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Variation in bioactive content in broccoli (*Brassica oleracea* var. *italica*) grown under conventional and organic production systems

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Abstract

BACKGROUND: Broccoli and other cruciferous vegetables contain a number of bioactive compounds - in particular glucosinolates and polyphenols, which are proposed to confer health benefits to the consumer. Demand for organic crops is at least partly based on a perception that organic crops may contain higher levels of bioactive compounds; however insufficient research has been carried out to either support or refute such claims.

RESULTS: In this study we examined the effect of conventional, organic, and mixed cultivation practices on the content of total phenolics, total flavonoids, and total and individual glucosinolates in two varieties of broccoli grown over two years in a split-plot factorial systems comparison trial. Levels of total phenolics and total flavonoids showed a significant year on year variation but were not significantly different between organic and conventional production systems. In contrast, levels of the indolyl glucosinolates glucobrassicin and neoglucobrassicin were significantly higher ($p<0.05$) under fully organic compared to fully conventional management.

CONCLUSION: Organic cultivation practices resulted in significantly higher levels of glucobrassicin and neoglucobrassicin in broccoli florets, however other investigated compounds were unaffected by production practices.

Keywords: *Brassica oleracea*; organic agriculture; glucosinolates; neoglucobrassicin; glucobrassicin; phenolic compounds.

INTRODUCTION

Over the last 10 years demand for organically produced food products has increased and this has been recognized as an important consumer trend in the USA and Europe.[1] Although the reasons behind this increasing demand differ among countries and consumer groups, the perception of improved animal welfare, environmental protection, and food quality characteristics (including health, nutritional and/or sensory attributes) are among some of the reasons cited for this trend.[1,2] Organic crop production in the European Union is carried out under strictly defined production practices. [3,4] Despite consumer presumption that organic fruits and vegetables are healthier, the reality is that previous studies on nutrient and phytochemical composition have shown contradictory results. [5-12] One of the main challenges for these types of studies is the design of a robust experiment that takes account of important factors which can contribute to variability between studies such as crop variety, geographical location, growth season, management practices used, experimental design and statistical power.

Levels of glucosinolates and phenolic compounds in broccoli appear to be affected primarily by variety although only a few studies have been carried out with a number of cultivars examined under uniform cultivation conditions. [13-17] Levels are also affected by factors such as nitrogen fertilization, environmental factors and season, [18-2] although levels of both phenolic compounds and glucosinolates appear to remain stable under post-harvest storage treatments designed to simulate commercial storage and marketing. [22]

Broadly three types of study have been used to examine differences in nutritional or phytochemical content between organic and conventional foods. The first type comprise basket surveys, in which sampling is made at retail points, by grouping samples according to production system e.g. [9] The second type of study uses matched paired farms where existing farms using either conventional or organic production systems are matched as far as possible

in terms of location and crop produced. [23] The last type of study is by performing replicated field trials. [24] In any of these types of study a number of uncontrolled factors can influence the results obtained. Consequently, contradictory results have been obtained between different studies. In an extensive and widely reported meta-analysis Dangour *et al.* [5] note the difficulties and variable quality of research in the area and suggest five criteria for a study of acceptable quality: firstly to clearly define the organic production methods including the name of the certification body; secondly to specify crop cultivar or animal breed; thirdly to state which nutritionally relevant substance was analysed; fourthly to state methods of analysis and lastly to state methods used for statistical analyses.

The aim of this study was to compare the content of glucosinolates and phenolic compounds in organically and conventionally grown broccoli (*Brassica oleracea* var. *italica*).

MATERIALS AND METHODS

Experimental design

Two varieties each of broccoli, carrot and onion were grown in a replicated split-plot systems comparison trial. The field trial has a factorial design which divides both organic and conventional production systems into component soil management and pest-control practices. It was designed to investigate the effect of, and any interaction between, production system components - a) soil management and b) pest-control measures, that differ between organic and conventional systems. In order to encompass seasonal variation, sampling and data analysis presented is on two years of crop production. The trial design and statistical analyses comply with the suggested quality criteria of Dangour *et al.* [5] The trial design includes fully organic, fully conventional and mixed treatments, which allows comparison of fully organic and fully conventional production, whilst allowing investigation of soil and pest-control

components which make up organic or conventional agricultural practices. Management practices used are summarised in Table 1.

Plant material

Broccoli samples for this study were grown in 2009 (year1) and 2010 (year 2). Varieties selected were cv. 'Belstar' and cv. 'Fiesta' both of which are commercial varieties commonly grown by conventional and organic growers in Ireland. Plants were grown according to cultivation practices in compliance with European and Irish standards for organic certification (Irish Organic Farmers and Growers Association (IOFGA) and Organic Trust standards, see details below) and/or following the Irish Agriculture and Food Development Authority (Teagasc) recommendations for conventional practices.[25, 26] The year 1 broccoli crop was sown on 24th April, transplanted on 25th June and harvested between 4th and 25th September 2009. The year 2 crop was sown on 23rd March, transplanted on 18th May and harvested between 27th July and 5th August 2010. Plants were produced as modular transplants in 216 trays and transplanted at 40 cm in-row spacing with 2 rows per 1.52 m bed. External rows were treated as guard rows with samples harvested from internal rows only. Specific applied inputs for broccoli propagation and cultivation are shown in Table 2. Climatic conditions during the growing season in both years are shown in supplementary material (Table S1). Additional information on the field trial is available at http://www.ipfn.ie/publications/agronomic/.

For each experimental plot broccoli was harvested at commercial maturity from the internal rows with guard rows excluded. The mean floret weight was calculated as the total weight of harvested broccoli florets divided by the number of florets. Samples for analysis were primary florets of similar size and were immediately refrigerated and then frozen at -20°C within 24 hours of harvest. Samples from each experimental plot were composite samples comprising three healthy, disease free florets of marketable quality.

Field trial

The field trial is located at Teagasc Kinsealy Research Centre, Kinsealy (53° 25' N Lat 6° 10' W), in north county Dublin, Ireland. Soil type was characterised as loam to clay loam belonging to the grey brown podzolic soil group and with a high base status (Altitude: 28 metres O.D. (ordnance datum), Slope: 1°, Drainage: Moderately well drained). Soil type was consistent across the experimental trial site as indicated by a previous detailed soil map of the area. [27] Equivalent rates of nitrogen (N), phosphorus (P) and potassium (K) were applied to both conventional and organic soil treatments for each crop and the rates applied were according to Teagasc published recommendations for the crop. [25] Although the amount of N, P and K supplied was identical between systems, plant availability and uptake is affected by fertilizer form (chemical vs. organic) due to differences in water solubility and the need for organic fertilizer to be broken down by soil microbes. The trial was established in spring 2009 on land which had previously been under grass set-aside for over 10 years. The same irrigation source was used for the whole trial.

The trial was a 2 x 2 x 2 factorial split-plot design, with 4 replicates (blocks). Variety was assigned as the main plot, with pest-control and soil treatment assigned as sub plots. Two varieties (V1, V2) of 3 crops (carrots, broccoli and onion) were grown in each year. There were two levels of soil treatment – an organic soil treatment (OS) and a conventional soil treatment (CS); and 2 levels of pest control – an organic pest-control treatment (OP) and a conventional pest-control treatment (CP).

The OS treatment comprised certified organic fertilizer inputs, a 4 year horticultural crop rotation including a red clover ley (*Trifolium repens*) and use of winter cover crops (Table 1). The CS treatment comprised use of mineral fertilizers, with no set crop rotation. Equivalent rates of nitrogen (N), phosphorus (P) and potassium (K) were applied to both CS

and OS treatments for each crop following a spring soil test and rates were according to Teagasc recommendations for the crop.[25] Fertilizer was applied as calcium ammonium nitrate (CAN), single super-phosphate and sulphate of potash for the CS treatment, or Greenvale (3:3:1) and ProKali (3:0:14) for the OS treatment. Specific inputs are shown in Table 2.

