruvylshikimate-3-phosphate synthase). The endogenous plant EPSPS is critically important for the production of certain essential aromatic amino acids. Glyphosate, the active ingredient of Roundup herbicide formulations, is able to bind to all known plant, weed and crop, EPSPS versions. The binding leads to the inactivation of the enzyme and consequently death for the plant. Glyphosate binds the CP4 EPSPS expressed in GM-soy cells in a condensed, non-inhibitory conformation. Hence plants engineered to express the CP4 EPSPS enzyme are tolerant to glyphosate. Accordingly, the farmer may eradicate all kinds of plant weeds by spraying with glyphosate, and not harm the GM crop plants. However, the extensive use of glyphosate over vast land areas may lead to shifts in weed populations and selection of glyphosate-tolerant weeds (Shaner, Lindenmeyer, & Ostlie, 2012). This, in turn, typically triggers the use of higher doses or more applications of glyphosate, which can further accelerate the evolution of glyphosate resistance in weed species (Binimelis, Pengue, & Monterroso, 2009). Such a spiral is clearly not sustainable for farmers, but may also affect the consumer through plant tissue accumulation of glyphosate residues. Evolution of resistance to glyphosate is unfortunately progressing, particularly in the US. System vulnerability to resistance development is enhanced where there is a low diversity in weed management practice coupled with crop and herbicide monoculture.

USDA data document dramatic increases in the use of glyphosate-based herbicides and GM soy is a major driver for this development (Benbrook, 2012). US GM soybeans thus represent a system that is influenced by glyphosate exposure and should be an ideal system in which to test whether crop management practices that include spraying with glyphosate might lead to accumulation of chemical residues, or other compositional differences, in the final soy product. Residue analysis is of particular interest, since there are no programmes in the EU, US or Canada designed to monitor the main herbicides used in transgenic crop production.

In contrast to real-life samples from the market, transgenic crops intended for scientific studies are often produced in well-controlled small experimental plots. In most research studies, application of herbicides has been omitted or has been done at doses lower than those typically used by farmers, giving test materials that are not representative of actual conditions existing in typical agricultural operation, e.g. with regard to glyphosate residues. The knowledge regarding links between glyphosate application rates and soybean nutrient composition is scarce. One study found links between glyphosate application on glyphosate-tolerant soybean and decreased levels of α-linolenic acid (ALA) and iron, and increased levels of oleic acid (Zobiole, Bonini, de Oliveira, Kremer, & Ferrarese, 2010). A 12-14 % reduction in phytoestrogen levels in GM soybean strains compared to isogenic conventional strains has been documented (Lappé, Bailey, Childress, & Setchell, 1998). However, Wei and co-workers showed that GM soybeans may have both a higher and lower content of isoflavones compared to conventional soy (Wei, Jone, & Fang, 2004).

Generally, the suggested key food and feed nutrients found in the OECD consensus documents, are considered in safety evaluations of new varieties of soybeans and risk assessment of GM plants has focused on allergenicity and toxicity resulting from the transgenic product itself, or from the possible unintended effects of the transformation process (Podevin & Jardin, 2012). However, little attention is given to the residues of herbicides and their metabolites that can potentially accumulate in the final product, and also whether exposure to these herbicides, or other functional alterations related to the genetic modification itself (such as alterations in intermediary metabolism of the GM plant), may affect nutrient and elemental composition.

In the present study, 31 samples of soybeans grown within a defined area within the state of Iowa in the US, were collected. The influence of agricultural practice on (i) residues of glyphosate, AMPA and other pesticide compounds, and (ii) the nutritional and elemental composition of "ready-to-market" soybeans was analysed. We used methods of multivariate analyses, such as cluster and discriminants analyses, and attempted to track differences (if any), both between individual samples and between the three management systems through which they were produced, namely GM, conventional and organic systems.

With H_0 as substantial equivalence between the categories of soy, the following hypotheses were tested:

H_1: The residues of pesticides in soybeans will be influenced by the agricultural practice they have been produced under, specifically:

a) GM-soybeans contain high residue levels of glyphosate and AMPA due to repeated spraying of the plants with glyphosate-based herbicides throughout the production season. Other pesticides may also be present according to use.

b) Conventional soybeans contain low residue levels of glyphosate and AMPA due to pre-planting applications. Other pesticides may also be present according to use.

c) Organic soybeans are expected to represent a control group with zero residues of glyphosate, AMPA and others chemical pesticides. Such pesticides are not allowed in organic farming.

H_2: The detailed nutritional composition and hence, the nutritional quality (i.e. total fat and protein, main sugars, ash, amino acids, fatty acids and micronutrients/ basic elements) of soybean samples will be influenced by the agricultural practices under which they have been produced.

2. Materials and Methods

2.1. Soy samples and characterisation

Three kg samples of whole soybeans were obtained from n=31 individual fields/sites in Iowa, USA. Seed type (genetic variety), agricultural practice, i.e. whether samples were ‘GM’ (n=10), ‘conventional’ (n=10) or ‘organic’ (n=11), and pesticide use was noted for all samples (Table 1). All individual soybean samples were analysed for their nutritional content, including total protein, total fat, dry matter, starch, ash, minerals, trace elements, vitamin B6, amino acid and fatty acid composition, in addition to the relevant pesticides.

2.2. Proximate composition of the soybeans

Dry matter was analysed by drying at 103ºC for 24 h, ash by weight after burning at 540ºC and lipid after extraction with ethyl-acetate. Nitrogen was measured with a nitrogen determinator (LECO, FP-428, Leco Corporation, St Joseph, MI, USA) according to the Association of Official Agricultural Chemists official methods of analysis and protein calculated as N X 6·25. Glycogen was measured after enzymatic degradation.

