

Journal of Organic Systems

Volume 9 Number 2

October 2014

www.organic-systems.org

ISSN 1177-4258

Contents

Editorial: Celebrating 2014 UN International Year of Family Farming: A reflection from New Zealand

Brendan Hoare, Jane Adams, Christine Dann, Richard Main and Kiera Jacobson 2

Genetically engineered crops, glyphosate and the deterioration of health in the United States of America

Nancy L. Swanson, Andre Leu, Jon Abrahamson and Bradley Wallet 6

Investigating the context of purchase choices to further understanding of switching behaviour

J. Henryks and D. Pearson 38

Control of root-burrowing nematode (*Radopholus similis*) in banana using extracts of *Azadirachta indica* and *Allium sativum*

Ezra S. Bartholomew, Richard A.I. Brathwaite and Wendy-Ann P. Isaac 49

The impact of peat moss and sheep manure compost extracts on marigold (*Calendula officinalis* L.) growth and flowering

Alaa E. Hasan, Karim M. Bhiah & Mushtaq T. H. Al-Zurfy 56

Phosphorus-use efficiency, growth and yield of spelt wheat (*Triticum aestivum* ssp. *spelta*) compared with standard wheat (*T. aestivum* ssp. *vulgare*) in south-eastern Australia

J. Evans, R. Neeson, V. Burnett, D.J. Luckett and N.A. Fettell 63

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Editorial: Celebrating 2014 UN International Year of Family Farming: A reflection from New Zealand

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It is a wonderful thing to be a part of something great from the beginning. Brendan was approached a few years back by the World Rural Forum (WRF)¹ with a request to support their idea of having 2014 as the International Year of Family Farming (IYFF)². I was, at that stage, their only contact point in Oceania. The request, while a little wild, seemed plausible and the correct thing to support; I backed the need and passion for this important initiative. Through such responses, their idea became a reality.

WRF's global supporters had a brief meeting in Rome which resulted in the formation of the World Consultative Committee and concluded in a simple and realistic strategy: take the message back down to the regions and to the national level.

With no funds and this simple approach Oceania has seen the spreading of the message of the importance of family farming using existing programmes; such as, in the Pacific, that of Karen Mapusua on "Cultivating Community"³, and through the use of new communication tools, as Jennie Clarke⁴ has developed in Australia.

To celebrate the IYFF in New Zealand I formed a national steering committee. We carried out surveys, and held a series of workshops and events, to explore the role of family farming in New Zealand, and to identify opportunities and issues facing the country's family farmers. We found that even though New Zealand had become highly urbanised, most New Zealanders still have a strong tie to the land, and identify with the values of family farming. The resilience and ingenuity of the family farmer were identified as being a core part of New Zealand's national identity.

Perhaps this is not surprising, as the development of New Zealand as a modern nation was built on the hard work of pioneering family farmers who modified the land and introduced the animal and plant species that formed the foundation of our economy. Pastoral, arable and horticultural farming were initially based on introduced species, the first brought by Maori from the Pacific, and then many more by settlers from Britain and Europe. In the late nineteenth century the Government supported family farming by purchasing and subdividing large pastoral leases and Maori land for sale to family farmers.

Family farming still dominates all farming systems, though recent times have seen a growing trend towards multiple farm ownership by farming families and, to a lesser extent, corporate farming business, particularly in dairy, sheep and beef farming. Most vegetable, arable and fruit production, beekeeping, amenity plant breeding and production and specialist nurseries, as well as some aquaculture and farm forestry, are still predominately family farming businesses.

An important element in our work in celebrating the IYFF is to ensure the global message that the year is not about the 'farm' or the 'farmer' but about the importance of 'farming'. 'The Global Dialogue on Family Farming' is inclusive of the whole community, farm labour and all methods of generating livelihood from nature.

New Zealand is unusual amongst OECD countries in that a high proportion of its overseas income is derived from the products of farming activities. According to NZ Statistics, in 2012 primary products accounted for 70 percent of all goods exported, and over 50% of New Zealand's total export earnings. Family farming also contributes to another major income source and growth industry, tourism. The country's scenery, natural environment and a range of outdoor activities make New Zealand a popular tourist destination; and farming activities provide an essential part of the iconic landscapes that attract visitors to this country.

¹ <http://www.ruralforum.net/Default.asp?id=en>

² www.fao.org/family-farming-2014/en and www.familyfarmingcampaign.net

³ http://lists.spc.int/mailman/listinfo/cultivating_community

⁴ familyfarms.enviroed4all.com.au

An important feature of New Zealand's primary sector is its huge agricultural diversity. Historically, entrepreneurial families have been responsible for the introduction and development of new products, some of which have become major industries, for example: kiwifruit, wine grapes, farmed venison, avocado and King salmon. The most famous of these is kiwifruit. While the fruit is originally from China, it was 'Kiwi entrepreneurial' behaviour that developed and bred the species to become a world renowned commercial fruit. The primary variety – 'Hayward' – bears the family name of the breeder and commercial developer of the fruit. This is also true for farm engineering and science best practices, including innovations in electric fencing and herd breeding.

The organic sector continues to grow at 8% per annum, largely driven by the fresh fruit sector; the vast majority of these operations being family owned. The uniqueness of the sector is that its organisational framework is based on representation that covers home gardeners, consumers, Maori organisations, retailers, exporters, and the certification capacity under one lead organisation to ensure it has a single voice to government and wider interests. This model has unfortunately not been replicated in the primary sector as a whole in NZ.

New Zealand also has an extensive coastline which supports thriving aquaculture and fisheries industries. New Zealand implements a sustainable fisheries policy with a quota system to regulate catches of wild species. Pacific oyster, green-lipped mussels and quinnat salmon are the three main species farmed in New Zealand; the first two are significantly supplied through family businesses.

Many family farming businesses value add to their farmed products, or support other local family businesses to do so. This has developed a rich tapestry of innovative food and consumer products that support rural economies and supply local and niche export markets. Examples include olive and avocado oils, artisan cheeses, beverages and preserves, natural cosmetics, nutraceuticals, and even high-end fashion brands. Local supply chains and farmers markets, local cafes and tourist activities (food festivals, wine and food tasting, etc.) are all well established and supported by regional economic strategies. The New Zealand family farming story is central to the marketing of these products.

Although the business environment is generally supportive of family farming, the IYFF national workshops and surveys identified some key issues that are impacting family farming in New Zealand; most notable were succession, debt, rural connectivity and connectivity in general, managing the environment and identity.

Strong and prolonged growth in land values has made affordable land ownership challenging. For example, in some areas general farmland has increased in value at a compounding rate of 10% per annum over the past 20 years. In the areas that are closest to urban centres, demand for 'lifestyle blocks' has pushed the price of land even higher. Such inflated land values have created a disconnect between the value of the land and its actual productive capacity. This has created a challenge for the next generation of farmers to be able to secure adequate entry capital and sufficient cash flow required to farm. Scale of farm ownership, rather than profitability, is still seen as a symbol of success; and in pastoral farming and some horticulture operations there is a trend toward larger farms and cooperative or corporate ownership. Although these farms are still managed by rural families, they are now tenants on the land rather than owners, and are likely to have a different relationship to the land and its stewardship.

Keeping the farm in the family therefore now requires a lot more thought and effort than it once did, and this is giving rise to a range of innovations in family farming structures. These include equity partnerships, community supported agriculture, land trusts, indigenous Maori ownership models, and other combinations and permutations designed to keep families in farming and close to the land. A common tension we identified in succession planning is that generations X and Y are more impatient than their baby boomer parents, and seek control and input into farm decision making 'now'. They lead a changing world where being responsive and quick to change is critical for commercial survival. In contrast, we found that many of the farmers who are close to retirement want to put off the decisions about succession until they are ready to leave the farming business.

Increasing land values and the push to increase farm size has created a need amongst family farmers to take on high level of debt to fund land purchases as well as new technologies. Our workshops identified that the problem of high debt levels is extensive and exacerbated by lack of financial literacy among some farmers, many yet to become confident with modern account keeping, forecasting and budgeting methods. In addition, it was felt that many credit managers lack a good understanding of

primary production systems, especially when it comes to assessing the financial risks associated with new or unfamiliar production practices. Furthermore, both parties were often misled into thinking that the route to financial success is increasing gross turnover, rather than assessing every aspect of primary production and consumption by how each contributes to net profitability.

Creating greater value from farming activities is seen to be an important part of reducing debt. Many family farms increase value through the development of local niche products and by connecting directly with consumers. However, supply chain structures that connect producers with consumers directly, and do it well, are currently weak, and poorly supported and, for many family businesses, the lack of scale has been identified as a major limitation to successful development of supply chains, processing capacity and marketing. It was felt that better farm scale models of risk management are needed, and policy needs to be developed accordingly, otherwise it will continue to be difficult for family farmers to significantly increase the value of their products.

Many programmes have been developed by the industry sector and voluntary organisations to assist rural families to improve their business skills, manage debt and plan for succession. Examples are the 'Beef + Lamb NZ' programme for farmers, facilitated by the Agri-Women's Development Trust (AWDT), to support business development and leadership in rural communities. Dairy NZ has run Farmers' Forum workshops on succession planning and farm management; and, as part of its celebration of IYFF, Rural Women NZ ran a series of road shows around the country at which seminar topics of succession and rural business management were presented.

Pressure to increase profits to service debt often creates real tensions that result in environmental degradation. Not all family farmers care for the land well; but history shows us that as a general rule it is dangerous to divorce ownership and management of the land from the families who live on it and care for it as their home. To this end, for nearly two centuries, New Zealand farming families have been building attractive homes, creating gardens around them, and planting trees and keeping animals for pleasure as well as utility. In contrast, absentee owners, tenant farmers and hired labour generally have less interest in creating a safe and attractive place to live. Most family farmers have a strong sense of land stewardship and practice sustainable land management. Many are actively involved in conservation through revegetation projects and pest control on their land, and work in conjunction with regional and central government departments to ensure these conservation projects are successful.

Technology is transforming agriculture and family farming; so that being connected to broadband is now as important to modern business as having access to a road or port. While technology and internet connectivity offer the opportunity to break down isolation barriers and boost family farm performance, access to high speed broadband is not universal throughout rural New Zealand. There are vast sectors of New Zealand that are not served well; some being as close as 30 minutes from our major cities. This electronic isolation is having a profound effect on rural families. Although a significant government initiative is aiming to achieve broadband coverage across the country, no telecommunication companies in New Zealand currently have strategies to effectively deliver broadband to the rural sector, and the lag in the provision of these internet services has created a digital capability gap within rural New Zealanders. Our research indicated that although there is interest and an understanding of these issues, while the private telecom companies see no profit in serving rural sector their focus will remain on the urban and under 35 year old markets.

Our surveys suggest these recent challenges to the viability of family farming have undermined the confidence of family farmers and their role in society. The need to make ends meet has drawn their focus of energy inward, and some have developed a siege mind-set, so that when discussions were held around the broader role of family farming (e.g., farmers being stewards of the land, stalwarts of community etc.), a number of them had an 'awakening' experience. Farming families not only provide economic support for other rural businesses, but also contribute to the social fabric of rural communities through volunteer activities (e.g., fire fighters, and governance and political roles); but several farmers confessed that they had not considered the wider roles of their businesses as 'anchors' within their communities.

We also found a significant difference between the views of younger and older farmers regarding the future of family farming. Many older farmers surveyed were pessimistic about the future of family farming, perceiving a loss in the family farming lifestyle and ability to own land. In contrast, younger farmers were generally very confident about the future of family farming, although they regarded the farm as a business rather than a lifestyle, and accepted that they would need to possibly seek outside investment to increase the sophistication of the business, ensure farming was conducted within

environmental limits, and fight for good connectivity to provide strong ties to the global economy. It appears that future family farmers will continue to be the innovators and the backbone of New Zealand's rural economy.

Participants in our 2014 IYFF workshops agreed that fostering discussion about and support for family farming's positive contribution to the community and the nation was an important activity that needs to be continued beyond the 2014 International Year of Family Farming.

Genetically engineered crops, glyphosate and the deterioration of health in the United States of America

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Abstract

A huge increase in the incidence and prevalence of chronic diseases has been reported in the United States (US) over the last 20 years. Similar increases have been seen globally. The herbicide glyphosate was introduced in 1974 and its use is accelerating with the advent of herbicide-tolerant genetically engineered (GE) crops. Evidence is mounting that glyphosate interferes with many metabolic processes in plants and animals and glyphosate residues have been detected in both. Glyphosate disrupts the endocrine system and the balance of gut bacteria, it damages DNA and is a driver of mutations that lead to cancer.

In the present study, US government databases were searched for GE crop data, glyphosate application data and disease epidemiological data. Correlation analyses were then performed on a total of 22 diseases in these time-series data sets. The Pearson correlation coefficients are highly significant ($< 10^{-5}$) between glyphosate applications and hypertension ($R = 0.923$), stroke ($R = 0.925$), diabetes prevalence ($R = 0.971$), diabetes incidence ($R = 0.935$), obesity ($R = 0.962$), lipoprotein metabolism disorder ($R = 0.973$), Alzheimer's ($R = 0.917$), senile dementia ($R = 0.994$), Parkinson's ($R = 0.875$), multiple sclerosis ($R = 0.828$), autism ($R = 0.989$), inflammatory bowel disease ($R = 0.938$), intestinal infections ($R = 0.974$), end stage renal disease ($R = 0.975$), acute kidney failure ($R = 0.978$), cancers of the thyroid ($R = 0.988$), liver ($R = 0.960$), bladder ($R = 0.981$), pancreas ($R = 0.918$), kidney ($R = 0.973$) and myeloid leukaemia ($R = 0.878$).

The Pearson correlation coefficients are highly significant ($< 10^{-4}$) between the percentage of GE corn and soy planted in the US and hypertension ($R = 0.961$), stroke ($R = 0.983$), diabetes prevalence ($R = 0.983$), diabetes incidence ($R = 0.955$), obesity ($R = 0.962$), lipoprotein metabolism disorder ($R = 0.955$), Alzheimer's ($R = 0.937$), Parkinson's ($R = 0.952$), multiple sclerosis ($R = 0.876$), hepatitis C ($R = 0.946$), end stage renal disease ($R = 0.958$), acute kidney failure ($R = 0.967$), cancers of the thyroid ($R = 0.938$), liver ($R = 0.911$), bladder ($R = 0.945$), pancreas ($R = 0.841$), kidney ($R = 0.940$) and myeloid leukaemia ($R = 0.889$). The significance and strength of the correlations show that the effects of glyphosate and GE crops on human health should be further investigated.