Pest-control measures were required for control of weeds, cabbage root-fly (*Delia radicum*), and caterpillars of Diamond-back moth (*Plutella xylostella*) and Large White butterfly (*Pieris brassicae*). Organic pest-control (OP) measures comprised mechanical (e.g. hand hoeing, brassica collars) and certified organic treatments as shown in Table 2. Conventional pest-control (CP) treatments involved a chemical spay programme and were in accordance with an Integrated Pest Management plan, in keeping with commercial growing practises in North Dublin.

Within each replicate (n=4) each crop is grown under 8 possible treatment combinations (V1+OS+OP, V1+OS+CP, V1+CS+OP, V1+CS+CP, V2+OS+OP, V2+OS+CP, V2+CS+OP, V2+CS+CP) giving a total of 32 plots per crop per year (Supplementary material Figures S1, S2). The organic cultivation practices used were in compliance with EC 834/2007 [3] and with national standards for organic certification set out by the Irish organic certification bodies (IOFGA and the Irish Organic Trust) with the exception that for experimental purposes the separation distance (generally 50 m) required between adjacent organic and conventional enterprises was not practised between organic and conventional treatment plots.

Each experimental plot comprised two 1.52 m beds of 5.5 m length (area 16.7 m^2). In order to prevent or reduce as far as possible any cross contamination between treatment plots, within each replicate each plot was separated from neighbouring treatment plots by a 1 m wide untreated grass inter-plot area, with 3 m grass areas between replicate blocks. Fertilizers were applied by hand and tractor tillage operations carried out such as to minimise movement

of soil between treatments. Pesticides were applied using a knapsack sprayer with hood to prevent spray drift between treatment plots. Although it was not practically feasible to completely prevent all movement between plots (e.g. arthropods, earthworms, microbes could be expected to move through the soil to some extent) statistically significant differences in soil microbial activity and functional diversity between plots under different management practices has been demonstrated in this trial [28] indicating that measures used were effective in allowing different soil biology in different plots.

Diagrams showing the plot allocation and crop rotation in years 1 and 2 are shown in supplementary material Figures S1 and S2.

Total phenolics and total flavonoids

For the determination of total phenolics a modification of the Folin-Ciocalteau method was used. Briefly, broccoli samples were ground to a fine powder under liquid nitrogen. Frozen tissue (0.50 g) was transferred to a falcon tube and 5 mL of 80 % methanol (v/v) was added. Tubes were vortexed thoroughly and allowed stand at room temperature for 20 minutes. Tubes were mixed by inversion and 1.5 mL aliquots of extract were transferred to a microfuge tube. Microfuge tubes were centrifuged at 12,000g for 5 minutes at 4 ^{o}C and the supernatant was transferred to a fresh tube. For each sample 150 µL of the methanolic extract, 150 µL 80 % (v/v) methanol, 150 µL Folin-Ciocalteau reagent and 1050 µL sodium carbonate solution (20 % w/v) were pipetted into a microfuge tube, votexed and placed in the dark at room temperature for 20 minutes. Tubes were centrifuged at 12,000g for 3 minutes and the supernatant was transferred to a fresh tube. The absorbance at 725 nm (A_{725}) was determined relative to a blank containing 80% (v/v) methanol instead of extract, and the concentration was determined from a calibration curve using gallic acid. Results are expressed as gallic acid equivalents on a fresh weight basis (GAE mg/100g FW).

Determination of flavonoids was according to Marinova *et al.*[29] For each sample 150 µL of the methanolic extract and 600 µL MilliQ water were added to a microfuge tube and mixed by inversion. To each tube 45 µL of 5% sodium nitrite ($NaNO_2$) was added and tubes were incubated at room temperature for 5 minutes. To each tube 45 µL of 10% aluminium chloride was added and tubes incubated for a further 1 minute. Subsequently 300 µL of 1M sodium hydroxide (NaOH) and 360 µL MilliQ water was added and tubes mixed vigorously. The absorbance at 510 nm (A_{510}) was measured relative to a blank containing 80% (v/v) methanol instead of extract, and flavonoid concentration was determined from a standard curve using catechin as a standard. Results are expressed as catechin equivalents (CE mg/100g FW).

Glucosinolate extraction

Frozen broccoli samples were freeze dried in a large scale freeze drier (*Frozen in Time Ltd.* United Kingdom). Once freeze dried, samples were milled, vacuum packed in polypropylene bags and kept at -80°C until analysis. Glucosinolates were extracted from freeze-dried broccoli powder using pressurised liquid extraction with an ASE 200 instrument (Dionex; Sunnyvale, CA, USA) with attached solvent controller. Glucosinolate extraction was carried out in 22 mL steel cartridges packed with a mixture of freeze-dried broccoli (1.00 g) and technical grade silica to disperse sample. Extraction conditions were as described in Hernandez-Hierro *et al.* [30]

Sulphatase extraction procedure

Sulphatase (Type H-1 from *Helix pomatia*, Sigma, MO, USA) was purified by dissolving the sulphatase powder (70 mg) in deionised water (3 mL) and adding ethanol (3 mL). This solution was centrifuged (12000 rpm, 10 min, room temperature) and to the supernatant ethanol (9 mL) was added after which the solution was centrifuged (12000 rpm, 10 min, room

temperature) again. The pellet was dissolved in deionised water (2 mL) and this sulphatase solution was subsequently passed through a 0.5 mL DEAE Sephadex A-25 and a 0.5 mL SP Sephadex C-25 column. This solution was collected in a vial and kept at -80 °C until use.

An aliquot (1mL) of glucosinolate extract was applied to a DEAE Sephadex A-25 column (0.5mL) and the unbound material was removed by washing with deionised water (2 x 1mL) and sodium acetate buffer (2 x 0.5mL, 20 mM, pH 5.0). After washing, purified sulfatase solution prepared as above was added and the columns were incubated overnight at room temperature. After overnight incubation, the desulphoglucosinolates (dGLS) were eluted from the columns with deionised water (3 x 1ml). The collected eluate was dried under constant N_2 flow and re-dissolved in deionised water (200µL)

Micellar Electrokinetic Capillary Chromatography

Analyses were performed using a CE capillary electrophoresis system (Agilent, Waldbronn, Germany) equipped with diode array detector. All separations were performed on a fused silica capillary (Agilent, Stevens Creek, CA; 75 lm ID, 64.5 cm total length, 56 cm effective length). Samples were injected from the anodic end of the capillary (vacuum injection, 50 mbar, 1 s). The separation buffer consisted of sodium chlorate (250 mM) and boric acid (200 mM) at pH 8.5; the separation was carried out at 12 kV and 60 °C. The capillary was conditioned between each run sequentially with 1.0 M NaOH (3 min), 0.1 M NaOH (1 min), water (1 min) and separation buffer (5 min). Detection was performed on column at 230 and 280 nm. Data processing was carried out with Chemstation software (Agilent Waldbronn, Germany). The quantity of desulfo-glucosinolates was estimated as the average of quantities calculated by comparison of normalized area under the curve (area under the curve divided by migration time) of each identified desulfo-glucosinolate peak with the normalized area under the curve of glucotropaeolin (internal standard, from the laboratory collection). Identification

of individual desulfo-glucosinolates was performed by calculating their migration time relative to the migration time of glucotropaeolin and their PDA profile

Statistical analysis

Statistical analysis was carried out using SAS 9.1 (Cary, NC). Floret weight, total phenolic, total flavonoid and glucosinolate data were analysed using an ANOVA mixed model containing a contrast code to compare the fully organic (OS+OP) and fully conventional (CS+CP) treatments as well the individual treatments and interactions (SAS 9.1). Pearson correlation coefficients were calculated between total phenolics, flavonoids and mean floret weights using SAS 9.1.

RESULTS AND DISCUSSION

Yield and quality

In crop production the main differences between organic and conventional growing systems involve the use of organic manures and crop rotations instead of inorganic fertilizers; and mechanical or biological methods (including naturally derived compounds) for pest-control instead of synthetic pesticides. Thus it can be considered that organic and conventional agriculture differ in two major respects – how the soil fertility is managed, and how pests are managed. Both factors may impact on crop yield and quality.