Amino acids and Vitamin B6 were determined by high pressure liquid chromatography (HPLC) methods and fatty acids by GLC (gas liquid chromatography). Multielement determination in the soybeans was carried out by inductively coupled plasma MS.

Eurofins laboratories GfA, Otto-Hahn-Str. 22, D-48161 Münster (Germany), performed analysis of organochlorine, organophosphorus, pyrethroides, PCBs, glyphosate and AMPA (aminomethylphosponic acid - the major degradation product of glyphosate) based on the list of pesticide brand names used by the farmers (see Table 1). The following Eurofins methods were used; LMBG L00.00-34, DFG S19, GC-ECD for organochlorine pesticides, pyrethroides, PCBs and LMBG L00.00-34, DFG S19, GC-FPD for organophosphorus pesticides. DFG 405, HPLC-FLD for glyphosate and AMPA.

Three pooled samples (equal amounts of all individual samples) representing each of the soy categories (GM, conventional and organic) were in addition analysed for the average values of monosaccharides, disaccharides and fibre at the Czech Agriculture and Food Inspection Authority (CAFIA), Za Opravnou 300/6, 150 00 Praha 5, (Czech Republic) and for selected organochloride pesticides OCPs (30 active components including their metabolites) at the National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway. Organochlorine pesticides (OCPs*)* were determined by GCMS on a Trace GC 2000 series and Trace DSQ single quadrupole (Thermo Fisher Scientific, Waltham, MA, USA).

2.3. Geographic distribution

All samples were collected in Iowa (USA) within a 200 km radius. There were examples of GM-soy and organic soy samples collected within the same town/village (the smallest distance between farms was 5 km). Nine out of ten samples from the conventional soy were sampled in a town or village where most of the GM-soy samples (six out of ten) were also collected. Organic soy and conventional soy samples were not from the same town/village.

2.4. Soy varieties

The ten samples of conventional soybeans were of four different varieties: Legend 2932 (4 samples), Legend 2375 (3 samples), Asgrow 2869 (2 samples) and Legend 2200. The GM samples were from 8-9 different varieties: Stine 2032 (2 samples), Stine [unnamed], Stine 2538-4, Stine 2602-4, Stine 2062-4, Latham 2158, PB 2217VNRR, PB 2421, Pioneer 92M76). The organic samples consisted of nine different varieties: Pioneer 9305 and ED 4315 (both 2 samples), Legend 2375, Stine 2686, US Soy 20333, Mark 0427, Mark 0431, PB291N and Pioneer 93M52. The conventional and organic varieties overlapped in the use of “Legend 2375” (n=3 conventional and n=1 organic sample). There was no overlap in varieties between the GM and either the conventional or organic varieties.

2.5. Multivariate analyses

Characteristics of the soy samples were analysed with the R-project software with library (vegan) for 35 variables: glycogen, all amino acids, sum of unsaturated, mono- and poly-unsaturated fats, omega3, omega6 and trace elements. Glyphosate and AMPA were first taken out of the primary analyses to look for differences beyond/because of these. In later analyses, concentrations of glyphosate or AMPA and soy variety were included to identify co-variation to other variables. GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA) and Statistica™ 7 (StatSoft Inc., Tulsa, OK, USA) was used to evaluate correlations between nutrient composition and residue levels of glyphosate and AMPA. Differences in nutrients between the soybean categories were analysed using a one-way ANOVA, and in cases when ANOVA showed significant differences, post hoc tests (Tukey HSD test) were used.

3. Results

3.1. Herbicides and pesticides

All individual samples of GM-soy contained residues of both glyphosate and AMPA. In contrast, no sample from the conventional or the organic soybeans showed any residues of these chemicals (Fig. 1). In the GM-soy samples, the concentration of AMPA (mean concentration = 5.74 mg/kg) was on average nearly twice as high as glyphosate (3.26 mg/kg). The minimum - maximum values for AMPA and glyphosate were 0.7 - 10.0 mg/kg and 0.4 - 8.8 mg/kg, respectively.

Fluazifop-P was found in a concentration of 0.078 mg/kg in one of the GM-soy samples, malathion was found in a concentration of 0.02 mg/kg in one of the conventional soy samples and Dieldrin was found in a concentration of 0.002 mg/kg in one of the organic soy samples. Other residues were not found. The additional testing for pesticide residues in pooled samples of GM, conventional and organic soybeans showed trace-levels of Alpha-endosulfane, Trans-nonachlor and Trans-chlordane, all close to the detection limit of 0.05 µg/kg and in all soy types. Dieldrin was also found in very low levels with 0.51, 0.45 and 0.6 µg/kg in GM, conventional and organic soybeans, respectively.

3.2. Main constituents of the soy – individual samples

The organic soybeans differed in nutrient composition compared to the conventional and GM soybeans in several variables (Table 2). The organic samples contained significantly more total protein compared to both the GM-soy and conventional soy ($p<0.01$, ANOVA, Tukey correction), which was also reflected with a higher content of the indispensable amino acids (IAAs). There was significantly lower content of 18:2n-6, and sum saturated fats in the organic soybean material. There were no significant differences in the 18:1n-9 (monounsaturated) or the 18:3n-3 (Omega 3) fatty acids between the three groups.

The content of Zn was significantly higher in the organic samples compared to the conventional and GM samples ($p=0.001$ and $p<0.001$, respectively, ANOVA, Tukey correction). Other differences were relatively small (Table 2). There was a significant positive correlation between the AMPA residue levels and iron ($p=0.028$, linear regression) and AMPA residue levels and 18:2n-6 content in the GM soybeans ($p=0.016$, linear regression).