Keywords: Glyphosate, GMO, GE corn, GE soy, toxicology, obesity, cancer, hypertension, diabetes, Alzheimer's disease, senile dementia, autism, Parkinson's disease, inflammatory bowel disease, intestinal infections, hepatitis C, end stage renal disease, kidney failure, thyroid cancer, liver cancer, bladder cancer, pancreatic cancer, kidney cancer.

Introduction

Within the last 20 years there has been an alarming increase in serious illnesses in the US, along with a marked decrease in life expectancy (Bezruchka, 2012). The Centers for Disease Control and Prevention (CDC) estimates that the cost of diabetes and diabetes-related treatment was approximately \$116 billion dollars in 2007. Estimated costs related to obesity were \$147 billion in 2008 and cardiovascular diseases and stroke were \$475.3 billion in 2009. Health care expenditures in the US totaled 2.2 trillion dollars in 2007 (CDC, 2013a). The onset of serious illness is appearing in increasingly younger cohorts. The US leads the world in the increase in deaths due to neurological diseases between 1979-81 and 2004-06 for the 55-65 age group (Pritchard et al., 2013). These mental disorder deaths are more typical of the over 65 age group. There have been similar findings for obesity, asthma, behavior and learning problems, and chronic disease in children and young adults (Van Cleave et al., 2010). Type II diabetes in youth is being called an epidemic (Rosenbloom et al., 1999). The rate of chronic disease in the entire US population has been dramatically increasing with an estimated 25% of the US population suffering from multiple chronic diseases (Autoimmunity Research Foundation, 2012). These findings suggest environmental triggers rather than genetic or age-related causes.

During this same time period, there has been an exponential increase in the amount of glyphosate applied to food crops and in the percentage of GE food crops planted (Benbrook, 2012). We undertook a study to see if correlations existed between the rise of GE crops, the associated glyphosate use and the rise in chronic disease in the US.

Genetic engineering

To genetically modify a plant for herbicide tolerance, genes are identified which convey tolerance of the active chemical in the herbicide to the organism. In the case of glyphosate, glyphosate-tolerant genes were isolated from a strain of *Agrobacterium*. These were inserted into the genome of the plant via a multi-step process resulting in a plant that can withstand the direct application of the herbicide. Genetic modification is also utilised for developing insect resistant plants by using insecticidal proteins from *Bacillus thuringiensis*, or Bt toxin. The promoter used to drive the expression of the foreign genes is generally the 35S promoter from the Cauliflower Mosaic Virus (CaMV). Not only are the virus and bacteria genes themselves potentially harmful (Ho, 2013; Ewen & Pusztai, 1999), but the plants are sprayed directly with herbicides. The herbicide-tolerant plants absorb the poisons and humans and domestic animals eat them.

The GMO industry claims that genetic engineering is no different than plant hybridisation, which has been practiced for centuries (FDA, 1992). It is the reason they gave, which the US Food and Drug Administration (FDA) accepted, for not having to submit GE food to rigorous safety testing to obtain FDA approval. This distortion of the facts needs to be corrected. One critical issue is that multiple genes are being transferred across taxonomical kingdoms in ways that do not occur by natural breeding methods (Bohn et al., 2014).

All living things are classified according to a ranking system that starts with species and sub species. Closely related species are grouped together under a rank that is called a genus. Closely related genera are grouped together under the rank of family. There are seven ranks. Starting with the highest they are: kingdom, phylum or division, class, order, family, genus, species.

Plants, animals, fungi, viruses and bacteria belong to separate kingdoms. Natural inter-breeding can take place between some species that belong to the same genus and very occasionally between species of different genera. However, species that belong to different families do not inter-breed and definitely species that belong to different kingdoms such as plants, animals, fungi, bacteria and viruses do not inter-breed in nature. Plants, for example, do not inter-breed with animals, bacteria or viruses. Genetic engineering allows for the transfer of genes between kingdoms in a way that does not occur naturally.

The other great misconception is that only one gene with the desired trait is inserted. At this stage, science is not sophisticated enough to insert a single gene and get it to work. To overcome this problem, scientists have to combine the gene with the desired trait (such as herbicide tolerance or pesticide production) with other genes that will make it work, such as promoter genes and marker genes. The result is a complex construction of transgenes that can come from bacterial, viral, fish, plant and other sources. This is completely different from natural hybridisation.

The stance taken by Monsanto, Dow, Bayer and the other purveyors of both chemicals and genetically engineered seeds is that GE food is “substantially equivalent” to non-GE products. According to the US FDA, “the substances expected to become components of food as a result of genetic modification of a plant will be the same as or substantially similar to substances commonly found in food, such as proteins, fats and oils, and carbohydrates” (FDA, 1992, Section I). The FDA maintains that it is up to the biotech companies that manufacture GE seeds to research and determine the safety of their products.

But Bohn et al. (2014) were able to discriminate between organic, conventional and GE soybeans without exception, based on vitamin, fat and protein content. Furthermore, they were able to distinguish GE soybeans from both conventional and organic by their glyphosate and AMPA (glyphosate degradation product) residues, as well as substantial non-equivalence in numerous compositional characteristics of soybeans. The researchers stated, “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” (p. 207).

Exponentially increasing use of glyphosate world-wide

Since glyphosate was introduced in 1974 as the active ingredient in Roundup® it has become the most widely used herbicide for urban, industrial, forest and farm use (Monsanto, 2010). Pre-harvest application of glyphosate to wheat and barley as a desiccant was suggested as early as 1980, and its use as a drying or ripening agent 7-10 days before harvest has since become routine. It is now used on grain crops, rice, seeds, dried beans and peas, sugar cane and sweet potatoes (Monsanto, 2010; Orgeron, 2012; Orson & Davies, 2007). According to the Canadian Pulse Growers Association (PGA pamphlet, 2012), “Desiccants are used worldwide by growers who are producing crops that require ‘drying down’ to create uniformity of plant material at harvest. These products may also assist in pre-harvest weed control. In Canada, products such as diquat (Reglone) and glyphosate (Roundup) have been used as desiccants in pulse crops in the past, and there are new products on the way.” In 2012, 98% of spring wheat, 99% of durum wheat and 61% of winter wheat were treated with glyphosate or glyphosate salts in the US (USDA:NASS, 2013c). The glyphosate plots in this study include all formulations of glyphosate.

Monsanto, the manufacturer of Roundup®, states, “Since its discovery in the early 1970’s the unique herbicidal active ingredient glyphosate has become the world’s most widely used herbicide because it is efficacious, economical and environmentally benign. These properties have enabled a plethora of uses which continue to expand to this day providing excellent weed control both in agricultural and non-crop uses to benefit mankind and the environment. Glyphosate has an excellent safety profile to operators, the public and the environment. ... It is approved for weed control in amenity, industrial, forestry and aquatic areas. Roundup Pro Biactive and ProBiactive 450 can be used at any time of the year as long as weeds are green and actively growing” (Monsanto, 2010, p.1).

The Monsanto document outlines use areas including vegetation control on agricultural land, on GE Roundup Ready Crops and on non-agricultural land. By 2006, glyphosate became used routinely for both agricultural and non-agricultural weed control and pre-harvest treatment. Since 1995, glyphosate use has rapidly increased with the planting of GE glyphosate-tolerant crops. Glyphosate and its degradation product, aminomethylphosphonic acid (AMPA) have been detected in air (Majewski et al., 2014, Chang et al., 2011), rain (Scribner et al., 2007, Majewski, 2014), groundwater (Scribner, 2007), surface water (Chang, 2011; Scribner, 2007; Coupe et al., 2012), soil (Scribner, 2007) and sea water (Mercurio et al., 2014). These studies show that glyphosate and AMPA persist in the soil and water, and the amounts detected are increasing over time with increasing agricultural use. Chang et al. (2011) reported that glyphosate was frequently detected in water, rain and air in the Mississippi River basin with concentrations as high as 2.5 µg/L in agricultural areas in Mississippi and Iowa.

Because glyphosate is in air, water and food, humans are likely to be accumulating it in low doses over time. Glyphosate residues of up to 4.4 parts per million (ppm) have been detected in stems, leaves and beans of glyphosate-resistant soy, indicating uptake of the herbicide into plant tissue

(Arregui et al., 2004). Reports from Germany of glyphosate in the urine of dairy cows (Kruger et al., 2013b), rabbits and humans (Kruger et al., 2014) ranged from 10-35 ppm. According to the study (Kruger, 2014, p. 212), "Chronically ill humans had significantly higher glyphosate residues in urine than healthy humans." Furthermore, the cows were dissected and glyphosate residues in the tissues of the kidney, liver, lung, spleen, muscles and intestines were comparable to that found in the urine. This means that the glyphosate is not being passed through the urine without affecting the organism and that meat and dairy are an additional source of dietary glyphosate for humans.

Industry and lobbyists claim that GE crops reduce the amount of pesticides used on crops, resulting in a more sustainable agriculture. This has proved not to be the case. Since the introduction of GE seeds in 1996 the amount of glyphosate used on crops in the US has increased from 27 million pounds in 1996 to 250 million pounds in 2009 (US Geological Survey pesticide use maps, 2013). Charles Benbrook (2012) showed that there was a 527 million pound (239 million kilogram) increase in herbicide use in the United States between 1996 and 2011. Furthermore, Benbrook states that the spread of glyphosate-resistant weeds has brought about substantial increases in the number and volume of herbicides applied. This has led to genetically engineered forms of corn and soybeans tolerant of 2,4-D, which he predicts will drive herbicide usage up by approximately 50% more.

In the US, glyphosate residues allowed in food are some of the highest in the world. In July of 2013 the Environmental Protection Agency (EPA, 2013) raised the maximum allowable residues of glyphosate. An abbreviated list is provided in Table 1 and Table 2.

Table 1. Glyphosate residues allowed in food from crops (EPA, 2013).

Crop	Maximum residue allowance for glyphosate (ppm)
Beet, sugar, dried pulp	25
Beet, sugar, roots	10
Beet, sugar, tops	10
Canola, seed	20
Corn, sweet, kernel plus cob	3.5
Grain, cereal, group 15	30
Oilseeds, except canola	40
Pea, dry	8
Peppermint, tops	200
Quinoa, grain	5
Shellfish	3
Soybean, seed	20
Spice subgroup 19B	7
Sugarcane, cane	2
Sugarcane, molasses	30
Sweet potatoes	3
Vegetable, legume, group 6 except soybean and dry pea	5

Table 2. Glyphosate residues allowed in livestock feed (EPA, 2013).

Animal feeds	Maximum residue allowance for glyphosate (ppm)
Grass, forage, fodder and hay, group 17	300
Grain, cereal, forage, fodder and straw	100
Soybean, forage	100
Soybean, hay	200
Soybean, hulls	120
Cattle, meat byproducts	5

Glyphosate and disease

The connection between glyphosate and chronic disease has been outlined in a recent review paper by Samsel & Seneff (2013a). The authors show how glyphosate disrupts the metabolic process by interfering with the Cytochrome P450 (CYP) pathways. The CYP is known as a super-family of enzymes that are present in most tissues of the body. They are responsible for around 75% of the reactions involved in drug metabolism and the oxidation of organic molecules. According to the authors, "glyphosate enhances the damaging effects of other food borne chemical residues and environmental toxins. Negative impact on the body is insidious and manifests slowly over time as inflammation damages cellular systems throughout the body. Here, we show how interference with CYP enzymes acts synergistically with disruption of the biosynthesis of aromatic amino acids by gut bacteria, as well as impairment in serum sulfate transport. Consequences are most of the diseases

and conditions associated with a Western diet, which include gastrointestinal disorders, obesity, diabetes, heart disease, depression, autism, infertility, cancer and Alzheimer's disease. We explain the documented effects of glyphosate and its ability to induce disease, and we show that glyphosate is the 'textbook example' of exogenous semiotic entropy: the disruption of homeostasis by environmental toxins" (p. 1416).

Séralini et al. (2011) reviewed 19 studies of animals fed with GE soy and corn. The studies covered more than 80% of the GE varieties that are widely cultivated around the world. Their review found significant levels of negative effects to the kidneys and livers of the animals that ingested GE feed.

In another review article, Samsel & Seneff (2013b) point out that glyphosate is patented as a biocide and, as such, it kills the beneficial bacteria in our gut, leading to the steep rise in intestinal diseases. This has also been reported in the microbiota of horses and cows (Kruger, 2013a) and poultry (Shehata et al., 2012) where it was found that, "highly pathogenic bacteria as *Salmonella Enteritidis*, *Salmonella Gallinarum*, *Salmonella Typhimurium*, *Clostridium perfringens* and *Clostridium botulinum* are highly resistant to glyphosate. However, most of beneficial bacteria such as *Enterococcus faecalis*, *Enterococcus faecium*, *Bacillus badius*, *Bifidobacterium adolescentis* and *Lacto-bacillus* spp. were found to be moderate to highly susceptible" (p. 350). The authors postulate that glyphosate is associated with the increase in *C. botulinum*-mediated diseases in these domestic farm animals. Carman et al. (2013) reported that a diet of GE corn and soy was associated with stomach inflammation in pigs.

In 2012, Antoniou et al. published a review of the evidence on the teratogenicity and reproductive toxicity of glyphosate on vertebrates. Gasnier et al. (2009) published evidence that glyphosate-based herbicides are endocrine disruptors in human cells. They reported toxic effects to liver cells at 5 ppm and endocrine disrupting actions starting at 0.5 ppm. They concluded that glyphosate damages DNA in human cells. Subsequent studies have also shown that glyphosate is an endocrine disruptor (Paganelli et al., 2010; Antoniou et al., 2012). A more recent study showed that glyphosate causes the multiplication of estrogen sensitive human breast cancer cells, which further confirms that it acts as an endocrine disruptor (Thongprakaisang et al., 2013).