Analysis of broccoli yield, total phenolic content and total flavonoid content is shown in Table 3. Year showed a significant ($p<0.01$) effect with some measures showing a year x treatment interaction therefore data for each year was analysed separately (Table 3). Floret weight, quality and yield was higher in year 2 than in year 1 for both varieties. Mean floret weight ranged from 230.6±20.3 g to 307.0±17.0 g in year 1 and was considerably higher at 247.1±78.5 g to 449.2±19.9 g in year 2. Climate data for the trial site (Supplementary

material, Table S1) shows the growing season in year 1 was overall slightly warmer but much wetter than year 2 with excessive rainfall during the summer especially in July. In year 1 a significant soil treatment effect on floret weight ($p<0.01$) was found, with crops grown with the conventional soil (CS) treatment showing higher floret weights, however this effect was not significant in year 2. This may be due to soil nutrient leaching and/or poor root establishment in year 1 as a result of heavy rainfall. Since nutrients in conventionally fertilized soil are more readily crop available, it is possible that rainfall effects were exacerbated in the organically fertilized soil. Year 1 was a poor year for crop growth with frequent heavy rain and this was reflected in lower mean floret weight across all treatments. Year 2 was an improved year for crop production with a better yield seen for both varieties. Variety 'Belstar' performed particularly well under improved climatic conditions with a more typical level of rainfall in year 2 as indicated by the significant main effect of variety ($p=0.0242$) in this year.

In both years infestations with caterpillars of Large White butterfly and Diamond-back moth occurred throughout the crop and were effectively treated with Pyrethrum in the organic pest-control (OP) plots and with Decis in the conventional pest-control (CP) plots. At harvest broccoli florets were scored for incidence of insect or other damage, and fungal and bacterial diseases prevalent in North Country Dublin including White Blister (*Albugo candida*) and Wet Rot (*Erwinia carotovora* and *Pseudemonas* spp.). Levels of insect damage and disease were generally low and were not different between treatments in either year.

Overall no significant differences in yield were found between fully organic (OS+OP) and fully conventional (CS+CP) management in any year (Table 3). However a significant soil treatment main effect was found for floret weight in year 1 only with lower weights recorded for broccoli grown under organic soil management (OS). These data indicate that soil treatment rather than pest-control was the primary driver of broccoli yield and quality. We

suggest that under favourable conditions such as year 2, both organic and conventional fertilizer performed well. But under stressful conditions (such as year 1) the conventional soil (CS) treatment provided readily plant available mineral fertilizers which enabled these plants to outperform those under organic soil (OS) treatment.

Total phenolic and total flavonoid content

Levels of total phenolics (Figure 1 and Table 3) in year 1 were in the range 267.0 ±18.4 GAE mg/100g FW to 376.5±19.0 GAE mg/100g FW, and were considerably lower in year 2, ranging from 69.3±11.5 to 106.8±7.5 GAE mg/100g FW. Thus levels in year 2 equate to around one third to one quarter of the levels found in year 1. Total flavonoid content showed the reverse trend (Figure 2 and Table 3) and ranged from 5.6±1.1 to 25.9±3.4 CE mg/100 FW in year 1, with higher levels in year 2 ranging from 23.8±3.5 to 35.4±5.2 CE mg/100 FW across treatments.

The major phenolic compounds found in broccoli include flavonols such as quercetin and kaempferol glycosides, hydroxycinnamoyl derivatives and chlorogenic acids.[22] Phenylalanine ammonia lyase (PAL) the key entry point enzyme for synthesis of phenolic compounds is well known to be up-regulated by stresses including UV light, low temperature, nutrient deficiency, wounding and pest or pathogen attack. [31] The branch pathway to flavonoid synthesis is controlled by chalcone synthase (CHS). Flavonoid synthesis is commonly reported to be up-regulated by light in several crops including broccoli. In a three year field study which examined levels of the flavonols kaempferol and quercetin in three broccoli varieties ('Marathon', 'Lord' and 'Fiesta') the level of total solar radiation over the growing period had a significant effect on both flavonols with higher levels under increased radiation. [20] We propose that the higher levels of total flavonoids in year 2 in this study are due to higher light levels; whilst the high overall content of total phenolics in year 1 reflect increased

production of phenolic acids in response to stress caused by heavy rainfall and associated waterlogging of soils.

As with yield no significant differences in total phenolic or flavonoid content were found between fully organic (OS+OP) and fully conventional (CS+CP) management in any year (Table 3). However a significant soil treatment main effect was found for total phenolics and total flavonoids in year 1 only. Data indicated that in a poor year for crop growth (year 1) broccoli floret total phenolic and flavonoid content was increased when crops were grown in organic soil (OS). We ascribe this result to nutrient stress of the plants grown in the OS treatment. There was a significant strong positive correlation ($p<0.001$) in both years between total phenolic content and flavonoid content with $r = 0.74$ in year 1 and $r = 0.69$ in year 2.

The levels of total phenolics and flavonoids reported here are in agreement with levels found in green broccoli varieties in other studies. [13-16] Relatively few studies have compared phenolic content in vegetable crops grown under conventional and organic systems and to our knowledge no previous field trial studies have examined phenolic content in organic and conventionally grown broccoli. A recent well controlled Danish study [7] measured levels of flavonoids in onions and phenolic acids in carrots and potatoes grown over two years using either conventional or two types of organic system (cover crop fertility building or animal manure based fertilizer regime). The predominant phenolic acid in potatoes (5-caffeoylquinic acid) was significantly higher in the cover crop based organic system than in the conventional system.[7] In contrast phenolic acids in carrot and flavonoids in onion showed a large year to year variation but were not affected by production system. Our data indicate that total phenolic and flavonoid content in broccoli shows significant year to year variation but is not significantly different in fully organic (OS+OP) compared to fully conventional (CS+CP) production systems.

Total and individual glucosinolate content

Glucosinolate contents determined in broccoli samples are shown in Table 4. Peaks from all samples were resolved and easy to identify (Figure 3). The desulfoglucosinolates from broccoli were characterised by the presence of aliphatic glucosinolates and indol-3-yl glucosinolates. Mainly aliphatic glucoinolates - glucoraphanin, glucobrassicin, and neoglucobrassicin were the major components in most samples. 4-MeO-glucobrassicin was found at lower levels and progoitrin and sinigrin were found inconsistently in trace amounts in some samples. No evidence of glucoiberin nor 4-OH-glucobrassicin was found. Total glucosinolate content ranged from 3.0 to 20.9 μmol/g DW and averages were in the range 8.1±0.7 to 10.1±0.4 μmol/g DW (± standard error, n = 4) depending on treatment. Previous studies have shown that either glucoraphanin or glucobrassicin is the predominant glucosinolate in broccoli. [13-20,32-35] It is not yet well understood why in some studies one is in higher proportion than the other and this may be due to genetic (i.e. differences between cultivars) and/or environmental conditions (for review see [36]). In this study, the average concentration (± standard error, n = 4) of major glucosinolates across production systems and over the two year period ranged from 1.8.±0.2 to 3.3±0.3 μmol/g DW for glucoraphanin, 2.9.±.0.2 to 4.7±0.3 μmol/g DW for glucobrassicin, 0.2±0.02 to 0.4±0.03 μmol/g DM for 4-MeO-glucobrassicin and 1.4±0.2 to 3.1±0.1 μmol/g DW for neoglucobrassicin respectively. This is in agreement with ranges of glucosinolates in broccoli previously reported.[13, 17] No significant interaction or main effect of year on total or individual glucosinolate content was observed across the two year period. This is important because a significant difference in the content of secondary metabolites between years would indicate significant seasonal environmental interactions in the glucosinolate profile. Variability in biochemical data between years is often observed and is normally considered to be due to the crops response to different climatic conditions.[32-34] Differences in broccoli glucosinolate content due to

environmental conditions, in particular temperature and irradiation levels, have been reported in other studies. [20, 33] In the two seasons reported here, mean temperatures, humidity and wind speed were similar in both years, but rainfall levels were almost double in year 1 relative to year 2. Therefore rainfall levels do not appear to impact glucosinolate content. A number of previous studies have indicated a significant genotype effect on glucosinolate profile in broccoli.[13,17,34,35] Our data indicated levels of sinigrin were significantly higher ($p<0.05$) in cv. 'Fiesta' than in cv. 'Belstar' across treatments and years. Conversely glucoraphanin and 4-MeO-glucobrassicin were significantly higher ($p<0.05$) in cv. 'Belstar' than in cv. 'Fiesta' across treatments and years (Figure 4 and Table 4). Glucoraphanin has been extensively studied due to the potential bioactivity of its isothiocyanate breakdown product sulforaphane.[37] The finding of consistently higher levels in 'Belstar' is therefore of relevance from a health perspective. Levels of 4-MeO-glucobrassicin were lower in both varieties and its potential as bioactive compound has been less explored.