3.3. Main constituents of the soy – pooled samples

Samples representing each of the three production systems, containing equal amounts of all individual samples produced using those production systems were analysed for monosaccharides, disaccharides and fibre. The GM-soy (pooled samples) contained on average less of all the main sugars (glucose, fructose, sucrose and maltose) compared to both the conventional and organic soy (Table 3). The organic soy contained more sugars than both conventional and GM-soy, but less fibre (Table 3).

3.4. Cluster analysis

Exploratory cluster analyses were used to group and differentiate the soy samples based on the 35 variables measured. Ten of the organic samples were grouped with 1 of the GM samples, while most of the GM and the conventional samples were intermixed (Fig. 2a). By including the variety name to the samples in the cluster tree (Fig. 2b), the role of the genetic background was highlighted. In some cases, the same agricultural practice in combination with the same soy variety, the outcome was a close grouping (e.g. for conventional Legend2375). However, a third sample of the same Legend2375, also grown under a conventional practice showed an intermediate distance to the mentioned samples, but grouped very closely to an organic sample of Legend2375. For other pairs of varieties grown under the same agricultural practice, samples grouped with an intermediate distance (GM Stine2032 and conventional Asgrow2869), yet other pairs showed a great distance between sample characteristics (organic ED4315, organic Pioneer 9305).

3.5. Discriminant analysis

Soy from the three different categories, GM, conventional and organic, could be well separated (Fig 3). The first axis of variation mainly separated organic samples from both the GM and conventional, while the second axis differentiated the GM from conventional.

3.6. Redundancy analysis (RDA)

GM soybeans were most strongly associated with saturated and mono-unsaturated fatty acids. Organic soybeans were associated with elements and amino acids Zn, Asp, Lys, Ala, Sr, Ba, Glu. Conventional soy were associated with the elements Mo and Cd (Fig. 4). The model accounted for 21.5 % of the total variation in the material (PC1=19.0 %, PC2=2.5 %).

4. Discussion

4.1. General

Our data demonstrate that different agricultural practices lead to markedly different end products, i.e. rejecting the null hypothesis (H_0) of substantial equivalence between the three management systems of herbicide tolerant GM, conventional and organic agriculture. Both the H_1 and H_2 hypotheses were supported due to the key results of high levels of glyphosate/AMPA residues in GM-soybeans, and that all the individual soy samples could be discriminated statistically (without exception) into their respective agricultural practice background – based on their measured compositional characteristics (Fig. 3). Notably, the multivariate analyses of the compositional results was performed excluding the factors glyphosate/AMPA residues, which obviously otherwise would have served as a strong grouping variable separating the GM soy from the two non-GM soy types.

Since different varieties of soy (different genetic backgrounds) from different fields (environments) grown using different agricultural practices were analysed, we need to acknowledge that variation in composition will come from all three of these sources. However, since 13 samples out of the 31 had at least one ‘sibling’ (same variety) to compare both within and across the different agricultural practices, how the same variety ‘performed’ (i.e. its nutritional and elemental composition) between different environments and agricultural practices could be compared. As some samples of the same variety were highly similar in the cluster analysis, but others were intermediate or even highly different (Fig. 2b), we argue that (i) there was a strong genotype x environment interaction within all three agricultural practices, (ii) the combination of a range of varieties on a range of different farms in a relatively well defined geographical region, and grown in the same climate zone in the same season, give us representative data regarding soy composition from that particular region. To test food products that are not experimentally matched, e.g. for different soil conditions, resembles the situation for a consumer in the store.

4.2. Residues of pesticides in the soy

In this study it was found that Roundup Ready GM-soybeans sprayed during the growing season take up and accumulated glyphosate and AMPA at concentration levels of 0.4 - 8.8 and 0.7 - 10 mg/kg, respectively. In contrast, conventional and organic soybeans did not contain these chemicals. We thus document what has been considered as a working hypothesis for herbicide tolerant crops, i.e. that: *"there is a theoretical possibility that also the level of residues of the herbicide and its metabolites may have increased"* (Kleter, Unsworth, & Harris, 2011) was actually happening.

Glyphosate is shown to be absorbed and translocated within the entire plant, and has been found in both leaf material and in the beans of glyphosate tolerant GM soy plants. However, FAO have not distinguished GM from non-GM plants in their consideration on glyphosate residues. Monsanto has claimed that residues of glyphosate in GM soy are lower than in conventional soybean, where glyphosate residues have been measured up to 16-17 mg/kg (Monsanto, 1999), which likely must have been due to spraying before harvest (desiccation). Another claim has been that documented maximum residue levels up to 5.6 mg/kg in GM-soy represent "*...extreme levels, and far higher than those typically found*" (Monsanto, 1999). Seven out of the 10 GM-soy samples tested surpassed this "extreme level" of glyphosate + AMPA residues, indicating a development towards higher residue levels. The increased use of glyphosate on Roundup Ready soybeans in the US (Benbrook, 2012), contributing to selection of glyphosate-tolerant weeds (Shaner et al., 2012) with a response of increased doses and/or more applications used per season, may explain the plant tissue accumulation of glyphosate.

A pesticide residue is the combination of the pesticide and its metabolites. According to FAO, the total glyphosate residues should be calculated as the sum of gly + 1.5x AMPA. Using this formula, the data set has on average 'glyphosate equivalents' of 11.9 mg/kg for the GM soybeans (max. 20.1 mg/kg). Clear residue definitions are required to establish the compound or compounds of interest, e.g. for estimating dietary intake risks.

This issue becomes more complex in the near future as new GM plants may: (i) be tolerant to other/additional herbicides (e.g. 2,4-D and/or dicamba), eventually several stacked in the same plant, (ii) have altered tolerance to glyphosate (likely higher), (iii) metabolise herbicides into new breakdown products having altered toxicity and requiring potentially altered methods of detection. The insertion of *GAT*-genes into maize and soy for example, makes the plant transform glyphosate into the non-herbicidal N-acetyl-glyphosate, requiring a re-consideration of definitions.