An endocrine disruptor is a chemical that either mimics or blocks hormones and disrupts the body's normal functions. This disruption can happen through altering normal hormone levels, halting or stimulating the production of hormones, or interacting directly with the organ the hormone was meant to regulate. Because hormones work at very small doses, endocrine disruption can occur from low-dose exposure to hormonally active chemicals (Vandenberg et al., 2012). Threshold doses of pesticides are set based on toxicology studies assuming the response is linear. But the response is not only non-linear, it is also dependent on the hormone level in the body at any given time. The meta study on endocrine disruption by the World Health Organisation and the United Nations Environment Program clearly makes this point (Bergman et al., 2013, p. 19): "Endocrine disruptors produce non linear dose responses both in vitro and in vivo; these non linear dose responses can be quite complex and often include non-monotonic dose responses. They can be due to a variety of mechanisms; because endogenous hormone levels fluctuate, no threshold can be assumed." Consequently, low doses over long periods of time may lead to very serious illnesses.

Endocrine disruptors can increase or decrease hormone production, imitate hormones or even transform one hormone into another. Endocrine disruptors can also tell cells to die prematurely, compete with essential nutrients and build up in hormone-producing organs. These imbalances and malfunctions of the endocrine system can lead to diabetes, hypertension, obesity, kidney disease, cancer (breast, prostate, liver, brain, thyroid, non-Hodgkin's lymphoma) (Marc et al., 2004; Thongprakaisang et al., 2013), osteoporosis, Cushing's syndrome, hypo- and hyperthyroidism, infertility, birth defects, erectile dysfunction (Soto & Sonnenschein, 2010), sexual development problems and neurological disorders such as: learning disabilities, attention deficit disorder (ADD) (de Cock et al., 2012), autism (Schulkin, 2007), dementia (Ghosh, 2010), Alzheimer's (Merlo et al., 2010), Parkinson's and schizophrenia (MacSweeney et al., 1978). Endocrine disruptors are especially damaging to organisms undergoing hormonal changes: fetuses, babies, children, adolescents and the elderly (Bergman et al., 2013).

Given that glyphosate disrupts gut bacteria balance, the metabolic process, the uptake of nutrients, the endocrine system, and damages DNA, it seemed likely that there would be correlations between the increase of these diseases and the exponential increase in the use of glyphosate, particularly with the advent of glyphosate-resistant food crops. To this end, we searched for epidemiological disease data, along with pesticide use on crops and the percentage of GE crops planted since first being

introduced in 1995. These were plotted and Pearson correlation coefficients were calculated. These data, provided by the US government, are readily available on the internet.

Methods

United States Government databases were searched for GE crop data, glyphosate application data and disease epidemiological data. Correlation analyses were then performed on these time-series data sets.

Crop data

The United States Department of Agriculture National Agricultural Statistics Service (USDA:NASS) maintains a database of US crops. Every year they randomly select fields of certain crops and send surveys to the persons who manage those fields. Among other things, they ask what herbicides were used, the application rate, how many times was it applied, and whether or not the field was planted with a GE variety. Surveys are only sent to the states that are the major producers of a given crop, usually accounting for about 90% of the total US acreage planted in that crop. They then perform a statistical analysis and report the total acreage planted, the percentage of acres that are GE, the Percentage of Acres Treated (PAT) with each herbicide for that crop and the application rate per acre per year. One can then calculate the total amount of an herbicide that was applied to that crop in the survey states for that year.

Data files from the USDA containing the information for GE varieties are available from 2000-2013 (USDA:NASS 2013a), but only corn, cotton and soy are tracked. Data for 1996-1999 were obtained from a USDA agricultural report (Fernandez-Cornejo & McBride, 2002). The survey states accounted for 85-90% of all corn, cotton and soy grown in the US. Sampling errors for the percentage of GE crops planted are given as 1-2%, varying by year and crop. The increase in the adoption of GE crops in the US from 1996-2010 is shown in Figure 1.

Data files containing the information for herbicide applications are available from 1990-2012 (USDA:NASS 2013b). Sampling errors (reported as standard errors) are small (<5%) in both the PAT and the application rate if the PAT is greater than 50%. Sampling errors are 5-10% if the PAT is between 10-50%, while the sampling errors are 10-100% if the PAT is <10%. We extracted the data for glyphosate applications to corn, cotton and soy. Data for cotton was not included in these results because, except for cottonseed oil in food and cottonseed meal in animal food, cotton is not generally considered a food crop. Though the manufacturers claim that there are no GE content or traits in processed foods (like oil), it has been reported that glyphosate residues up to 0.350 ppm have been detected in refined soy oil (GEAC, 2006).

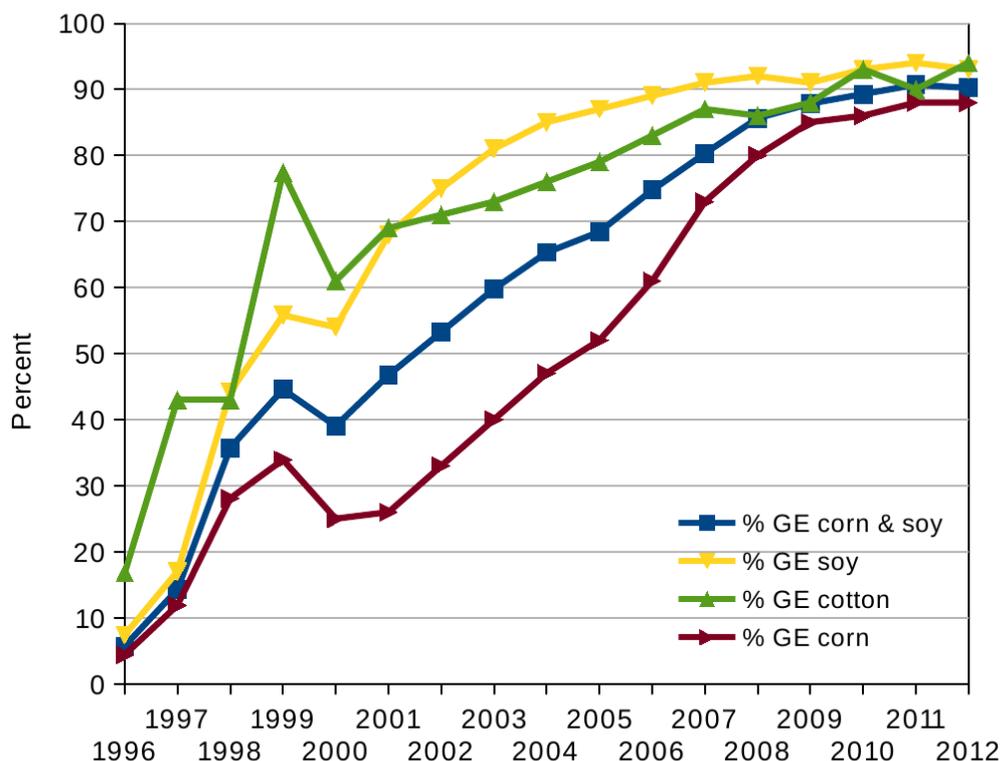


Figure 1. Adoption of GE crops in US.

From 1990-2002, glyphosate data were available for all three crops, but beginning in 2003 data were not collected for all three crops in any given year. Data on the application rates were interpolated for the missing years by plotting and calculating a best fit curve. Results for the application rates for soy and corn are shown in Figures 2 and 3. Because the PAT was relatively small prior to about 1995, the sampling errors are much larger for pre-1995 data, more so for corn than for soy. Also, data were not missing until 2003 for soy and 2004 for corn. For these reasons, the interpolated curves begin in 1996 for soy and 1997 for corn in Figures 2 and 3.

To calculate the amount of glyphosate applied, it was also necessary to interpolate the PAT for both corn and soy. This was easier because they followed almost exactly the curves for the percentage of acres planted in GE crops. GE soy crops are only herbicide tolerant (HT), which nicely tracked with the PAT for glyphosate, as shown in Figure 4. GE corn crops can be either insecticide resistant (Bt) or HT or both (stacked). The HT and stacked trait percentages, reported separately in the USDA files for corn, were plotted with the PAT for glyphosate as shown in Figure 5.

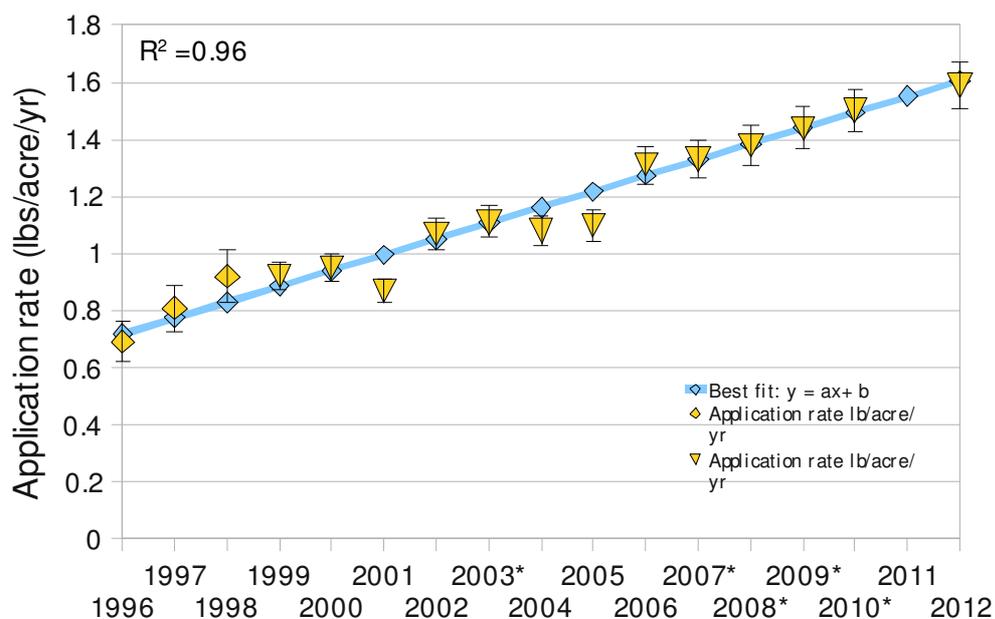


Figure 2. Application rate of glyphosate on soy. Best fit $y=ax+b$, interpolated values indicated with * next to the year. Error bars are from reported standard error from USDA. The residual standard error from the linear fit is 0.07 lb/acre/year.

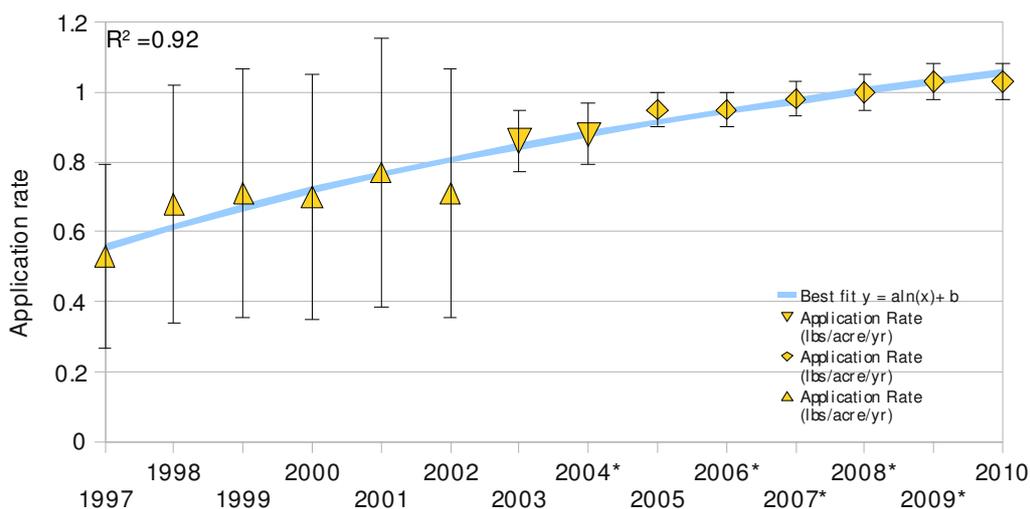


Figure 3. Application rate of glyphosate on corn. Best fit $y=a \ln(x)+b$, interpolated values indicated with * next to the year. Error bars are from reported standard error from USDA. The residual standard error from the linear fit is 0.06 lb/acre/year. Large errors for the earlier years are because of the smaller PAT with glyphosate.

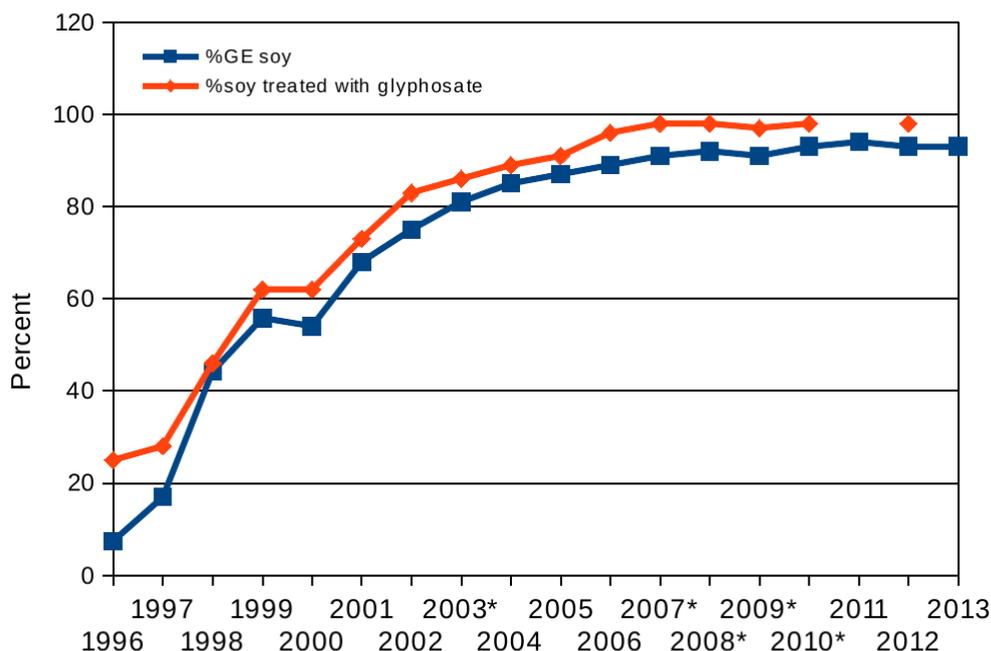


Figure 4. Percentage of GE soy crops planted and PAT with glyphosate. Interpolated values are indicated with an * next to the year. Data were not available for glyphosate applications to soy from 2007-2011. Data were only used through 2010; therefore the data point at 2011 was not interpolated. Data for 2012 are shown for reference.