Glucobrassicin and neoglucobrassicin content was significantly ($p<0.05$) higher in samples grown under fully organic treatment (organic soil and organic pest-control; OS+OP) compared to samples grown under completely conventional treatment (CS+CP). Mixed model ANOVA showed that no significant main or interaction effects were observed for different soil treatments (Table 4). For neoglucobrassicin a significant variety by pest-control interaction was seen. It is important to highlight that although glucobrassicin contents were not significantly different for pest-control, values were near the 95% confidence threshold ($p=0.067$).

In terms of potential bioactivity the breakdown product of indolyl glucosinolates including glucobrassicin and neoglucobrassicin is indole-3-carbinol (I3C). I3C and its condensation product 3,3' diindolylmethane (DIM) have recently received considerable attention as anti-carcinogenic compounds. They exhibit potent anti-tumor activity with low levels of toxicity

in a wide range of human cancer cell lines.[38,39] The finding of statistically significant higher levels of glucobrassicin and neoglucobrassicin in broccoli grown under organic management practices would appear to be especially robust since similar results were obtained in an earlier basket study. [9] In the study of Meyer *et al.* [9] levels of individual glucosinolates were profiled in market purchased broccoli obtained at monthly intervals over a 1 year period in Germany. Results indicated no significant differences in glucoraphanin content, whilst neoglucobrassicin and glucobrassicin were significantly higher ($p<0.01$) in organic than in conventional broccoli. The finding of similar results in two different types of study (market study and field trial) strengthen the conclusion that the indolyl glucosinolates glucobrassicin and neoglucobrassicin consistently occur at higher levels in organically grown broccoli across different varieties and geographical locations.

CONCLUSIONS

Our data indicated that yield (mean floret weight), total phenolic and flavonoid content in broccoli shows significant year on year variation, but is not significantly different in organic (OS+OP) compared to conventional (CS+CP) production systems. We hypothesise that in year 1 increased stress caused a generalised increase in total phenolic content via up-regulation of PAL – the key enzyme controlling entry of metabolites into central phenylpropanoid metabolism. We further hypothesise that since in year 2 environmental conditions were more favourable, PAL was not up-regulated, however the chalcone synthase controlled branch pathway into flavonoid synthesis, would be up-regulated by light. Thus under these conditions a greater proportion of phenolic synthesis would be shunted towards flavonoid synthesis. Further studies to investigate this hypothesis would be of interest.

In contrast total and individual glucosinolate content was unaffected by season, but levels of two specific glucosinolates – glucobrassicin and neoglucobrassicin - were

significantly higher in the fully organic production system. Levels of glucorophanin were also consistently higher in variety 'Belstar' than in 'Fiesta'.

These data underscore the nuanced regulation of levels of bioactive compounds in crop plants. It is becoming clear that plant foods contain a wide diversity of bioactive compounds which may be affected by genotype, and also respond differently to the plant's environment depending on the specific metabolite involved. We suggest that specific bioactive compounds will be responsive to production practices used in either organic or conventional agriculture whilst others will not. This complexity may account for much of the variability seen in literature reviews and meta-analyses (e.g. [5, 40, 41]) comparing organic and conventional food, and highlights the need for further well designed studies focussed on specific metabolites or groups of metabolities in known crop varieties.

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FIGURES

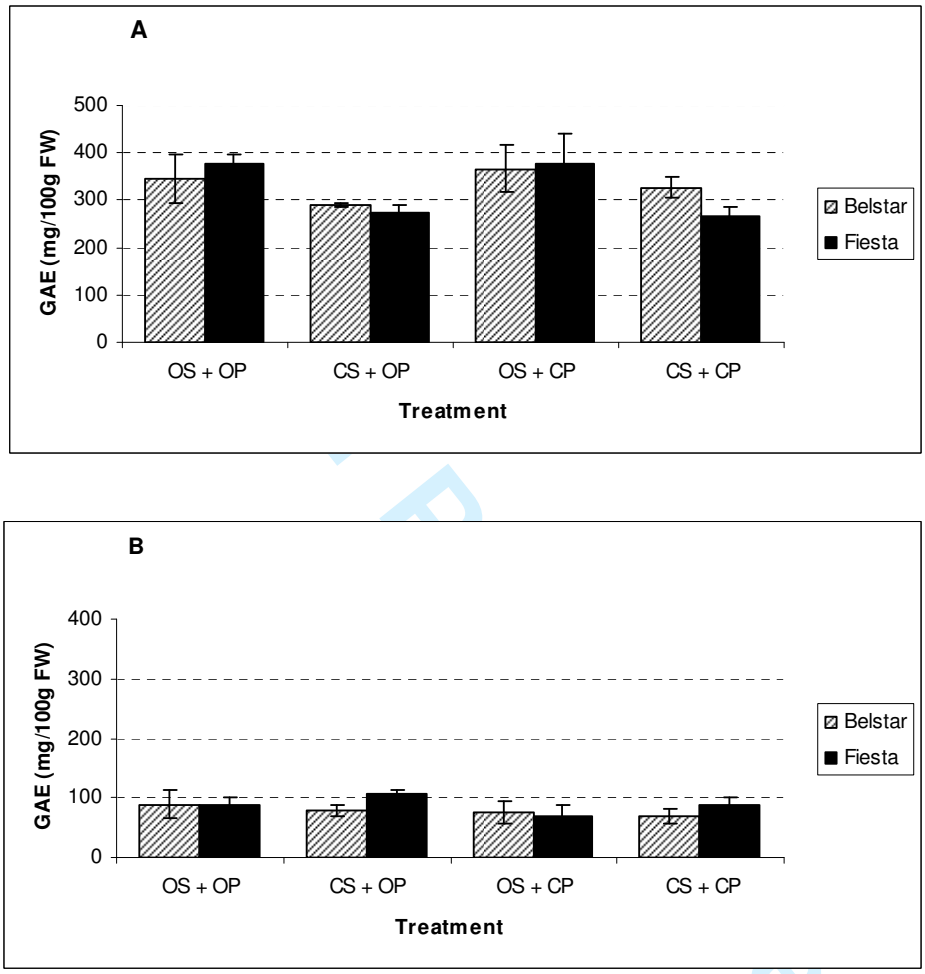

Figure 1. Total phenolic content (GAE mg/100g FW) in broccoli cv. 'Belstar' and cv. 'Fiesta' under different treatment combinations and in two harvest years (Panel A: year 1 and panel B: year 2). OS+OP means fully organic treatment and CS+CP means fully conventional treatment. Treatment codes: OS = organic soil treatment, CS = conventional soil treatment, OP = organic pest-control, CP = conventional pest-control. Bars show the mean and standard error of field replicates (n = 4).