Residues of agrochemicals must be expected to increase when repeated applications are carried out and when application takes place later in the growing season. Duke and co-workers showed that GM-soybeans sprayed at full bloom of the plant contained about 5-10 times more glyphosate and 10-25 times more AMPA than plants sprayed only early in the growing season (Duke, Rimando, Pace, Reddy, & Smeda, 2003). With early spraying, the levels of glyphosate and AMPA were 0.2-0.6 and 0.5-0.9 mg/kg, respectively. Spraying at full bloom gave substantially higher residue levels of glyphosate and AMPA, 2.2-3.1 and 7.3-25 mg/kg, respectively (Duke et al., 2003). The samples in the study showed residue levels comparable to these (i.e. somewhat higher in glyphosate and lower in AMPA), indicating that spraying later in the season has become common practice in the sampled area. This provides strong support for hypothesis (1a) of high residue levels in GM soy.

Even soybeans grown on areas with no application of glyphosate, have been shown to contain glyphosate and AMPA, e.g. 0.1-0.2 mg/kg (Duke et al., 2003), possibly due to herbicide drift or indicating plant uptake from a soil reservoir of the herbicide. Our samples from conventional soybean farmers did not contain any glyphosate or AMPA.

This was not surprising as the use of pre-plant herbicides did not include glyphosate-based chemicals. We thus find no support for hypothesis (1b) in our data set.

Under all three agricultural practices trace levels of pesticides other than glyphosate were detected (see results), but we consider these pesticide residues of little practical significance for the tested soy materials. Presumably, they are due to residual levels of persistent pesticides in the soil, even in organic fields.

4.3. Nutritional components

Soybean nutritional quality is determined by many factors but the protein level, the mineral content and fatty acid (FA) composition are essential components. Our results clearly show that different agricultural practices affect the quality of soybeans. The organic soybeans had significantly higher levels of total protein and lower levels of linoleic acid LA (18:2n-6) and palmitic acid PA (16:0). Soybeans are a major dietary source of LA and although LA is an essential FA, a high and unbalanced intake (high omega 6 and low omega 3) is emerging as a risk factor for developing obesity. We also show that GM-soy had a significantly higher level of PA, a saturated FA, compared to organic soybeans. EFSA has concluded that saturated fatty acids intake should be as low as possible within the context of nutritionally adequate diets.

Conventional soybeans were observed to have superior nutrient and dry matter composition compared to glyphosate-treated GM-soybeans (Zobiole et al., 2012). In a review on this topic, however, conflicting results were found, with most studies indicating that mineral nutrition is not affected by glyphosate tolerance trait or application of glyphosate (Duke et al., 2012).

4.4.Direct and indirect effects of glyphosate application on soy nutrition and plant environment

Glyphosate has been shown to reduce photosynthesis and nutrient uptake in GM-soy, in greenhouse and field trials, both for first and second generation of glyphosate resistant soy plants. High glyphosate application rates have been shown to reduce alfa-linolenic acid (ALA, 18:3n-3) but increase oleic acid (OL, 18:1n-9) (Bellaloui, Zablotowicz, Reddy, & Abel, 2008), i.e. producing a less healthy profile of fatty acids.

Glyphosate may also, depending on soil type, alter micronutrient status, in particular Mn and Zn. Our data showed significantly higher Zn concentrations in organic soy samples (mean 37.0 mg/kg), but no differences between GM and conventional soy samples (mean 30.4 and 31.7 mg/kg respectively). This indicates that factors other than glyphosate may be relevant, such as the use of organic versus synthetic fertiliser or long-term accumulated differences in soil treatment and quality. Status of the micronutrient Mn was not affected by the production system in our samples.

In general, a healthy microbial community, 'the plant microbiome', in the soil of the rhizosphere is an important contributing factor for plant trait characteristics and plant health (Lundberg et al., 2012). Glyphosate has the potential to adversely affect microbial communities present in soils into which plants are rooted, i.e. increased colonisation by *Fusarium* (Kremer & Means, 2009).

AMPA is mildly phytotoxic, and leads to reduced photosynthesis ('yellowing') and transpiration rates in soy plants (Ding, Reddy, Zablotowicz, Bellaloui, & Bruns, 2011). Other ingredients of glyphosate-based herbicides have also been described as detrimental to GM-soy. We found a significant positive correlation between AMPA residue levels in the GM soybeans and increasing levels of LA and iron (Fe).

4.5. Maximum residue level (MRL) of glyphosate in food and feed

The acceptance level of glyphosate in food and feed, i.e. the maximum residue level (MRL) has been increased by authorities in countries where Roundup-Ready GM crops are produced or where such commodities are imported. In Brazil, the MRL in soybean in 2004 was increased from 0.2 mg/kg to 10 mg/kg: a 50-fold increase, but only for GM-soy. The MRL for glyphosate in soybeans has also increased in the US and Europe. In Europe, it was raised from 0.1 mg/kg to 20 mg/kg in 1999, and the same MRL of 20 mg/kg was adopted by the US based on recommendations of the Codex Alimentarius Commission. In all of these cases, MRL values appear to have been adjusted, not based on new evidence indicating glyphosate toxicity was less than previously understood, but pragmatically in response to actual observed increases in the content of residues in glyphosate-tolerant GM soybeans.

In the US and Canada, there is a practice of using glyphosate to desiccate crops by spraying the maturing plants, in order to speed up and make the “maturation” of the crop more uniform, thereby facilitating harvest. This may add to the residue levels of glyphosate and AMPA, as shown in field pea, barley and flax seed. Particularly if the plant is still growing, translocation of glyphosate within the plant may result in accumulation of glyphosate residues in the seed, both for GM and unmodified soy.