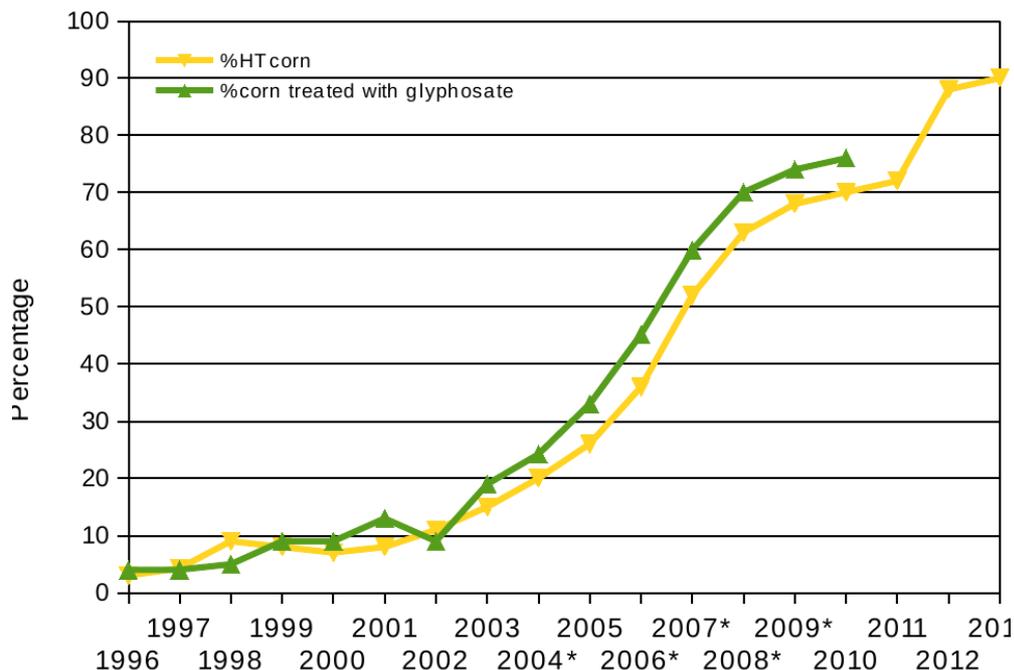


Figure 5. Percent GE corn crops and PAT with glyphosate; interpolated values indicated with *.

From these data, along with the total acreage planted in the survey states, the amount of glyphosate (in tons) applied to corn and soy crops in those states for each year from 1990-2010 was calculated and is shown in Figure 6. The calculation is: application rate (lbs/acre/year)*PAT/100*total acres planted. The contribution of glyphosate on soy is about twice that for corn. While both corn and soy are major US food crops (75-80 million acres planted annually), GE corn was more slowly adopted (Figure 1) and some of the earlier GE corn is Bt only. The curve for glyphosate applied to all three crops is included only to show that the shape of the curve is unchanged, so it is doubtful there would have been much change in the results had cotton been included.

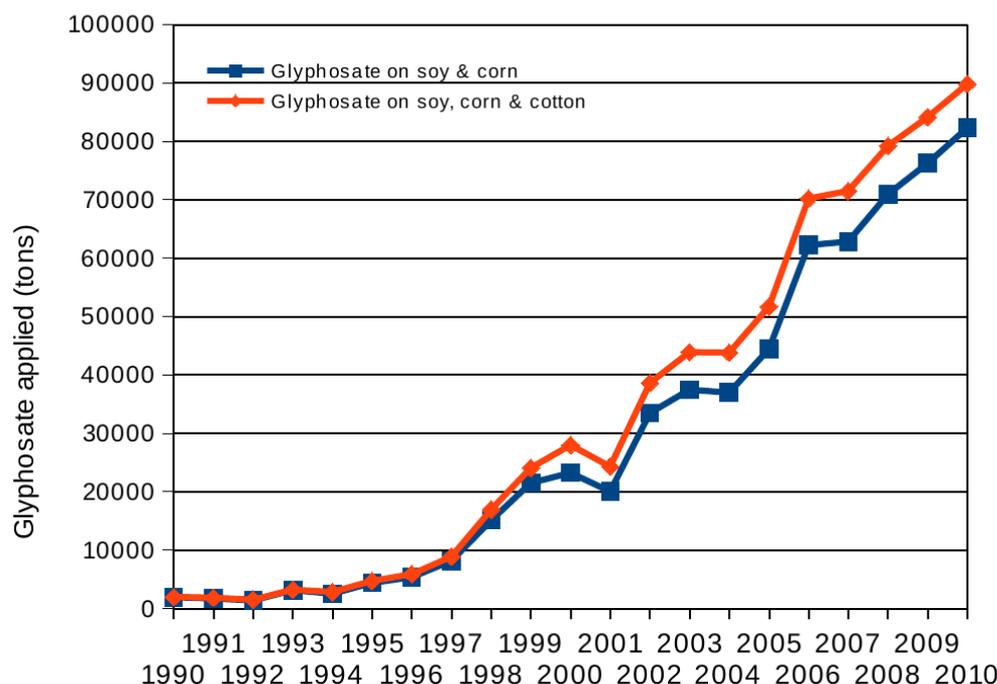


Figure 6. Glyphosate applied to corn, cotton and soy crops in the surveyed states. Standard errors (corn & soy): 2007-2010: 3-10%; 2000-2006: 7-16%; 1998-2000: 16-62%; 1992-1997: 16-100%; 1990-1991: 22-100%. Errors calculated from the USDA reported standard errors.

Epidemiological disease data

Databases were searched for epidemiological data on diseases that might have a correlation to glyphosate use and/or GE crop growth based on information given in the introduction. The primary source for these data was the Centers for Disease Control and Prevention (CDC). These data were plotted against the amount of glyphosate applied to corn and soy from Figure 6 and the total %GE corn and soy crops planted from Figure 1. The percentage of GE corn and soy planted is given by: (total estimated number of acres of GE soy + total estimated number of acres of GE corn)/(total estimated acres of soy + total estimated acres of corn) \times 100, where the estimated numbers were obtained from the USDA as outlined above.

Statistical analyses

A statistical analysis was performed on each of the data sets. A standard analysis for correlating two sets of data is to calculate the Pearson correlation coefficients. The Pearson correlation coefficient is based on the linear least-squares formulation, which in turn is based on the assumption that each of the individual variables is normally distributed. All of the US government data, both crop data and disease data, were gathered from surveys and census data. These data were statistically analysed and the results reported as an average with an associated error (standard deviation of the mean), indicating that normal distributions were assumed in the statistical methods used.

We generated scatter plots for each set of data (disease vs. glyphosate applications and disease vs. percentage of GE crops) to determine whether or not the Pearson correlation method (i.e. linear least-squares method) was appropriate. The scatter plots showed a strong linear relationship between the two data sets in all cases. Plots of the residuals were checked to confirm homogeneity.

The Pearson's correlation coefficient, R , is a determination of how closely correlated the two data sets are, i.e., how close the scatter plot is to a line. For N pairs of (x, y) data, the correlation coefficient is given by $R = \sigma_{xy} / (\sigma_x \sigma_y)$ where σ_{xy} is the covariance, $\sigma_{xy} = 1/N \sum (x_i - \bar{x})(y_i - \bar{y})$ and σ_x and σ_y are the standard deviations of the x and y variables.

When the individual standard deviations are not known, but calculated from the data sets themselves, the statistic $t = R\sqrt{(N-2)/(1-R^2)}$ can be used to test the claim that there is a positive correlation by calculating the probability that a value of R greater than or equal to that observed would have been

obtained if R were in fact 0 (the null hypothesis). If this probability, the P -value, is less than 5%, the correlation is deemed to be significant. If it is less than 1% it is described as highly significant. The probabilities obtained here are very small, so we may confidently reject the null hypothesis that $R = 0$. After verifying the accuracy of the results, we performed the correlation calculations using the online statistical package from the University of Amsterdam (UA, 2014).

Much of the CDC data is stored and retrieved according to the International Classification of Disease (ICD) codes. These codes changed from 1998 to 1999, causing some concern that there would be a discontinuity in the graphs between those years due to improper coding or added or subtracted categories. This only showed up in one graph, Alzheimer's. It is unclear whether the jump in the data on this graph is real or an artefact from the code change.

Results and Discussion

The plots are loosely grouped into related disease categories. If the disease data were linearly increasing prior to the 1990s, a linear trend line was overlaid on the plot in green. The error bars on the green trend lines are the residual standard errors from the least squares fit. In some cases, the axes have been adjusted to better illustrate the correlation; otherwise the data are plotted as is. In all cases, the left vertical axis is the prevalence or the rate of incidence or death from the disease. The right vertical axis is both the percentage of GE corn and soy planted and the amount (in 1,000 tons) of glyphosate applied to the corn and soy crops.

Correlations of cancers of the liver, kidney, bladder, and thyroid with the planting of GE crops and glyphosate applications

Epidemiology data for cancer incidence were obtained from the National Cancer Institute Surveillance, Epidemiology and End Results (SEER) database (National Cancer Institute, 2013). Based on published reports on endocrine disruptors, we expected but did not find correlations for: non-Hodgkin's lymphoma (slightly rising), prostate (oscillating), testicular (slightly rising), colon (slightly decreasing) and breast (slightly decreasing) cancers. The decrease in breast cancer may be attributable to reduced use of hormone replacement therapy (Chlebowski, 2012).

We found strong correlations for cancers of the liver, kidney, bladder/urinary and thyroid. Results are shown in Figures 7-10. Thyroid and bladder cancers especially seem to track with the advent of GE crops and associated glyphosate applications. Thyroid cancer seems to affect females more, while males are more susceptible to liver and kidney cancers (not shown in graphs). We found weaker correlations between pancreatic cancer incidence ($R = 0.84$ with %GE crops & $R = 0.92$ with glyphosate applications) and deaths from acute myeloid leukaemia ($R = 0.89$ with %GE crops & $R = 0.88$ with glyphosate applications). Both of these peaked in the 1980s, then decreased and are now rising again. Pancreatic cancer incidence began rising again in 1996 and myeloid leukaemia deaths in 1989.

Liver and Intrahepatic Bile Duct Cancer Incidence (age adjusted)

plotted against glyphosate applied to corn & soy ($R = 0.9596$, $p \leq 4.624e-08$)
 along with %GE corn & soy planted in U.S. ($R = 0.9107$, $p \leq 5.402e-05$)
 sources: USDA:NAS; SEER

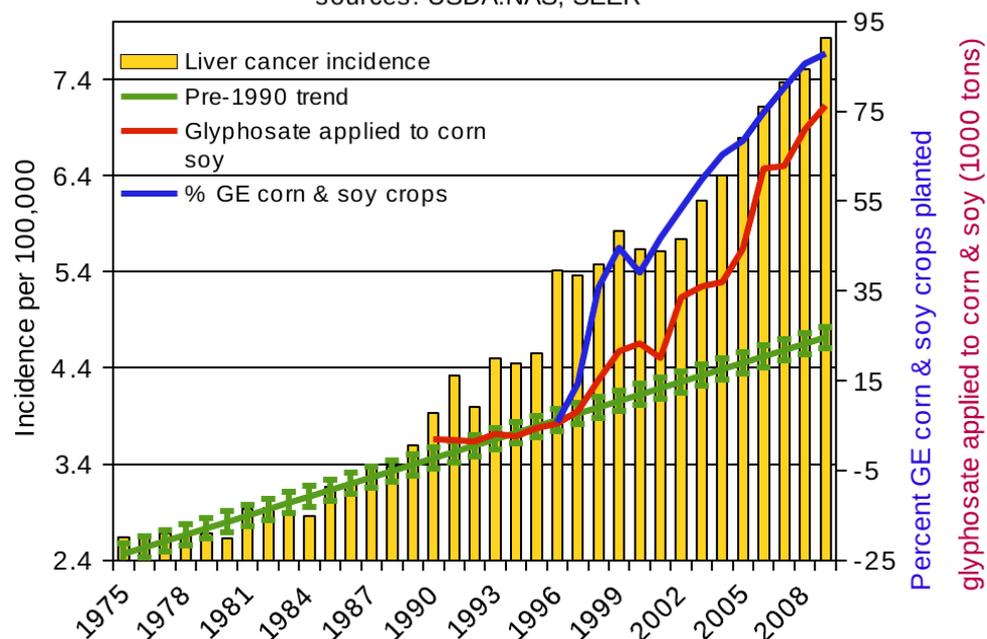


Figure 7. Correlation between age-adjusted liver cancer incidence and glyphosate applications and percentage of US corn and soy crops that are GE.