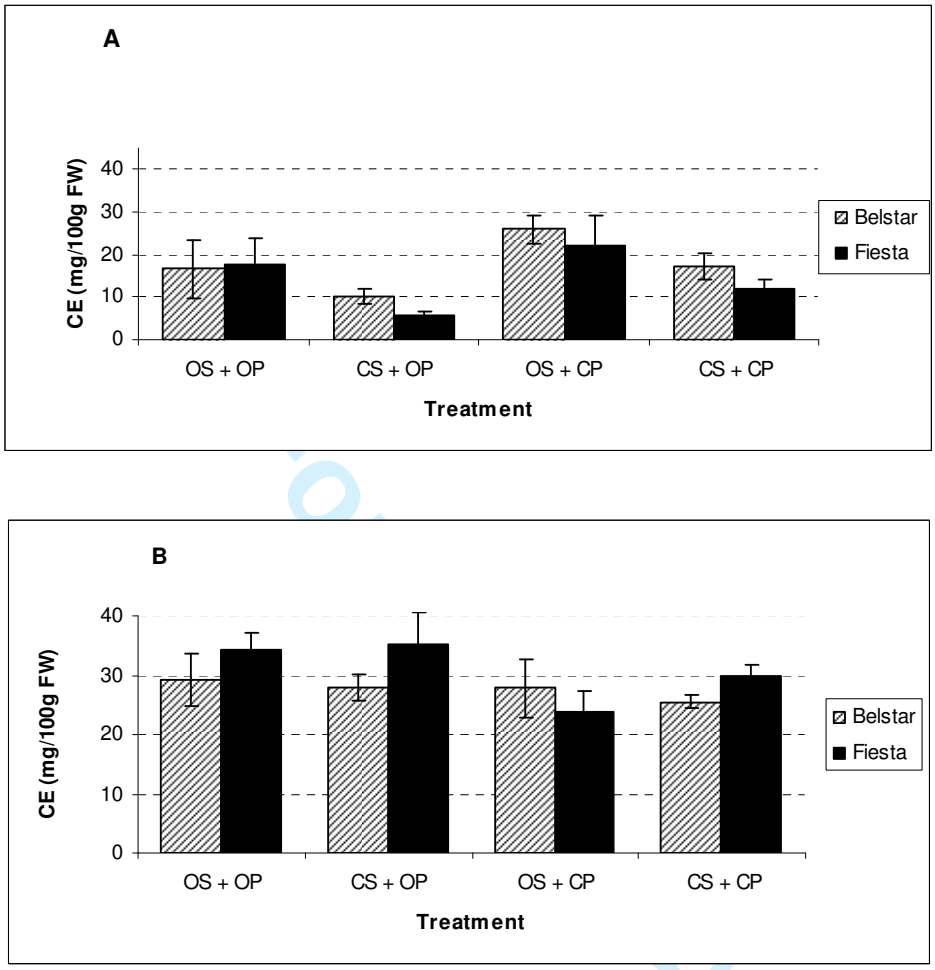

Figure 2. Total flavonoid content (CE mg/100g FW) in broccoli cv. 'Belstar' and cv. 'Fiesta' under different treatment combinations and in two harvest years (Panel A: year 1 and panel B: year 2). OS+OP means fully organic treatment and CS+CP means fully conventional treatment. Treatment codes: OS = organic soil treatment, CS = conventional soil treatment, OP = organic pest-control, CP = conventional pest-control. Bars show the mean and standard error of field replicates (n = 4).

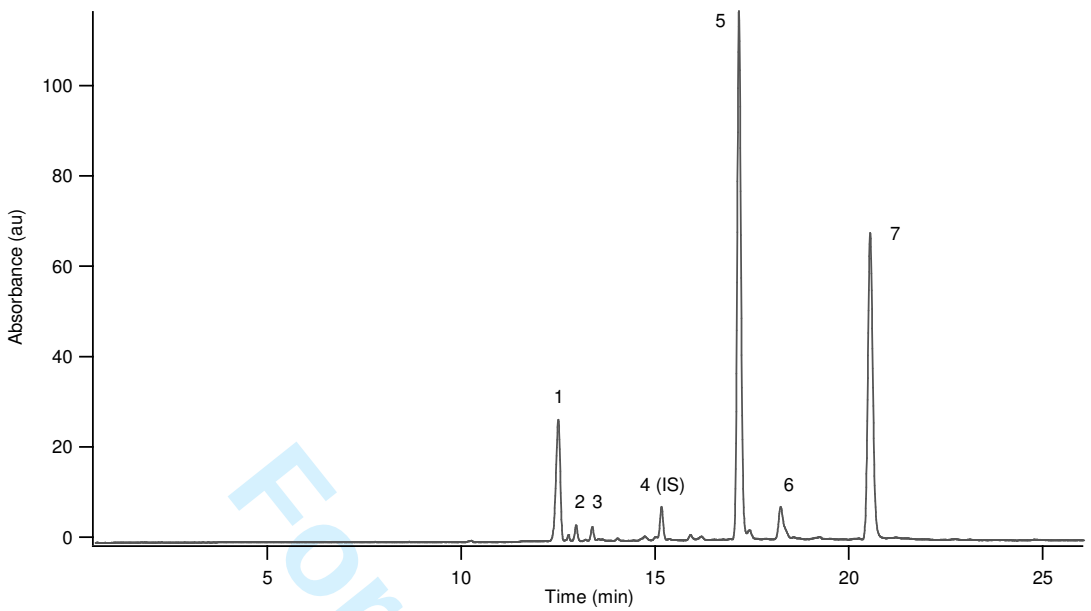

Figure 3. Representative electrochromatogram of desulfo-glucosinolates from broccoli. Each number above the peak represents a major identified desulfo-glucosinolate. **1**- desulfo-glucoraphanin; **2**-;desulfo-protoitrin; **3**- desulfo-sinigrin; **4**- desulfo-glucotropaeolin (used as internal standard); **5**- desulfo-glucobrassicin; **6**- desulfo-4MeO-glucobrassicin; **7**- desulfo-neoglucobrassicin.