4.6. Toxicity and health relevance of pesticide/glyphosate residues

It is the full, formulated herbicide (typically one of the many Roundup formulations) that is used in the field, and, thus, it is relevant to consider, not only the active ingredient glyphosate and its breakdown product AMPA, but also the other compounds present in the herbicide formulation. For example, herbicide formulations containing glyphosate commonly also contain adjuvants and surfactants to help stabilise the herbicide and to facilitate its penetration into the plant tissue. Polyoxyethylene amine (POEA) and polyethoxylated tallowamine (POE-15) are common ingredients in Roundup formulations, and have been shown to contribute significantly to the toxicity of Roundup formulations (Moore et al., 2012). However, glyphosate alone has been shown to interfere with molecular mechanisms that regulate early development in frogs and chickens, with deformities of embryos as a consequence and the retinoic acid signaling pathway as the affected mediator (Paganelli, Gnazzo, Acosta, Lopez, & Carrasco, 2010).

In human cells, Roundup may induce endocrine disturbances at concentrations far below the MRLs cited by authorities in the EU and US (Benachour & Seralini, 2009). A life-cycle feeding study in rats reported negative health effects and found significantly altered blood parameters in animals that were fed Roundup Ready GM maize or were given extremely small amounts of Roundup in the drinking water (Seralini et al., 2012). The authors emphasised the role of pesticide residues in edible herbicide tolerant GM plants and argued that these must be evaluated very carefully to accurately assess potential toxic effects. This study has been criticised for its methods, analysis and reporting by EFSA, which initially rejected the central conclusion of this study, that long term (life-time) toxicity and carcinogenicity studies are needed. However, EFSA as well as regulatory authorities from multiple EU states are now acknowledging that this study flagged up the need for long term studies. A recent study in the model organism *Daphnia magna* demonstrated that chronic exposure to glyphosate and a formulation of Roundup resulted in negative effects on several life-history traits, in particular reproductive aberrations like reduced fecundity and increased abortion rate at environmental concentrations of 0.45-1.35 mg/l (active ingredient), i.e. below accepted environmental tolerance limits set in the US (Cuhra, Traavik, & Bøhn, 2013). A reduced body size of juveniles was even observed at an exposure to Roundup at 0.05 mg/l. These results are strikingly different from data reported by a study funded by the European Commission which indicated a NOEC (No Observed Effect Concentration) in *D. magna* of 455 mg/l and 30 mg/l for glyphosate-IPA and glyphosate acid, respectively (EC, 2002).

The importance of pesticide residuals is recognised by EFSA in feeding studies for risk assessment. For glyphosate-tolerant GM soybeans, EFSA has argued that (i) the levels of glyphosate should be analysed as part of the testing, and (ii) both glyphosate-treated and untreated soybeans should be used in order to separate effects of the plant and the herbicide (van Haver et al., 2008).

The toxicity and health relevance of glyphosate and Roundup have been debated widely. Other studies claim that glyphosate is not linked to developmental or reproductive effects in animals and humans, but that surfactants may cause some toxic effects (Williams, Watson, & DeSesso, 2012). This controversy has been reviewed in depth in (Antoniou, Robinson, & Fagan, 2012), with the conclusion that the weight of evidence indicates that glyphosate itself is a teratogen and that adjuvants commonly used in conjunction with glyphosate amplify this effect.

4.7. Organic vs conventional vs GM agriculture

Comparisons between organic and conventional agriculture have not reached consistent conclusions on nutritional quality, but a review of 223 compositional studies of nutrients and contaminants found that organic foods have significantly lower levels of pesticide residues (Smith-Spangler et al., 2012). A recent feeding study that compared organic and conventional food products concluded that organic foods may be more nutritionally balanced than conventional foods, or that they contain higher levels of nutrients, since the fruit fly *Drosophila melanogaster* lived longer and produced more offspring when fed organic soybeans (or potatoes, raisins, bananas) compared to conventional produce (Chhabra, Kolli, & Bauer, 2013). Organic crops may be more variable than industrially produced plant products, but are in general richer in some nutritionally important elements, in antioxidant phytochemicals and lower in pesticide residues. Our data support these conclusions. Organic crops have also been reported to contain a higher content of selenium. This was however not supported by our data, where the selenium content was significantly lower in the organic soybeans compared to the GM and conventional soybeans.

5. Conclusion

This study demonstrated that Roundup Ready GM-soy may have high residue levels of glyphosate and AMPA, and also that different agricultural practices may result in a markedly different nutritional composition of soybeans. In the present study organic soybean samples had a more profitable nutritional profile than industrial conventional and GM soybeans. We argue that pesticide residues should have been a part of the compositional analyses of herbicide tolerant GM plants from the beginning. Lack of data on pesticide residues in major crop plants is a serious gap of knowledge with potential consequences for human and animal health. We therefore recommend (i) increased effort on sampling and testing crop material from the market; (ii) testing for possible dose-response effects of chemical residues in long-term feeding studies; (iii) inclusion of pesticide residue measurements and safety testing in the regulatory system for risk-assessment and (iv) further research on the indirect ecological effects of herbicides and pesticides, i.e. on ecological interactions in the soil community with possible effects on nutrient uptake and plant composition.

6. Acknowledgements

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Table 1. Soy varieties for Roundup Ready (RR) GM, conventional and organic soybeans tested. Farmer information on chemicals used in their soy production is also given. No chemicals were applied to organic soybeans.