Age Adjusted Kidney and Renal Pelvis Cancer Incidence

Plotted against glyphosate applied to corn & soy ($R = 0.9734$, $p \leq 1.98e-08$)
 along with %GE corn and soy planted in U.S. ($R = 0.94$, $p \leq 1.978e-05$)
 sources: USDA:NASS; SEER

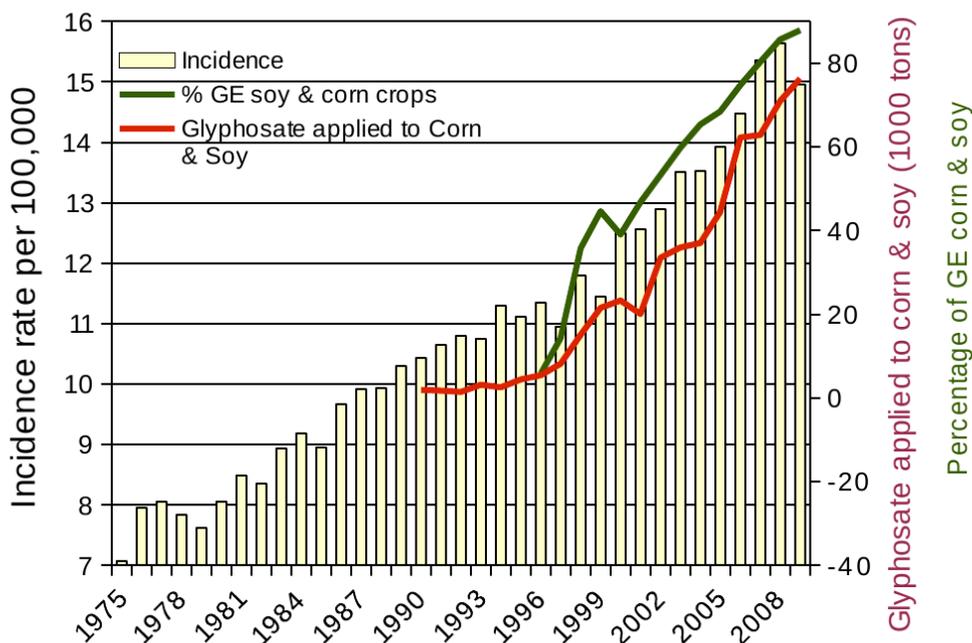


Figure 8. Correlation between age-adjusted kidney cancer incidence and glyphosate applications and percentage of US corn and soy crops that are GE.

Age Adjusted Urinary/Bladder Cancer Incidence

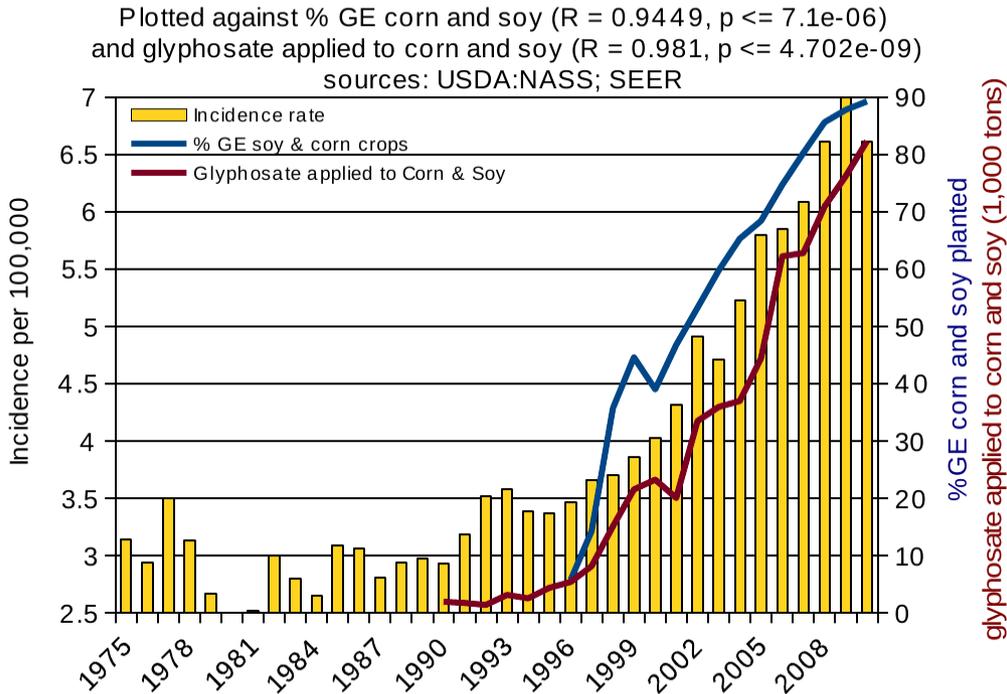


Figure 9. Correlation between age-adjusted bladder/urinary tract cancer and glyphosate applications and percentage of US corn and soy crops that are GE.

Thyroid Cancer Incidence Rate (age adjusted)

plotted against glyphosate applied to U.S. corn & soy ($R = 0.988$, $p \leq 7.612e-09$)
 along with %GE corn & soy crops $R = 0.9377$, $p \leq 2.152e-05$
 sources: USDA:NASS; SEER

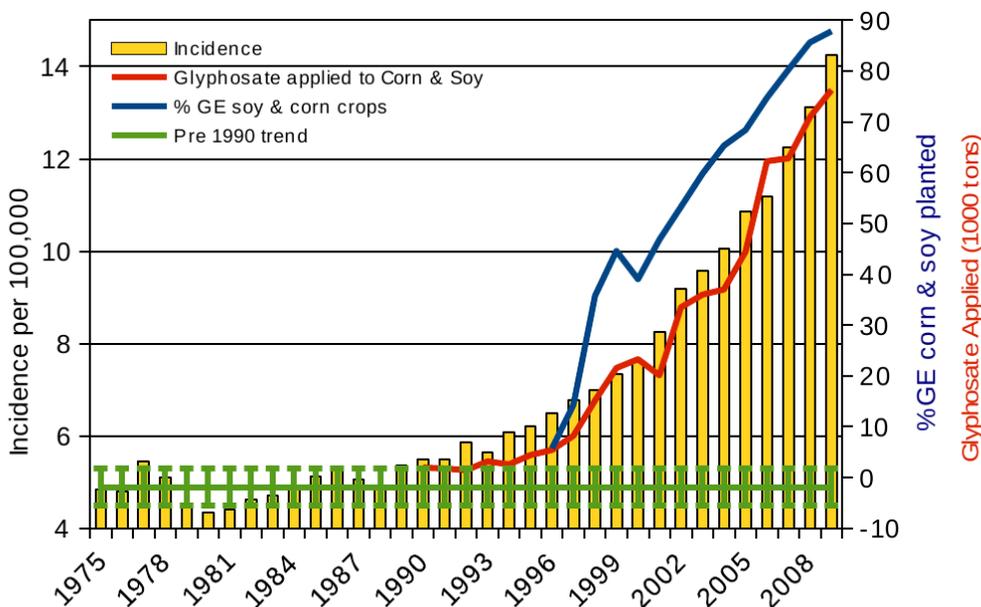


Figure 10. Correlation between age-adjusted thyroid cancer incidence and glyphosate applications and percentage of US corn and soy crops that are GE.

Correlations between hypertension and hemorrhagic strokes with the planting of GE crops and glyphosate applications

Correlations for deaths due to hypertension and hemorrhagic stroke are shown in Figures 11 & 12. Death data were obtained from the CDC mortality files (CDC, 2013b). Data for hypertensive heart disease suffered from a discontinuity between the years 1998 and 1999, most likely due to the change in ICD codes at that time (Joyner-Grantham, 2010). After adjusting the latter data (multiplying by a

constant factor) to remove the discontinuity, we found $R = 0.93$ with glyphosate applications and $R = 0.94$ with %GE crops, but the results are not presented here due to the necessary manipulation of those data.

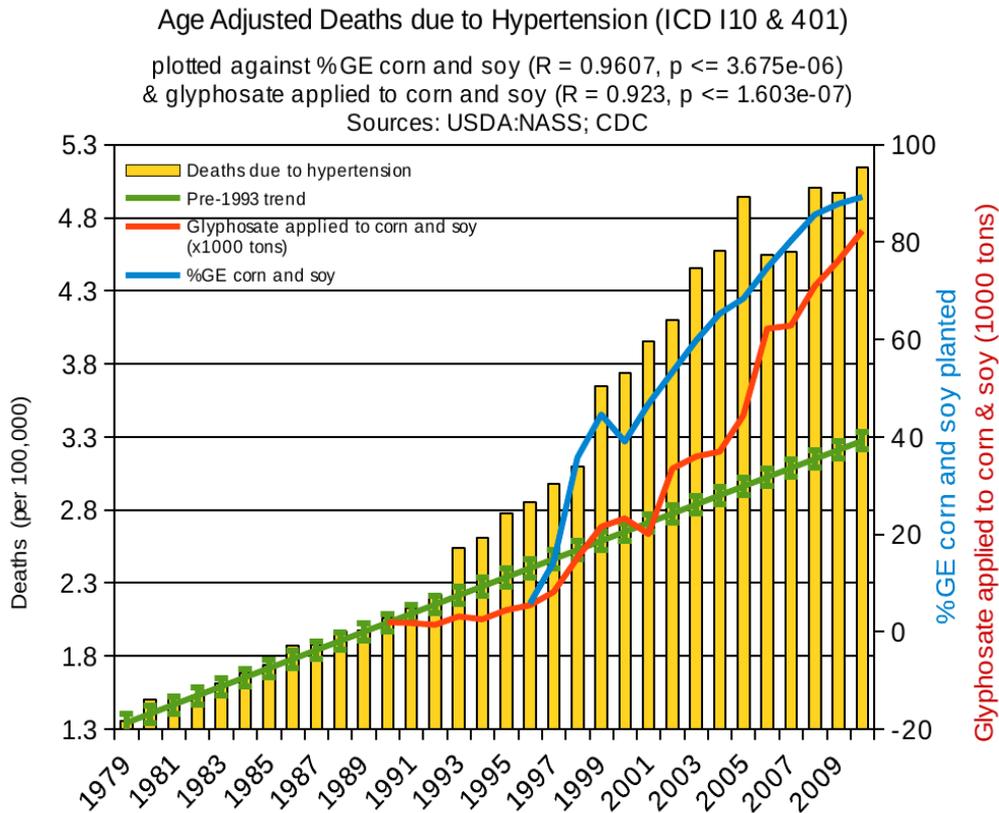


Figure 11. Correlation between age-adjusted hypertension deaths and glyphosate applications and percentage of US corn and soy crops that are GE.

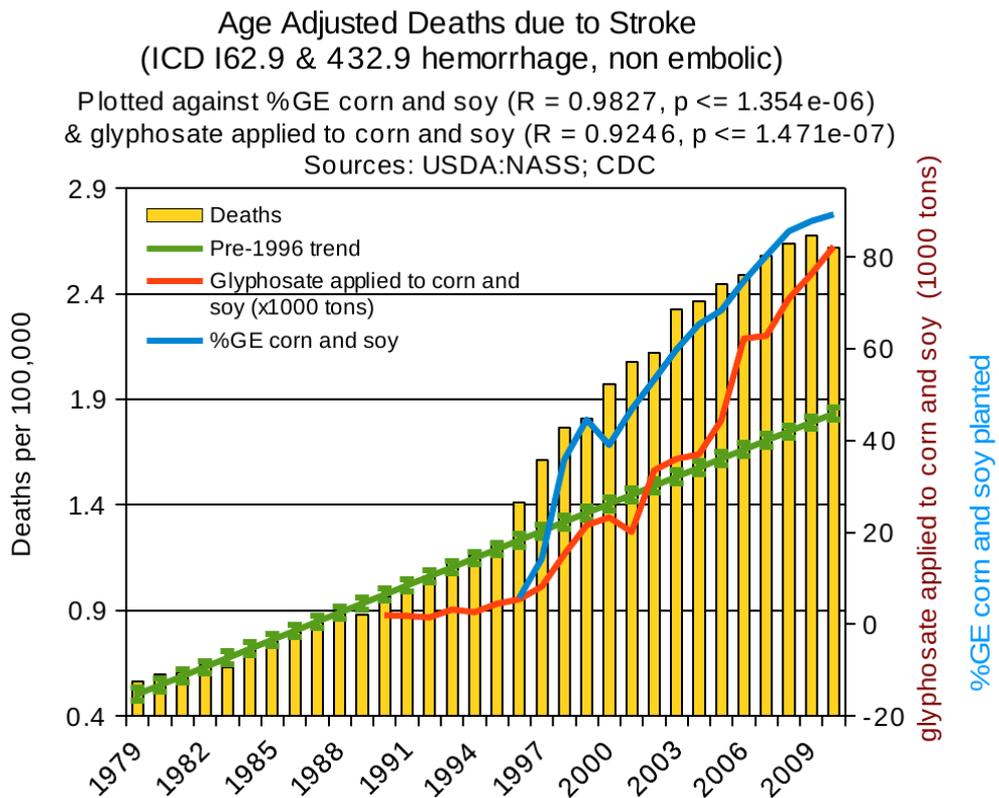


Figure 12. Correlation between age-adjusted hemorrhagic stroke deaths and glyphosate applications and percentage of US corn and soy crops that are GE.

Correlations of obesity, lipoprotein metabolism disorder and diabetes with the planting of GE crops and glyphosate applications

Epidemiological data for obesity deaths, lipoprotein metabolism (hyperlipidemia & hypercholesterolemia) disorder deaths, and diabetes incidence and prevalence also showed strong correlations with glyphosate use and GE crop growth. Death data were again obtained from the CDC mortality files (CDC, 2013b). Diabetes prevalence (CDC, 2013c) and incidence (CDC, 2013d) data were obtained from CDC National Center for Health Statistics. Results are shown in Figures 13-16.

According to the CDC, approximately one third of people with diabetes have not been diagnosed. Therefore, the National Health Interview Survey underestimates the true incidence and prevalence of diabetes. Because diabetes and obesity are associated with sugar consumption, we present the per capita sweetener delivery for US consumption (USDA, 2013) in Figure 17. The majority of the sugar consumed is from corn, sugar beets and sugar cane. In 2011, 88% of the corn (USDA:NASS, 2013a) and 90% of sugar beets (ISAAA, 2011) planted in the US were GE. Glyphosate is routinely used for sugar cane crop ripening and desiccation (Orgeron, 2012).

Hyperlipidemia is characterised by inflammation of the pancreas (pancreatitis), abdominal pain, enlargement of the liver and spleen (hepatosplenomegaly), and small yellow skin lesions called eruptive xanthomas (Raphael, 1993; Berglund, 2012). Diseases associated with secondary hyperlipidemias include obesity, diabetes mellitus (type I and type II), hypothyroidism, Cushing's syndrome, chronic kidney disease, nephrotic syndrome, and cholestatic disorder, a major risk factor for atherosclerosis and cardiovascular disease.