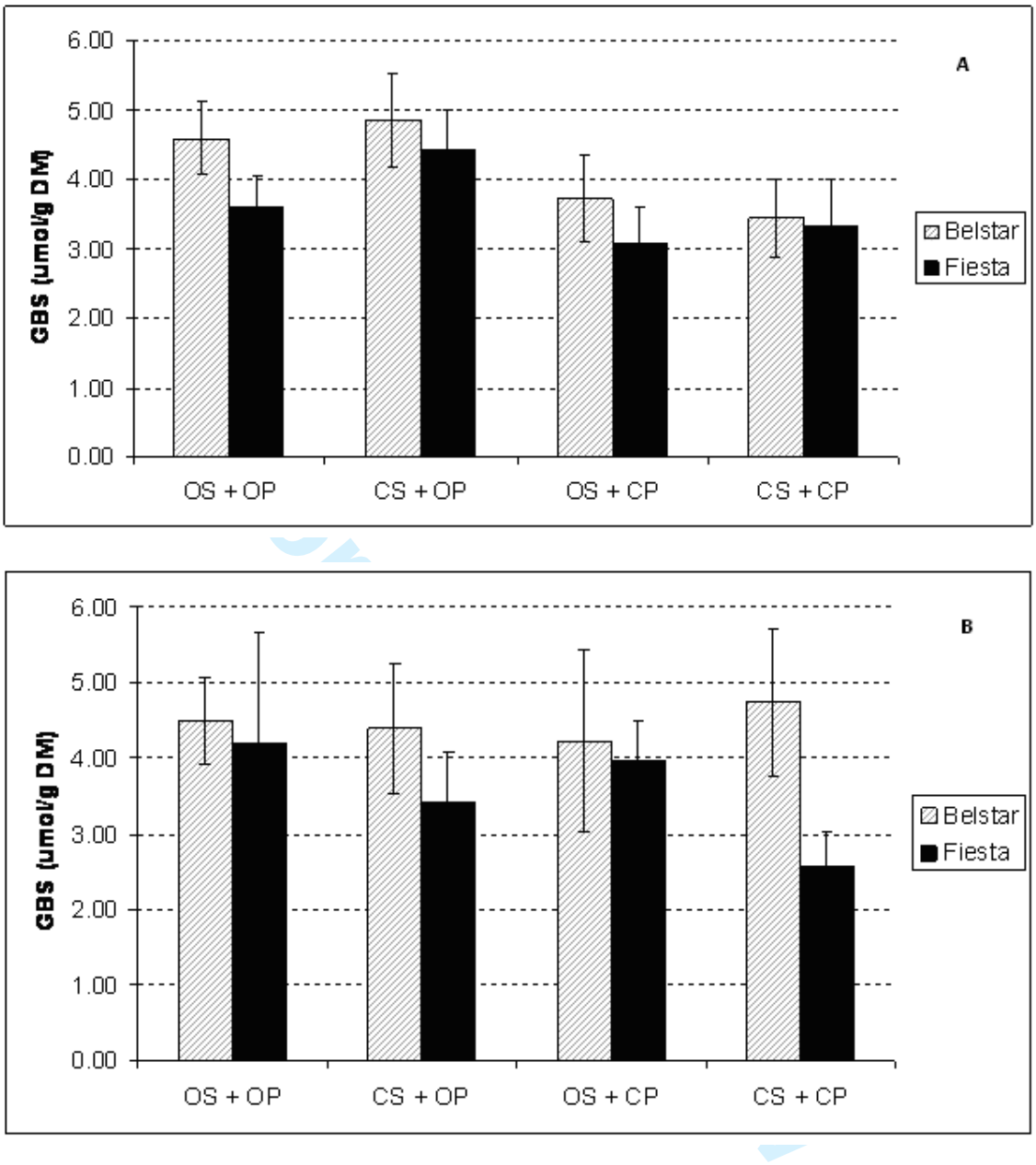

Figure 4. Average concentrations (µmol/g DW) of desulfo-glucobrassicin under different treatment combinations and two harvest years (Panel A: year 1 and panel B: year 2). OS+OP means fully organic treatment and CS+CP means fully conventional treatment. Treatment codes: OS = organic soil treatment, CS = conventional soil treatment, OP = organic pest-control, CP = conventional pest-control. Bars show the mean and standard error of field replicates (n = 4).

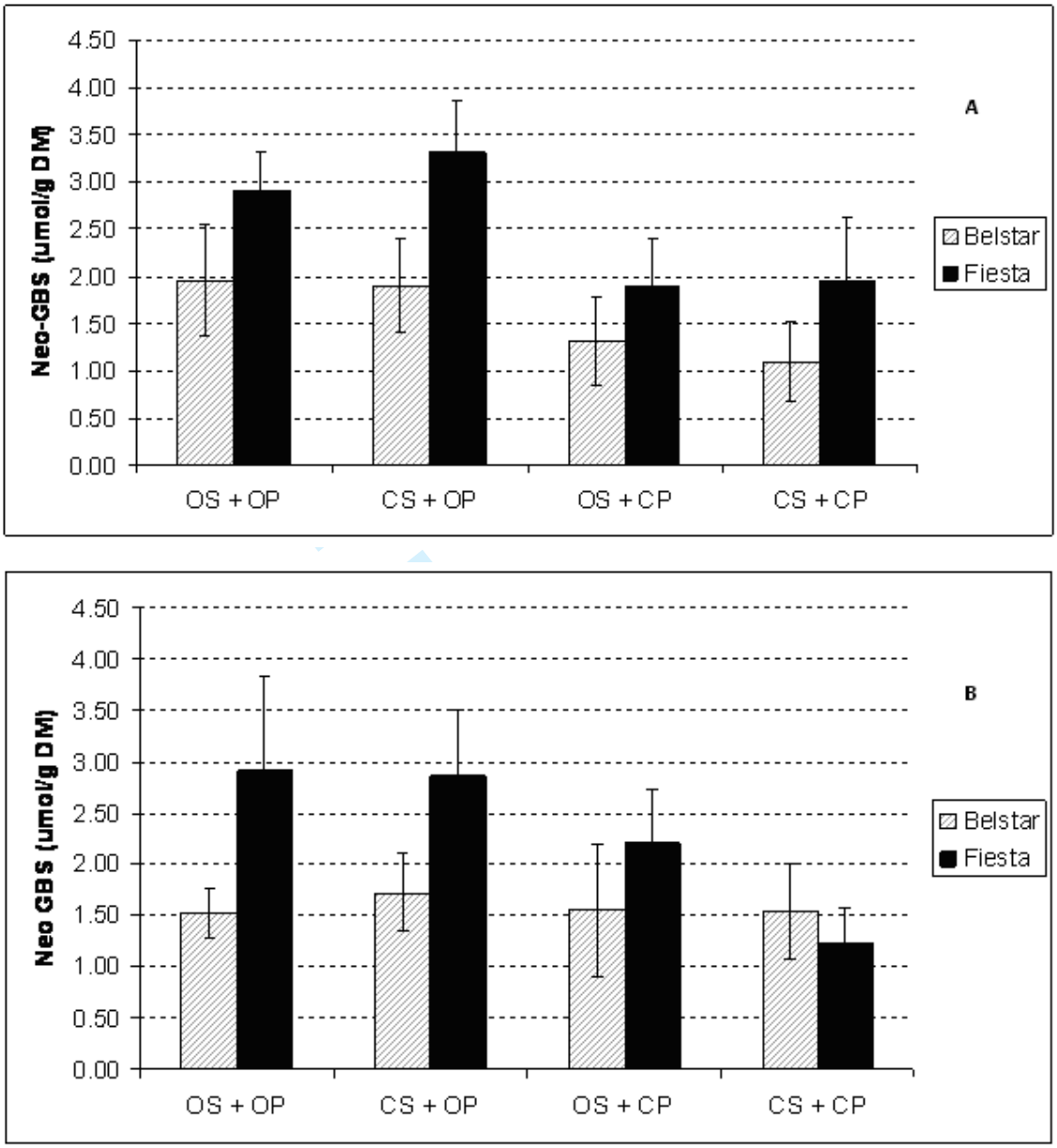

Figure 5. Average concentrations (µmol/g DW) of desulfo-neoglucobrassicin under different treatment combinations and two harvest years (Panel A: year 1 and panel B: year 2). OS+OP means fully organic treatment and CS+CP means fully conventional treatment. Treatment codes: OS = organic soil treatment, CS = conventional soil treatment, OP = organic pest-control, CP = conventional pest-control. Bars show the mean and standard error of field replicates (n = 4).

TABLES

Table 1. Different agricultural management treatments applied in this study.

	Organic	**Conventional**
Soil treatment	• Four year rotation: ley (red clover) → broccoli→ onion→ carrot • Additional organic fertilization as indicated by soil test • Winter cover crop	• No set rotation, plots randomly allocated each year • Mineral fertilizers as indicated by soil test • No ley crop • No winter cover crop
Pest-control treatment	• Certified organic seed • Refuge area • Mechanical pest-control • Weed control by mechanical methods • Certified organic treatments (e.g. garlic spray)	• Chemically treated seed • Chemical weed control (herbicides) • chemical pest – control (fungicides and insecticides)

Table 2. Specific pest-control and soil treatments used for broccoli cultivation in the Teagasc Kinsealy Systems Comparison trial in 2009 (year1) and 2010 (year 2).

	YEAR 1 (2009)	YEAR 2 (2010)
PEST-CONTROL TREATMENT		
Organic Pest-control (OP)	ECOguard garlic spray modular drench[3] (4%v/v at 2L/m^2) Brassica collars Mechanical weeding (hand hoeing). Pyrethrum 5EC [3] (1.1L/ha)	ECOguard garlic spray modular drench[3] (4%v/v at 2L/m^2) Brassica collars Mechanical weeding (hand hoeing). Pyrethrum 5EC [3] (1.1L/ha)
Conventional Pest-control (CP)	Dursban modular drench[3] (50ml per 5000 modules), Roundup[1] (4L/ha), Stomp (3.3L/ha), Butisan S[1] (1.5L/ha), Aramo [1] (1.5L/ha), Decimate[1] 20L/ha, Decis[3] (300ml/ha)	Proplant modular drench (2.4ml in 0.8L per 216 tray), Dursban modular drench[3] (50ml per 5000 modules), Roundup[1] (4L/ha), Stomp (3.3L/ha), Butisan S[1] (1.5L/ha), Stratos Ultra[1] (3L/ha), Decis[3] (300ml/ha)
SOIL TREATMENT		
Organic Soil treatment (OS)	Previous crop – grass set aside >10 years N 115 kg/ha P 64 kg/ha K 180 kg/ha B 11kg/ha Applied as Greenvale plant food (4.5:3:3) (pelleted chicken manure + calcified seaweed), ProKali (3:0:14) and Solubor. A top dress equivalent to 20kg/ha N was applied on 14th August.	Previous crop – Red clover (2009) N 115 kg/ha P 64 kg/ha K 180 kg/ha B 11kg/ha Applied as Greenvale plant food (4.5:3:3) (pelleted chicken manure + calcified seaweed), ProKali (3:0:14) and Solubor.
Conventional Soil treatment (CS)	Previous crop – grass set aside >10 years N 115 kg/ha P 64 kg/ha K 180 kg/ha B 11kg/ha Applied as CAN (27% N), Single superphosphate (7.8%P), Sulphate of potash (42% K) and Solubor. A top dress equivalent to 20kg/ha N was applied on 14th August.	Previous crop – Any (2009) N 115 kg/ha P 64 kg/ha K 180 kg/ha B 11kg/ha Applied as CAN (27% N), Single superphosphate (7.8%P), Sulphate of potash (42% K) and Solubor.