Type of soy	Variety	Seed treatment	Preplant	Postplant	Insecticide	Fungicide
RR GM	Latham 2158			Touchdown	Warhawk, Silencer	
RR GM	PB 2217VNRR			Roundup Power Max	Warrior, Lorsban	
RR GM	PB 2421			Roundup Power Max	Warrior	
RR GM	Pioneer 92M76	Cruiser Maxx		Touchdown	Cobalt	
RR GM	Stine		Trifluralin	Roundup		Apron Max
RR GM	Stine 2032	Cruiser Extreme		Roundup		
RR GM	Stine 2032			Roundup		
RR GM	Stine 2062-4			Touchdown	Warhawk, Silencer	Headline
RR GM	Stine 2538-4	Warden		Roundup (original Max), Durango	Leverage	Domark
RR GM	Stine 2602-4	Warden		Roundup (original Max), Durango	Leverage	Domark
Conventional	Asgrow 2869			Pursuit plus, Select, Flexstar	Lorsban, Warrior	Headline
Conventional	Asgrow 2869	Cruiser Maxx	Trust	Select, Flexstar, First Rate	Lorsban	
Conventional	Legend 2200			Pursuit plus, Select, Flexstar	Lorsban, Warrior	Headline
Conventional	Legend 2375	Cruiser Maxx	Treflan	Pursuit plus, Flexstar, First Rate	Cobalt	
Conventional	Legend 2375	Cruiser Maxx	Trust	Flexstar, Fusion, First Rate	Lorsban	
Conventional	Legend 2375	Cruiser Maxx	Prowl, Python	Pursuit plus	Cobalt	Headline
Conventional	Legend 2932	Cruiser Maxx	Prowl	Pursuit, Flexstar, Fusion	Lorsban	
Conventional	Legend 2932	Cruiser Maxx	Trust	Select, Flexstar, First Rate	Lorsban	
Conventional	Legend 2932	Cruiser Maxx	Trust	Flexstar, Fusion, First Rate	Lorsban	
Conventional	Legend 2932	Cruiser Maxx	Prowl, Python	Pursuit plus	Cobalt	Headline
Organic	ED 4315					
Organic	ED 4315					
Organic	Legend 2375					
Organic	Mark 0427					
Organic	Mark 0431					
Organic	PB291N					
Organic	Pioneer 9305					
Organic	Pioneer 9305					
Organic	Pioneer 93M52					
Organic	Stine 2686					
Organic	US Soy 20333					

Table 2. Composition of nutrients and elements in the different soybean types. Results are given as mean ± SD, based on measurement on individual samples. Significant differences (p<0.05) are indicated.

	GM	SD	Conv.	SD	Organic	SD	Anova
Proximate composition							
Dry matter (%)	89.4	1.4	88.1	2.0	88.2	2.6	ns
Protein (%)	34.6 [b]	1.3	34.3 [b]	1.5	36.3 [a]	1.1	p=0.003
Fat (%)	19.0	0.8	19.1	1.3	18.3	0.9	ns
Ash (%)	4.6 [ab]	0.2	4.5 [b]	0.2	4.7 [a]	0.2	p=0.005
Amino acids (mg/g)							
Methionine	4.2	0.3	4.0	0.3	4.0	0.4	ns
Lysine	22.1 [b]	1.5	22.2 [b]	1.3	24.2 [a]	0.9	p=0.002
Histidine	8.9	0.3	8.9	0.4	9.0	0.6	ns
Isoleucine	15.2	0.7	15.0	0.7	15.6	0.5	ns
Leucine	26.3 [ab]	0.9	26.2 [b]	1.1	27.4 [a]	1.0	p=0.02
Phenylalanine	18.0	0.6	17.7	0.7	18.0	1.2	ns
Threonine	13.8	0.4	13.8	0.5	14.3	0.6	ns
Valine	15.9	0.7	15.7	0.7	16.3	0.6	ns
Arginine	24.0 [ab]	0.9	23.4 [b]	1.1	24.9 [a]	1.8	p=0.04
Sum of IAAs [1]	142.3	5.4	140.8	5.2	147.1	5.8	p=0.037
Vitamins (mg/kg)							
Vitamin B6	15.7	1.5	14.9	1.2	14.9	1.4	ns
Fatty acids (mg/g)							
16:0 (palmitic acid)	22.6 [a]	1.2	21.1 [ab]	1.1	21.0 [b]	1.9	p=0.046
Sum Saturated	33.0 [a]	1.4	31.0 [ab]	1.6	29.7 [b]	2.3	p=0.001
18:1n-9 (oleic acid)	41.1	3.0	38.5	2.9	38.5	4.3	ns
Sum Monounsaturated	44.4	3.2	41.5	3.1	41.5	4.5	ns
18:2n-6 (linoleic acid)	115.7 [ab]	5.2	117.8 [a]	5.8	108.4 [b]	9.3	p=0.01
18:3n-3 (linolenic acid)	19.1	4.4	19.6	0.8	18.0	1.6	ns
Elements mg/kg							
Barium (Ba)	6.4 [b]	2.2	6.2 [b]	1.7	11.0 [a]	3.3	p=0.0005
Copper (Cu)	10.4	1.1	10.8	1.1	11.3	1.7	ns
Iron (Fe)	86.8	7.2	84.4	8.7	84.7	11.3	ns
Manganese (Mn)	24.1	2.8	22.8	1.7	24.5	2.3	ns
Molybdenum (Mo)	1.9	1.0	4.5	4.0	2.1	1.1	ns
Selenium (Se)	0.7 [b]	0.1	0.8 [a]	0.2	0.2 [b]	0.2	p=0.0003
Zinc (Zn)	30.4 [b]	2.4	31.7 [b]	2.8	37.0 [a]	3.4	p=0.0002

[1] IAAs Indispensible amino acids (except tryptophan).