According to Samsel & Seneff (2013a) glyphosate disrupts the CYP enzymes that are heavily involved in producing bile acids. Ordinarily, the liver exports a lot of cholesterol as cholesterol sulfate into the bile acids. This allows the digestive system to digest fats, which are then packaged up into the chylomicron with the cholesterol sulfate packed into its outer shell to deliver cholesterol to all the tissues. When the liver cannot make bile acids, it is forced to divert the cholesterol into LDL, so the LDL rises, resulting in hypercholesterolemia.

Furthermore, lipoprotein metabolism disorder has been associated with Alzheimer's (AD) and Parkinson's diseases (Merlo, 2010). According to Merlo, "Recent evidence suggests a strict link between metabolic disorders and AD. In the last decade much attention has focused specifically on the connection between dysfunction of lipid metabolism and AD. Here we discuss aspects of lipid regulation, including changes in cholesterol levels, function of apolipoproteins and leptin, and how these relate to AD pathogenesis. Despite the vast literature available, many aspects still need clarification. Nevertheless, the route is already delineated to directly connect aspects of lipid regulation to AD" (p. 537).

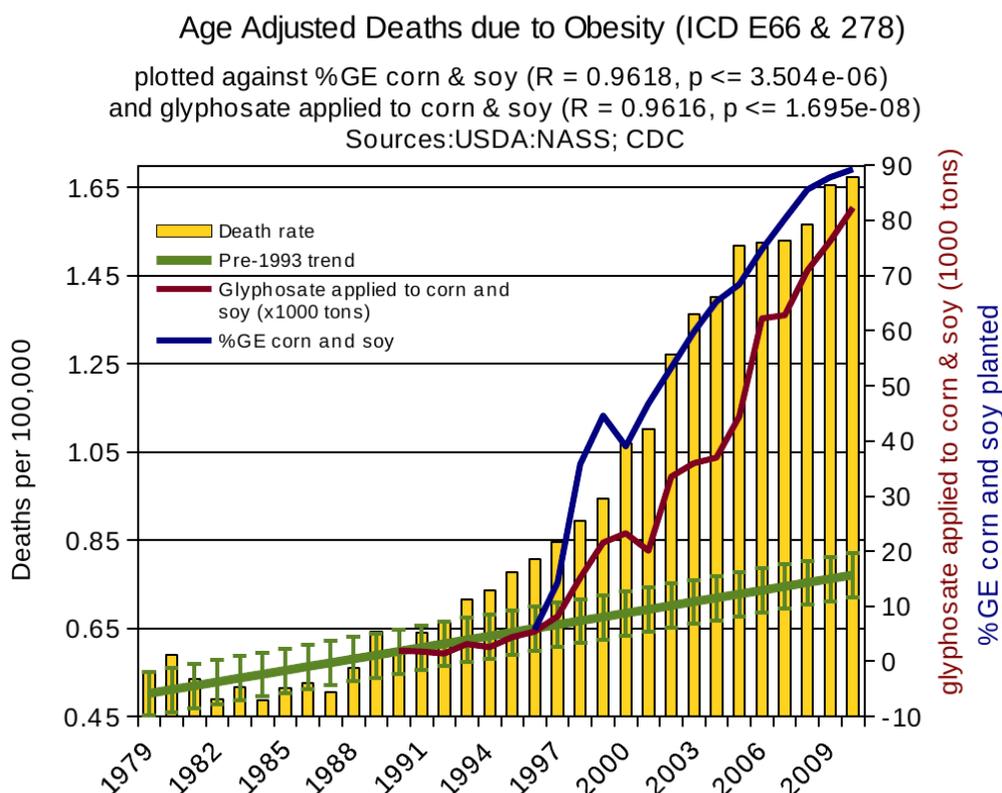


Figure 13. Correlation between age-adjusted obesity deaths and glyphosate applications and percentage of US corn and soy crops that are GE.

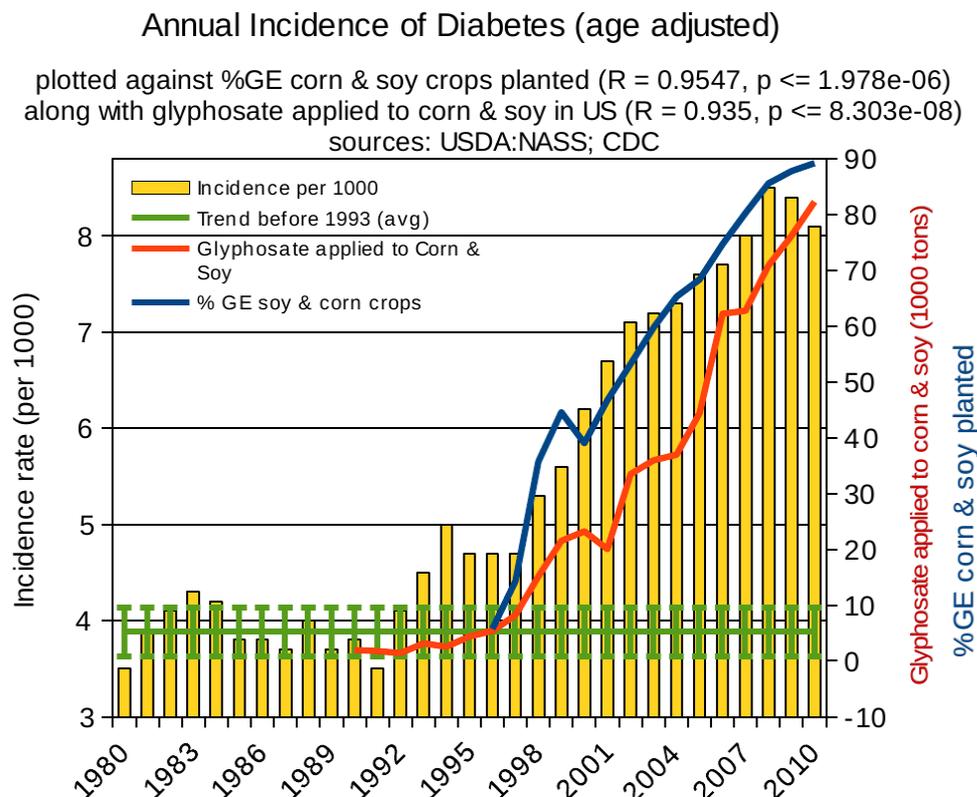


Figure 14. Correlation between age-adjusted diabetes incidence and glyphosate applications and percentage of US corn and soy crops that are GE.

Prevalence of Diabetes in US (age adjusted)
 plotted against glyphosate applied to corn & soy ($R = 0.971$, $p \leq 9.24e-09$)
 along with %GE corn & soy grown in US ($R=0.9826$, $p \leq 5.169e-07$)
 sources: USDA:NASS; CDC

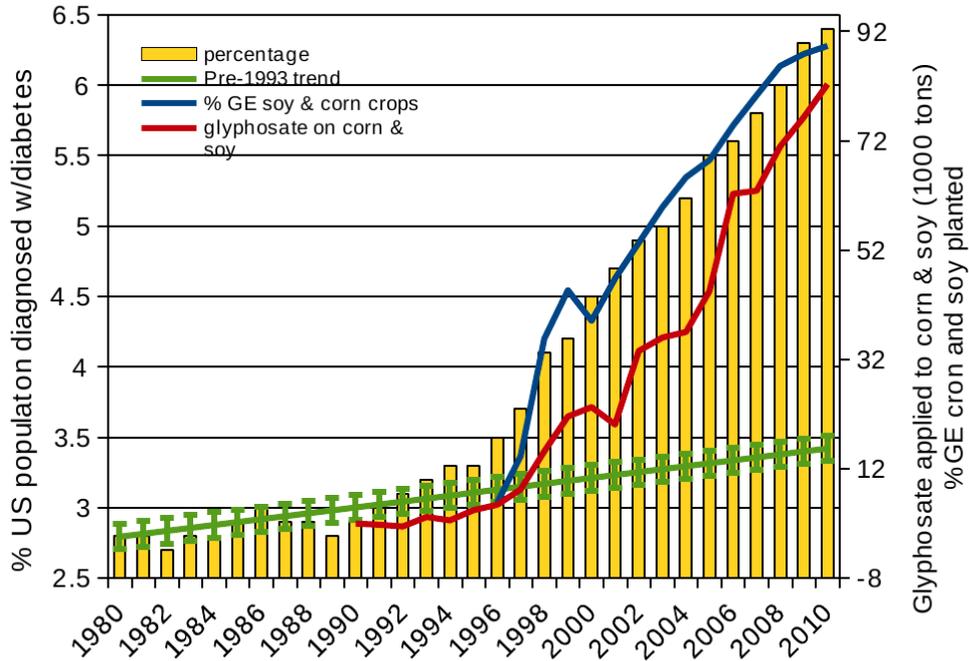


Figure 15. Correlation between age-adjusted diabetes prevalence and glyphosate applications and percentage of US corn and soy crops that are GE.

Age Adjusted Deaths due to Disorders of Lipoprotein Metabolism
 (ICD E78.5 hyperlipoproteinemia & E78.0 hypercholesterolemia)

plotted against %GE corn & soy ($R = 0.9549$, $p \leq 4.694e-06$)
 and glyphosate applied to corn & soy ($R = 0.9734$, $p \leq 7.886e-09$)
 Sources: USDA:NASS; CDC

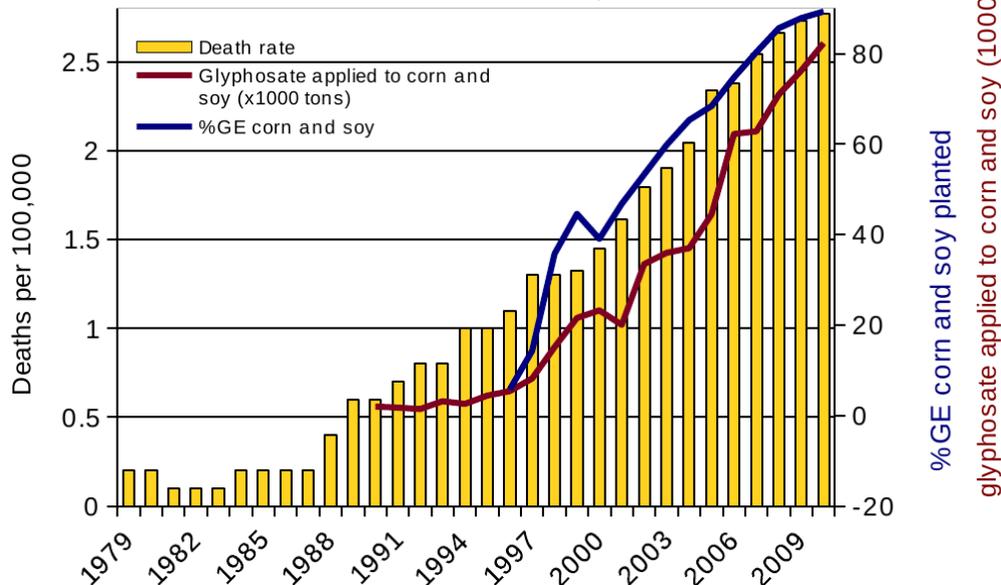


Figure 16. Correlation between age-adjusted lipoprotein disorder deaths and glyphosate applications and percentage of US corn and soy crops that are GE.

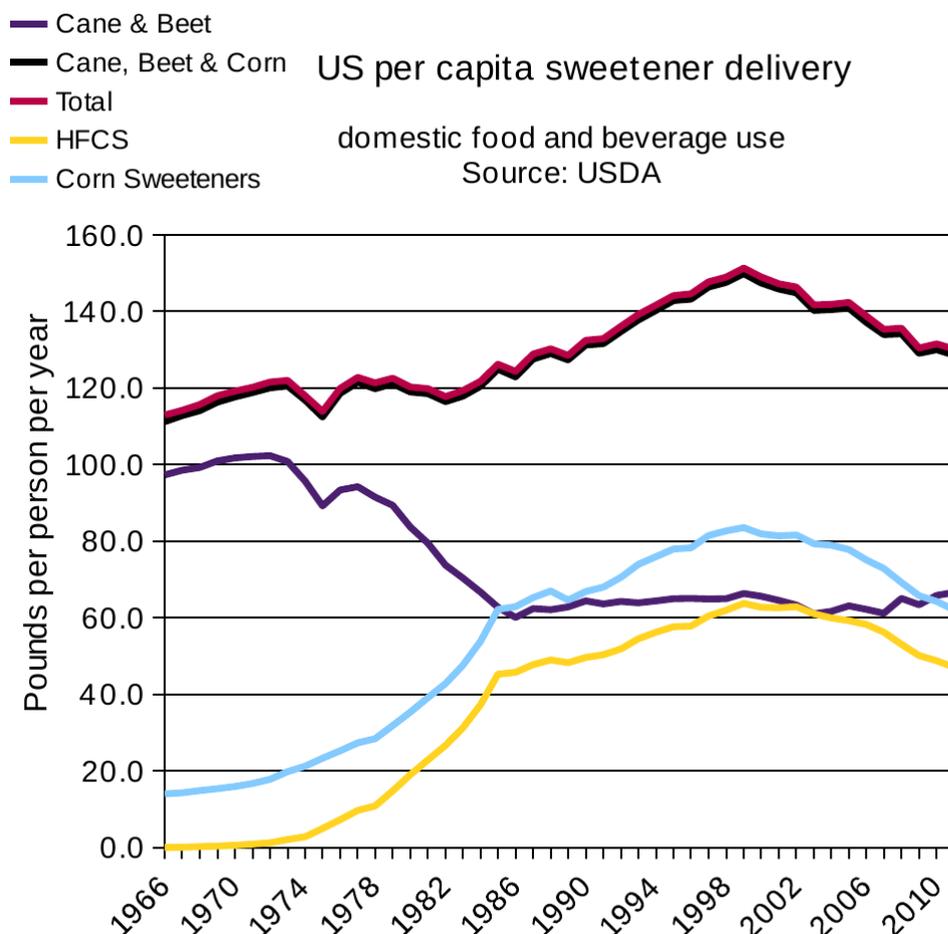


Figure 17. Sugar consumption in the US. The black line is sugar from cane, beet and corn combined; the red line is the total of all sources, including honey and syrups.