[1] Herbicide, [2] Fungicide, [3] Insecticide.

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Table 3. Broccoli floret weight, total phenolic and total flavonoid content under different management practices.

	Mean floret weight (g)		Total phenolic content (GAE mg/100g FW)		Total flavonoid content (CE mg/100g FW)	
Treatment:	**Year 1**	**Year 2**	**Year 1**	**Year 2**	**Year 1**	**Year 2**
V1 + OS + OP	252.9±27.2	442.3±19.9	345.7±51.3	88.5±24.0	16.6±6.9	29.2±4.5
V1 + CS + OP	268.4±22.3	383.3±20.8	290.8±3.9	79.3±9.6	10.2±1.8	27.9±2.2
V1 + OS + CP	235.8±12.3	449.2±19.9	365.9±50.2	74.9±19.1	25.9±3.4	27.8±4.9
V1 + CS + CP	293.2±33.7	363.1±19.5	326.8±22.4	69.3±11.5	17.2±3.1	25.6±1.2
V2 + OS + OP	230.6±20.3	300.0±7.3	376.5±19.0	87.6±13.5	17.8±6.0	34.3±2.8
V2 + CS + OP	307.±17.0	247.1±78.5	273.4±16.0	106.8±7.5	5.6±1.1	35.4±5.2
V2 + OS + CP	253.7±8.5	327.2±24.3	375.6±63.0	70.2±19.1	22.0±7.3	23.8±3.5
V2 + CS + CP	293.9±41.4	363.9±15.2	267.0±18.4	86.7±15.4	11.9±2.1	29.8±2.0
Statistical significance:						
ANOVA p values:						
Replicate	0.9501	0.7573	0.9645	0.1610	0.4220	0.5511
variety	0.5924	**0.0242**	0.7736	0.3971	0.5049	0.4315
soil	**0.0060**	0.0775	**0.0135**	0.5927	**0.0152**	0.6748
pest-control	0.9044	0.3604	0.7419	0.2070	0.1627	0.1025
variety x soil	0.4692	0.1507	0.2991	0.2031	0.6107	0.2284
variety x pest-control	0.9785	0.0845	0.5692	0.7178	0.6720	0.1655
soil x pest-control	0.9329	0.4742	0.9263	0.9814	0.9793	0.6376
variety x soil x pest-control	0.2049	0.1905	0.8488	0.8701	0.7489	0.4929
fully conventional vs. fully organic	0.1698	0.8379	0.1600	0.4662	0.6037	0.1995

Data shown are mean ± standard error of the mean (n =4). OS+OP means fully organic treatment and CS+CP means fully conventional treatment. Since the difference between years was significant data for individual years is shown separately. Treatment codes: V1= cv. 'Belstar', V2 = cv. 'Fiesta' OS = organic soil treatment, CS = conventional soil treatment, OP = organic pest-control, CP = conventional pest-control. ANOVA p values in bold are significant at $p<0.05$.

Table 4. Broccoli total and individual glucosinolate content (μmol/g DW) under different management practices.

Treatment	Total glucosinolates	Glucoraphanin	Sinigrin	Glucobrassicin	4-MeOGlucobrassicin	Neoglucobrassicin
V1 + OS + OP	9.26±0.57	3.22±0.21	0.37±0.05	4.54±0.21	0.36±0.03	1.73±0.13
V1 + CS + OP	10.09±0.41	3.04±0.22	0.38±0.03	4.66±0.29	0.35±0.02	1.82±0.16
V1 + OS + CP	9.01±0.63	3.30±0.25	0.40±0.06	3.96±0.36	0.33±0.03	1.43±0.18
V1 + CS + CP	9.06±0.46	3.15±0.28	0.40±0.03	4.04±0.30	0.35±0.02	1.30±0.13
V2 + OS + OP	9.99±0.70	2.59±0.26	0.94±0.55	3.90±0.39	0.26±0.03	2.91±0.28
V2 + CS + OP	9.15±0.41	1.78±0.19	0.65±0.16	3.99±0.24	0.25±0.02	3.10±0.21
V2 + OS + CP	8.38±0.35	2.79±0.32	0.50±0.12	3.43±0.20	0.19±0.02	2.02±0.18
V2 + CS + CP	8.09±0.67	2.83±0.34	1.64±1.10	2.92±0.23	0.19±0.02	1.58±0.16
Statistical significance:						
ANOVA p values:						
year	0.8272	0.0636	0.4388	0.8331	0.1054	0.2903
block	0.7676	0.0898	0.5193	0.6320	0.6335	0.7252
variety	0.2270	**0.0329**	**0.0466**	0.1872	**0.0183**	0.1223
soil	0.8776	0.2880	0.4852	0.5301	0.6759	0.2416
pest-control	0.2083	0.1182	0.6225	0.0670	0.3352	**0.0197**
variety x soil	0.2194	0.8252	0.5883	0.4062	0.5748	0.3861
variety x pest-control	0.4112	0.2318	0.5160	0.9482	0.3734	**0.0145**
soil x pest-control	0.9133	0.3195	0.0927	0.6916	0.3135	0.2475
variety x soil x pest-control	0.3401	0.3399	0.0501	0.5361	0.7351	0.6208
fully conventional vs. fully organic	0.1847	0.6651	0.3407	**0.0187**	0.2501	**<.0001**

Data shown are mean ± standard error of total and individual glucosinolates over two trial years ((μmol/g DW). OS+OP means fully organic treatment and CS+ CP means fully conventional treatment. Treatment codes: Variety V1= 'Belstar', V2 = 'Fiesta' ; OS = organic soil treatment, CS = conventional soil treatment; OP = organic pest-control, CP = conventional pest-control. ANOVA p values shown in bold are significant at $p<0.05$

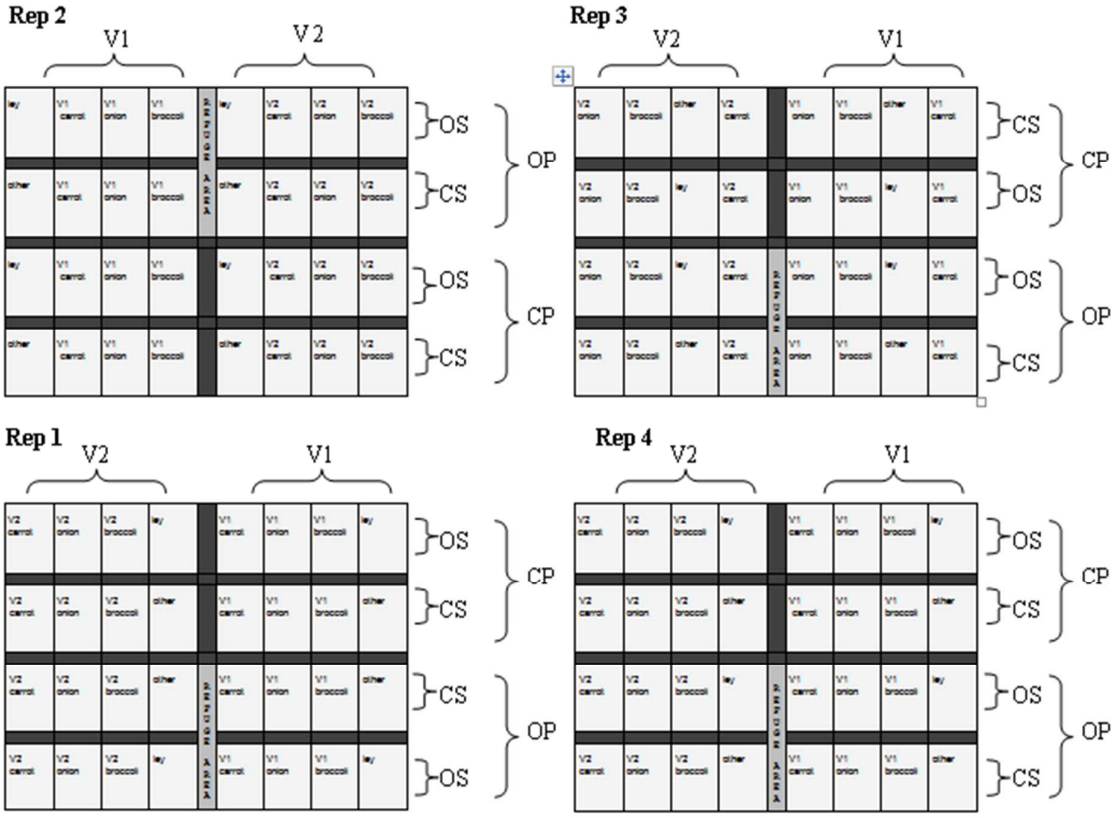
Supplementary material. Table S1. Climatic conditions during broccoli crop production in 2009 and 2010. T = Mean temperature (°C), TM = Mean maximum temperature (°C), Tm = Mean minimum temperature (°C), H = Mean humidity (%), V = Mean wind speed (Km/h), RA = Daily indicator for occurrence of rain or drizzle (total days), PP = Total monthly precipitation amount (mm).