Table 3. Composition of sugars and fibre (g/100 g fresh sample) in pooled soybean samples, i.e. mixing of all samples from GM (n=10), conventional (n=10), and organic (n=11) origin.

	Glucose	Fructose	Sucrose	Maltose	Fibre
GM	0,37	0,20	3,24	0,02	27,1
Conv.	0,62	0,31	4,18	0,02	28,4
Organic	1,04	0,62	4,82	0,54	24,7

Figure captions:

Figure 1. Residues of glyphosate and AMPA in individual soybean samples (n=31).

Figure 2. (a) Cluster dendrogram for GM, conventional and organic soy samples, based on 35 variables after standardisation of the data (mean = 0 and SD = 1). Glyphosate/AMPA residues were not included (would have separated the GM soy from non-GM soy). (b) Same as (a) but including information on the genetic line of soy grown.

Figure 3. Discriminant analysis for GM, conventional and organic soy samples based on 35 variables. Data were standardised (mean = 0 and SD = 1). Glyphosate/AMPA residues were not included (would have separated the GM soy from non-GM soy)

Figure 4. Redundancy analysis for the first two axes of variation (RDA1 and RDA2), based on standardised data. The positions of individual variables are indicated and the direction of the different soy types shown in arrows. The red lines indicate increasing levels of glyphosate + AMPA residuals (increasing towards 'gm').

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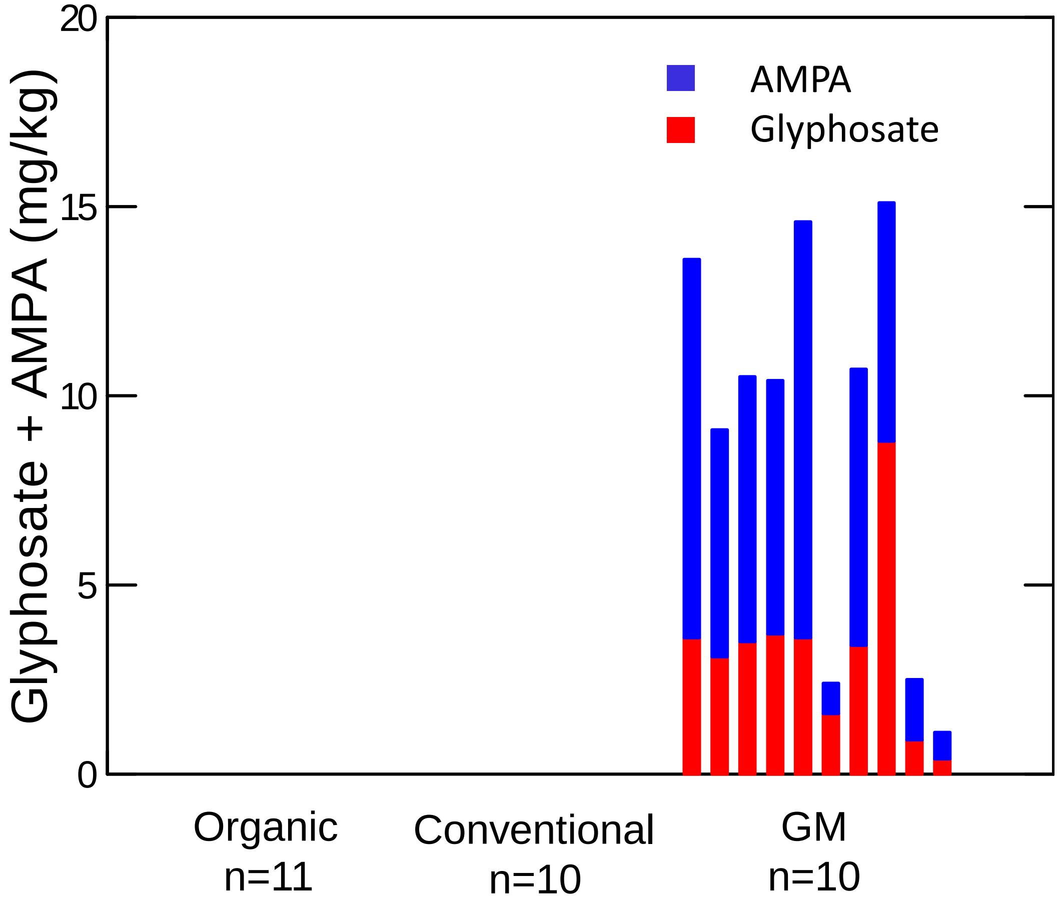

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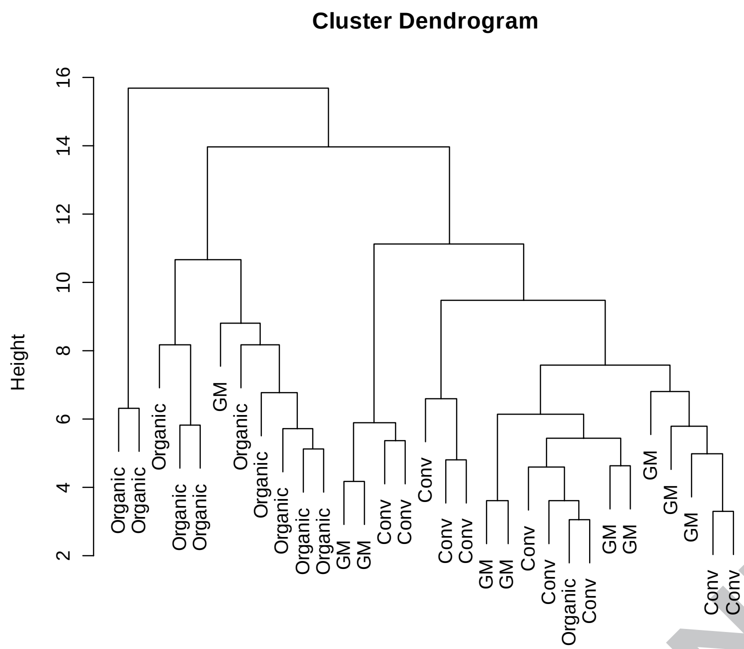