Correlations of renal failure with the planting of GE crops and glyphosate applications

Deaths from end stage renal disease (ESRD) and acute renal failure showed strong correlations with glyphosate use and GE crop growth. Death data were obtained from the CDC mortality files (CDC 2013b). Results are shown in Figures 18 and 19. Both of these have a peak in the mid-1980s, then decline and start rising again in the mid-1990s. The slight jump in ESRD deaths from 1998 to 1999 could be due to the ICD code changes at that time, but this was not apparent in the crude death rate data.

Researchers in Sri Lanka reported massive kidney failure in rice paddy workers exposed to glyphosate in combination with minerals in hard water. According to Jayasumana et al. (2014) glyphosate's strong chelating properties allow it to combine with heavy metals and arsenic in hard water resulting in damage to renal tissues, thereby causing chronic kidney diseases. The authors concluded that, "The GMA [Glyphosate-metal/arsenic complex] lattice hypothesis gives rational and consistent explanations to the many observations and unanswered questions associated with the mysterious kidney disease in rural Sri Lanka. Furthermore, it may explain the similar epidemics of CKDu [Chronic Kidney Disease of Unknown etiology] observed in Andhra Pradesh, India and Central America" (p. 2139).

An earlier study found that a 96 hour exposure to low levels of Roundup in water caused oxidative stress to the cells in the kidneys of goldfish (Lushchak et al., 2009). Studies by El-Shenawy (2009) and de Liz Oliveira Cavalli et al. (2013) confirm that Roundup and its active ingredient, glyphosate, caused oxidative stress and necrosis in the hepatic cells of rats.

The only lifetime feeding trial of rats with GE maize, Roundup, and GE maize combined with Roundup, compared to the controls fed the non-GE isogenic line of the maize, found very significant chronic kidney deficiencies, for all treatments compared to the controls. Seralini et al. (2014) reported that, "In treated males, liver congestions and necrosis were 2.5 to 5.5 times higher. Marked and severe nephropathies were also generally 1.3 to 2.3 times greater. In females, all treatment groups showed a two- to threefold increase in mortality, and deaths were earlier." (p.1)

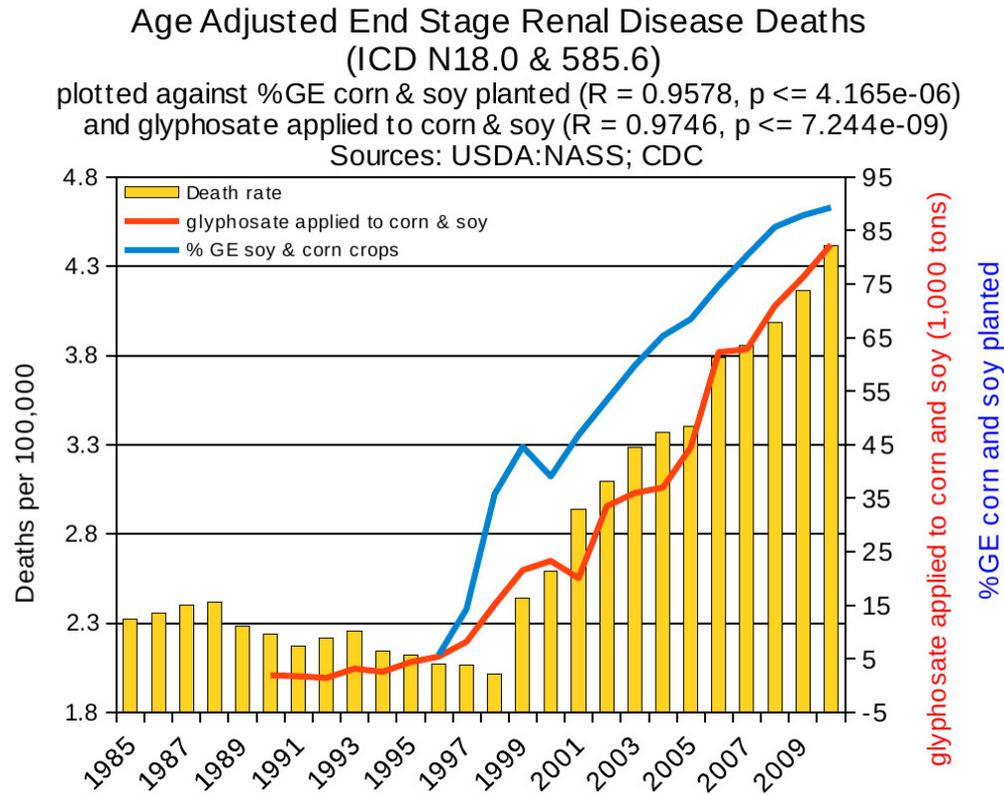


Figure 18. Correlation between age-adjusted End Stage Renal Disease deaths and glyphosate applications and percentage of US corn and soy crops that are GE.

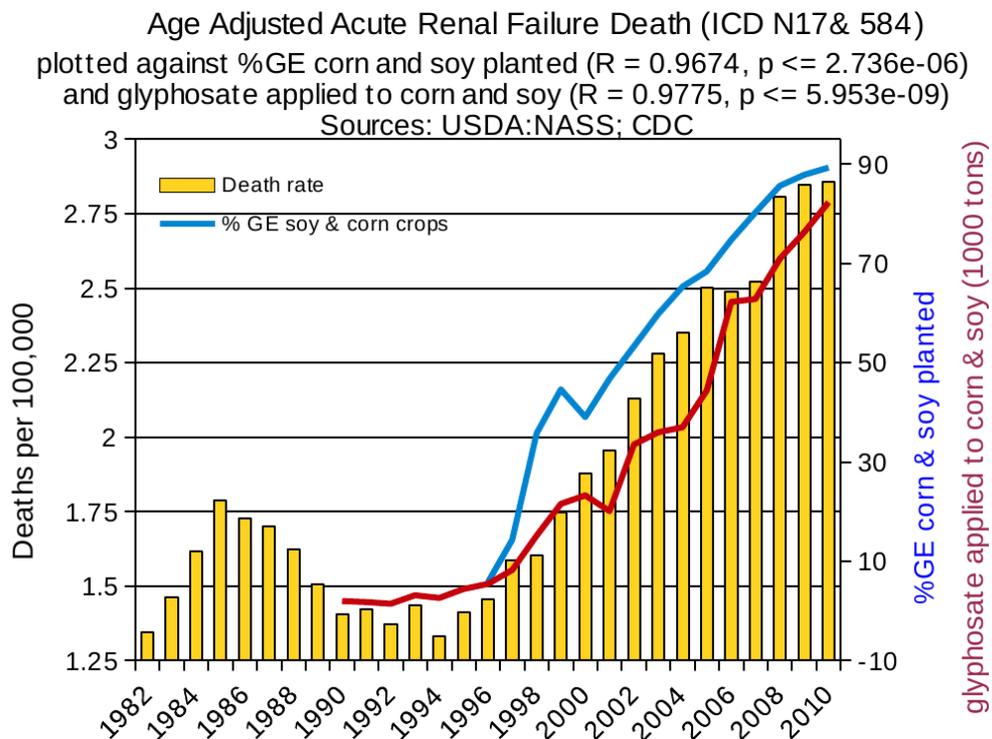


Figure 19. Correlation between age-adjusted renal failure deaths and glyphosate applications and percentage of US corn and soy crops that are GE.

Correlations of gastrointestinal disorders, (inflammatory bowel disease, intestinal infections and liver disorders) with the planting of GE crops and glyphosate applications

It is well-known that autistic children and people who suffer from neurological diseases also suffer intestinal problems (Anderson, 2012; Kang, 2013; Ashwood, 2003). According to Samsel & Seneff (2013b), glyphosate also disrupts the gut microbial balance. Data for inflammatory bowel disease were

obtained from the CDC hospital discharge data and are plotted in Figure 20 (CDC, 2013e). Data for deaths due to intestinal infection were obtained from the CDC mortality files (CDC, 2013b) and are shown in Figure 21.

While retrieving these data, we stumbled upon a startling increase in hospital discharges for viral hepatitis C. At first this was puzzling. We do not imply that hepatitis is transmitted by food, but that the CaMV is very similar to hepatitis and HIV and if those are already dormant in the body, introduction of the CaMV through the food could activate them. Ho (2013, p. 4760) has stated that, "insertion mutations [can occur] including those leading to cancer, activation of dormant viruses, and recombination with viral sequences in the genome to generate new viruses; all of which have been demonstrated in gene therapy experiments". And also, "New evidence raises the possibility that the CaMV 35S promoter in practically all transgenic crops grown commercially may enhance multiplication of disease-associated viruses including HIV through induction of proteins required for their transcription" (Ho et al., 2009, p. 172).

Furthermore, recent evidence (Furuta, 2013) suggests that cholesterol sulfate is an inhibitor of the hepatitis C virus and, according to Samsel & Seneff (2013a), glyphosate also interferes with the uptake of nutrients, particularly sulfates. We searched the hospital discharge data from the CDC (CDC, 2013e) for diagnoses of hepatitis C. We found a correlation between those data and the percent of GE soy crops planted in the US. Results are shown in Figure 22. We also looked at the data for deaths from HIV, but found that they have been steadily decreasing.

Hospital discharge diagnoses (any) of Inflammatory Bowel disease (Crohn's and Ulcerative Colitis ICD 555 & 556)

plotted against glyphosate applied to corn & soy ($R = 0.9378$, $p \leq 7.068e-08$)
Sources: USDA & CDC

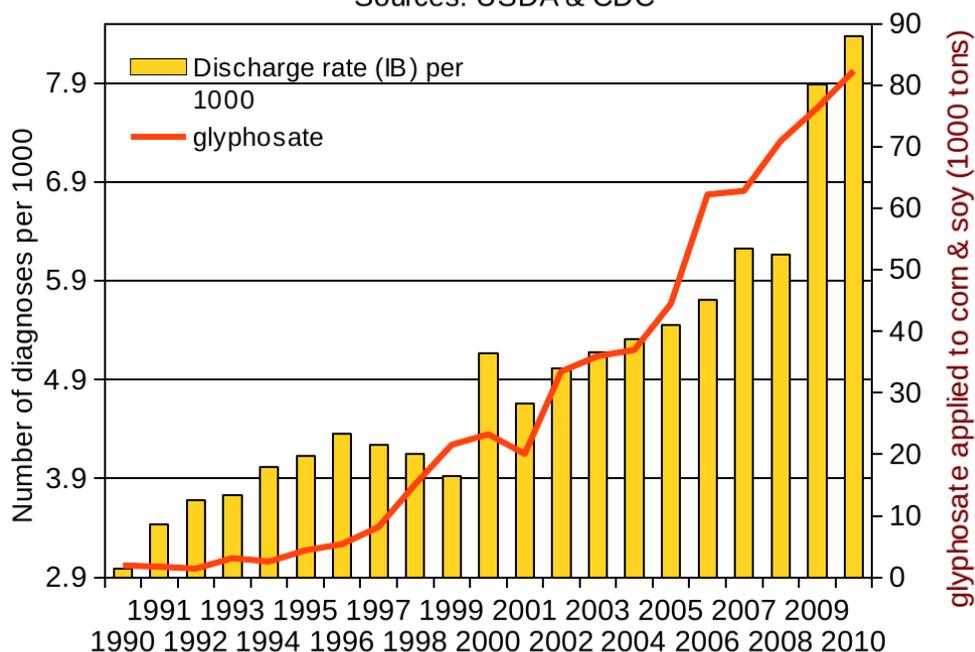


Figure 20. Correlation between inflammatory bowel disease and glyphosate applications to US corn and soy crops.

Age Adjusted Deaths due to Intestinal Infection
(ICD A04, A09; 008, 009)

plotted against glyphosate applied to corn & soy (R = 0.9738, p <= 7.632e-09)
Sources USDA:NASS; CDC

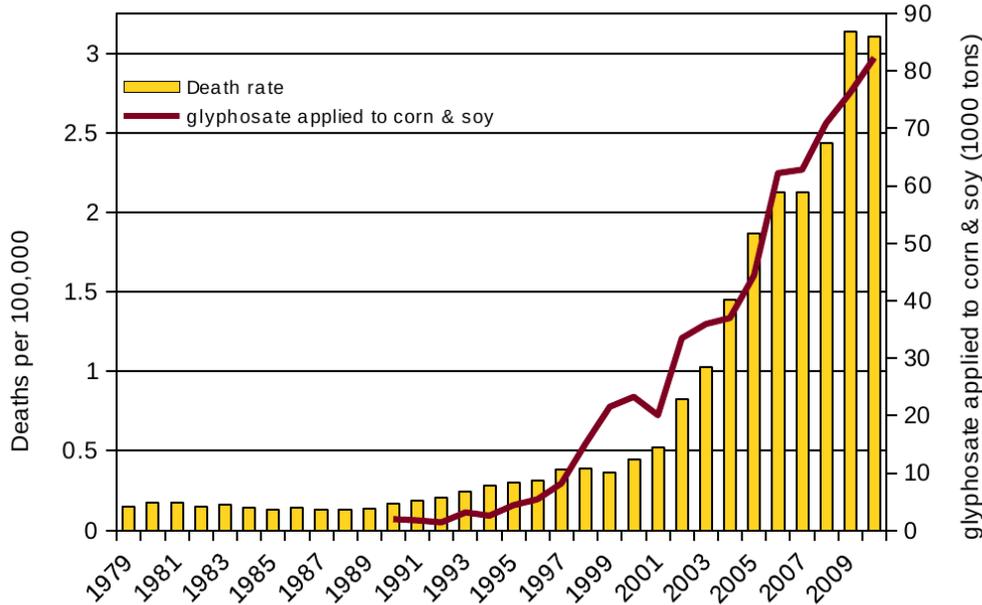


Figure 21. Correlation between age-adjusted intestinal infection deaths and glyphosate applications to US corn and soy crops.