	Month	T	TM	Tm	H	V	RA	PP
2009	April	8.8	12.4	4.3	84.0	17.9	22	70.1
	May	11.0	15.1	6.9	80.4	22.5	24	74.7
	June	13.8	18.2	8.4	78.6	16.6	19	65.0
	July	14.7	18.8	10.8	82.6	18.4	30	162.8
	August	15.4	19.3	11.4	81.4	20.7	28	69.3
	September	12.6	16.7	8.5	84.0	18.8	18	23.1
	Season (mean or total)	**12.7**	**16.8**	**8.4**	**81.8**	**19.2**	**141**	**465.1**

	Month	T	TM	Tm	H	V	RA	PP
2010	March	5.2	9.7	0.1	81.1	17.2	17	55.6
	April	8.1	12.9	2.5	78.1	15.6	14	26.7
	May	10.2	14.7	4.8	78.3	15.3	20	29.2
	June	14.5	19.2	8.9	78.8	14.5	17	57.4
	July	15.7	19.6	12.1	80.4	19.5	28	76.7
	August	14.0	18.3	9.6	80.8	17	22	47.5
	Season (mean or total)	**11.3**	**15.7**	**6.3**	**79.6**	**16.5**	**118**	**293.1**

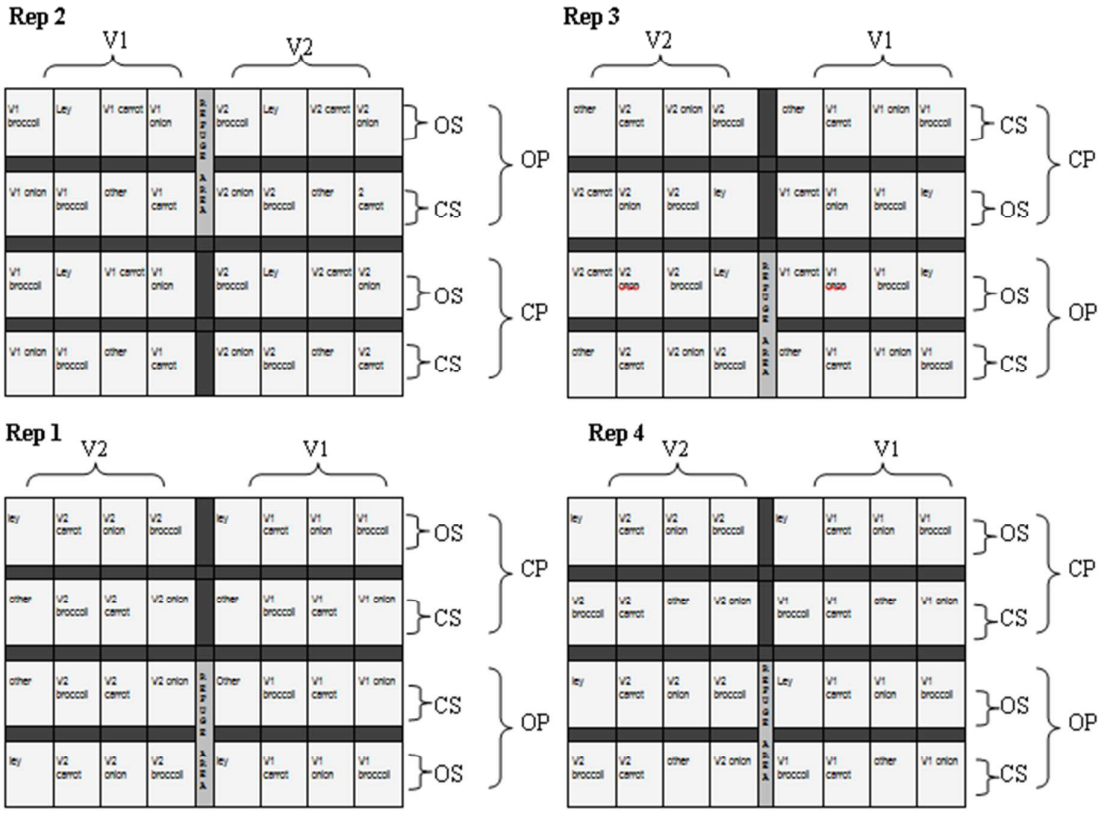
9

Year 1: Kinsealy Systems Comparison Trial



Supplementary material. Figure S1. Diagram showing the Kinsealy Systems Comparison Trial in Year 1. V1 = Variety 1, V2 = Variety 2 (for broccoli V1 = 'Belstar', V2 = 'Fiesta'). CP = Conventional pest-control treatment, OP = Organic pest-control treatment. CS= Conventional soil treatment, OS= Organic soil treatment. Dark grey areas indicate 1 m wide grass inter-plot areas. Light grey indicates permanent refuge areas planted with buckwheat, vetch, borage, sunflower, coriander, fennel, cornflower, corn marigold, cocksfoot grass to encourage beneficial insects as part of the OP treatment. Note: Crops within the organic soil treatment (OS) move each year according to a set rotation (Ley → broccoli→onion→carrot). Crops within conventional soil treatment (CS) do not follow a set rotation pattern and move randomly, taking into account practices of commercial growers e.g. onion 4 year gap before returning to same land (1 in every 4). The "other" crop used in CS plots in year 1 was wheat (*Triticum aestivum*) and in year 2 was lettuce (*Lactuca sativa*).

Year 2: Kinsealy Systems Comparison Trial



Supplementary material. Figure S2. Diagram showing the Kinsealy Systems Comparison Trial in Year 2. V1 = Variety 1, V2 = Variety 2 (for broccoli V1 = 'Belstar', V2 = 'Fiesta'). CP = Conventional pest-control treatment, OP = Organic pest-control treatment. CS= Conventional soil treatment, OS= Organic soil treatment. Dark grey areas indicate 1 m wide grass inter-plot areas. Light grey indicates permanent refuge areas planted with buckwheat, vetch, borage, sunflower, coriander, fennel, cornflower, corn marigold, cocksfoot grass to encourage beneficial insects as part of the OP treatment. Note: Crops within the organic soil treatment (OS) move each year according to a set rotation (Ley → broccoli→onion→carrot). Crops within conventional soil treatment (CS) do not follow a set rotation pattern and move randomly, taking into account practices of commercial growers e.g. onion 4 year gap before returning to same land (1 in every 4). The "other" crop used in CS plots in year 1 was wheat (*Triticum aestivum*) and in year 2 was lettuce (*Lactuca sativa*).