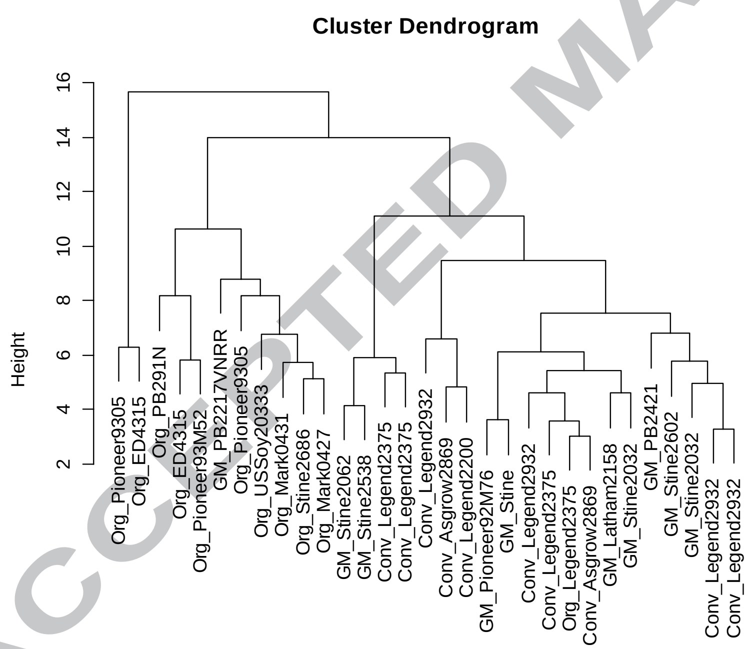

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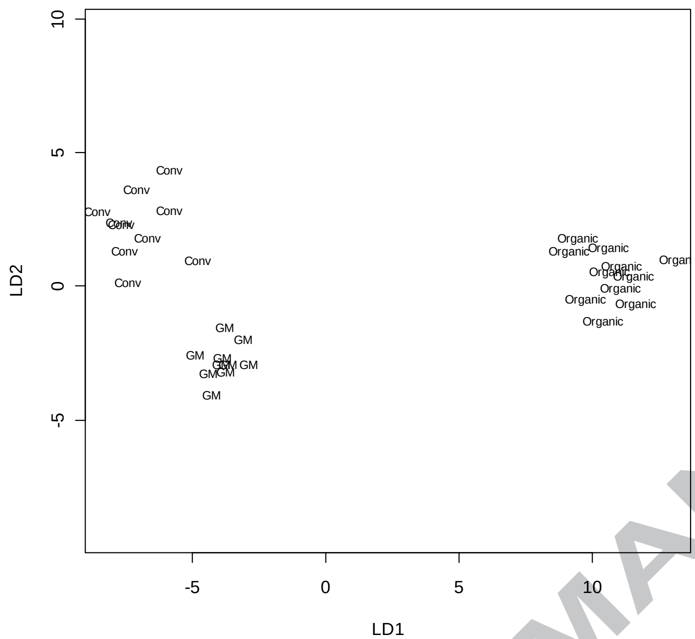

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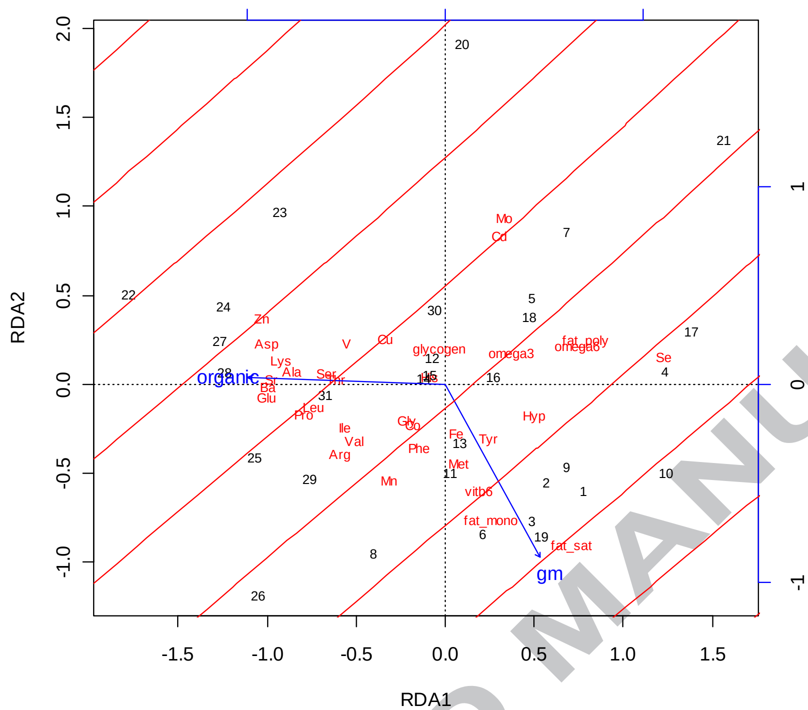

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This paper show the following interesting data:

1. Glyphosate tolerant GM soybeans contain high residues of glyphosate and AMPA
2. Soybeans from different agricultural practices differ in nutritional quality
3. Organic soybeans showed a more healthy nutritional profile than other soybeans
4. Organic soy contained more sugars, protein and zinc, but less fibre and omega-6
5. This study rejects that GM soy is "substantially equivalent" to non-GM soybeans