(ICD 070.4-070.7)
plotted against % acres planted in GE soy
(R = 0.9456, p <= 6.914e-06)

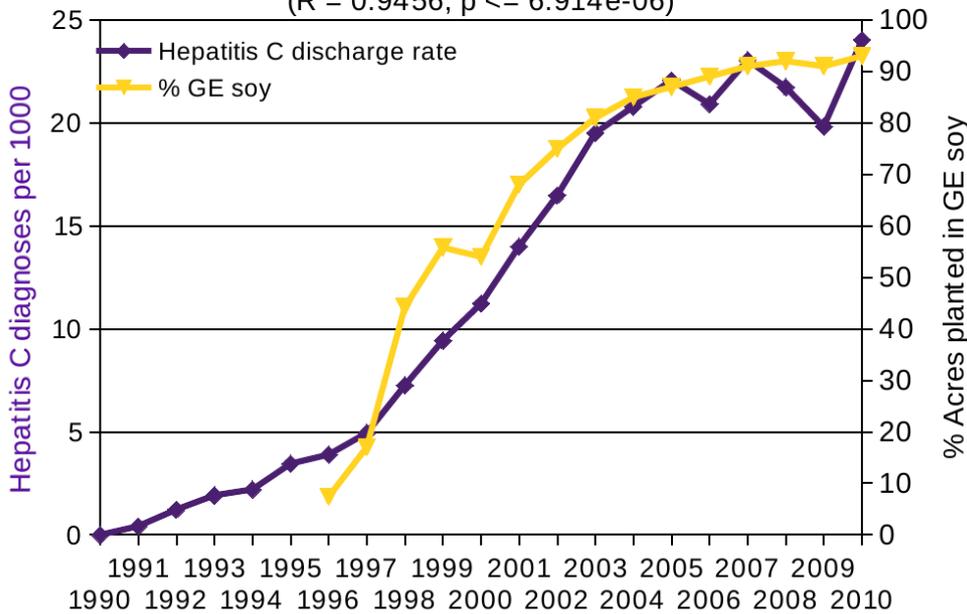


Figure 22. Correlation between diagnoses of hepatitis C and GE soy crop growth.

Correlations of neurological disorders (autism, Alzheimer's, senile dementia and Parkinson's) with the planting of GE crops and glyphosate applications

The incidence and prevalence of neurological disorders are not readily available for two reasons: they are not as well-studied as other diseases (cancer, diabetes etc.), and the diagnostic methods keep changing. Researchers argue over whether the increases are real, or a by-product of changes in diagnostics, along with greater attention given to these disorders in recent times. For example, a former diagnosis of mental retardation might now result in a diagnosis of autism. Furthermore, there is a large degree of overlap in symptoms. Typical manifestations of ADHD, such as distractibility or hyperactivity, are also present in pediatric bipolar disorder. However, the increases have been so

great in recent years that most experts now agree that they are real and must be environmentally induced (Weintraub, 2011).

We found data for autism from the US Department of Education, Individuals with Disabilities Education Act (USDE:IDEA) (Gallup, 2002; Snyder, 2012). These data are for autistic children 6-21 years old served under IDEA. In the plot in Figure 23, the numbers for the year correspond to the beginning of the school year in the fall.

According to the University of Washington Institute for Health Metrics and Evaluation (UW, 2012), Alzheimer's disease went from number 32 in 1990 to number 9 in 2010 in the ranking of leading causes of premature death in the US. Senile dementia and its care costs have also skyrocketed in the last two decades. Prevalence and incidence data were sparse, but data on death rates were available from 1979. Death data were again obtained from the CDC for senile dementia, Parkinson's & Alzheimer's diseases (CDC, 2013b). These are presented in Figures 24-26. A weaker correlation was found for multiple sclerosis deaths ($R = 0.88$ for %GE crops and $R = 0.83$ for glyphosate applications).

Cattani et al. (2014) found that both acute and chronic exposure to Roundup induced oxidative stress resulting in neural cell death and neurotoxic effects in the hippocampus of immature rats. Lushchak et al. (2009) found that a 96-hour exposure to low levels of Roundup in water caused oxidative stress to the cells in the brains, livers and kidneys of goldfish.

Number of children (6-21yrs) with autism served by IDEA
plotted against glyphosate use on corn & soy ($R = 0.9893$, $p \leq 3.629e-07$)
Sources: USDA:NASS; USDE:IDEA

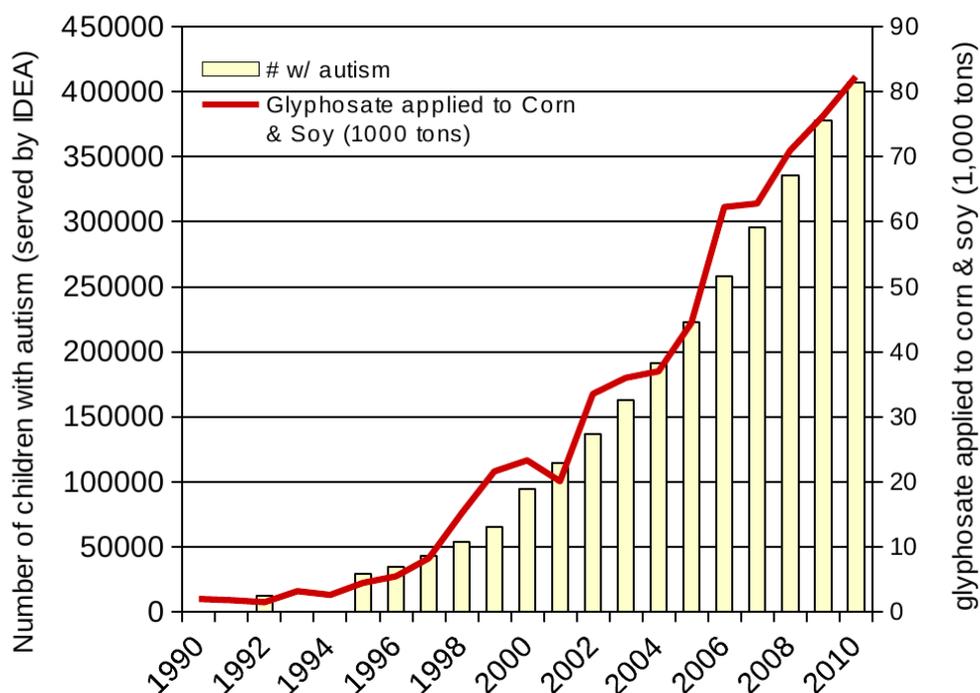


Figure 23. Correlation between children with autism and glyphosate applications.

Age Adjusted Deaths from Senile Dementia (ICD F01, F03 & 290)

Plotted against glyphosate use on corn & soy
($R = 0.9942$, $p \leq 1.822e-09$)
Sources: USDA:NASS; CDC

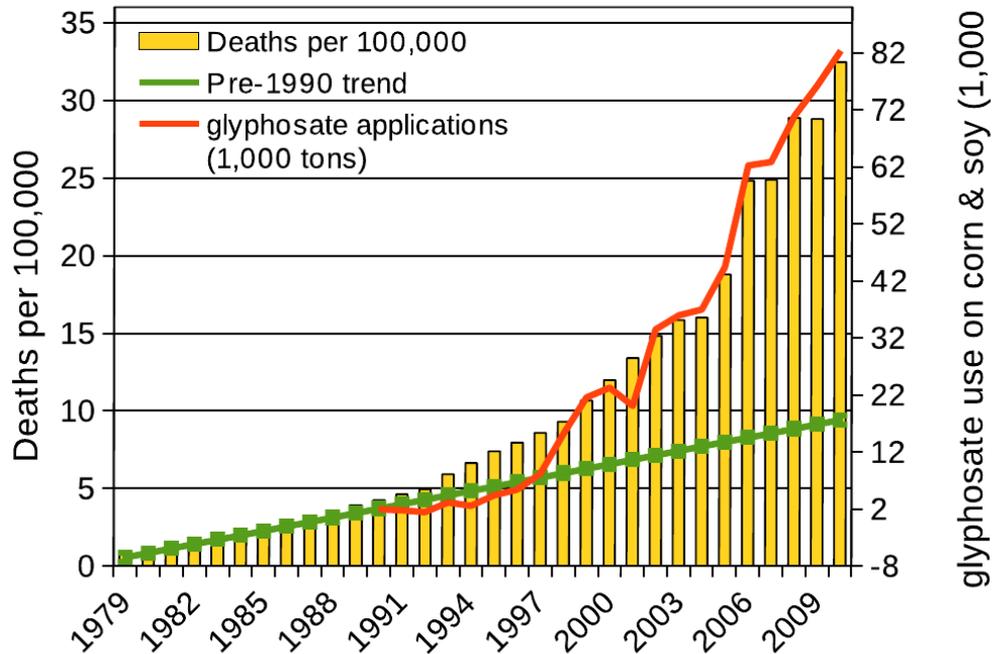


Figure 24. Correlation between age-adjusted dementia deaths and glyphosate applications.

Age Adjusted Deaths from Alzheimer's (ICD G30.9 & 331.0)

Plotted against glyphosate use ($R = 0.917$, $p \leq 2.205e-07$) &
%GE crops planted ($R = 0.9373$, $p \leq 9.604e-06$)
sources: USDA:NASS; CDC

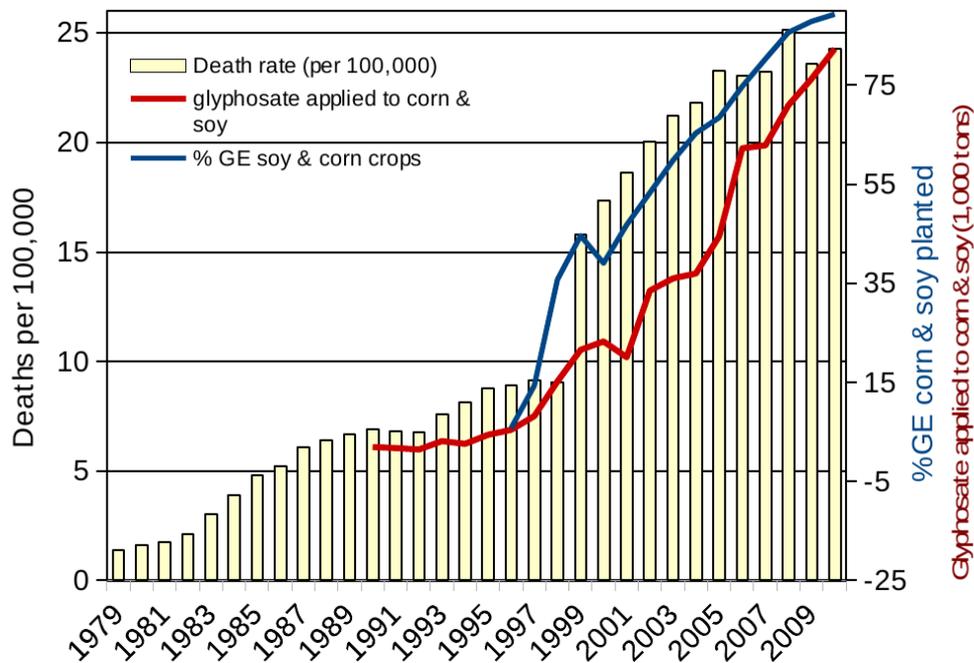


Figure 25. Correlation between age-adjusted Alzheimer's disease deaths and glyphosate applications and percentage of US corn and soy crops that are GE.

Age Adjusted Deaths from Parkinson's disease (ICD G20 & 332.0)

plotted against glyphosate use on corn & soy ($R = 0.8754$, $p \leq 1.631e-06$)
and percent GE corn & soy planted ($R = 0.9516$, $p \leq 5.398e-06$)
sources: USDA:NASS; CDC

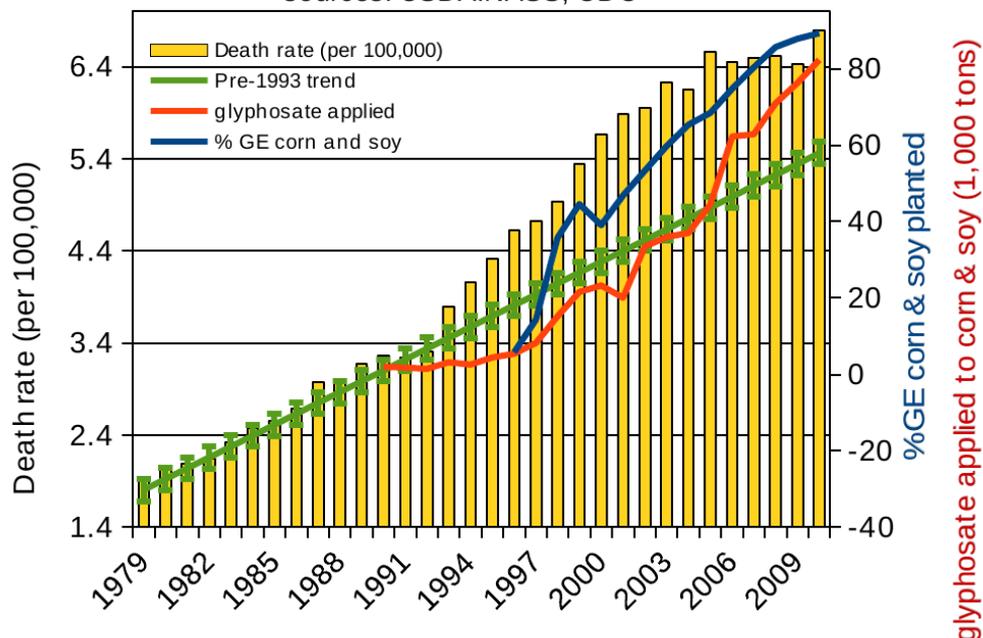


Figure 26. Correlation between age-adjusted Parkinson's disease deaths and glyphosate applications and percentage of US corn and soy crops that are GE.

Statistical summary of disease data correlations with GE crops planted and glyphosate applications

The Pearson correlation coefficient is a measure of the linear relation between two variables, X and Y . The correlation coefficient, R lies between -1 and 1 , and the coefficient of determination, R^2 is the proportion of the variation in Y that can be accounted for by the linear part of its relation with X . If, for example, $R = 0.9$, then 81% of the variation in Y can be accounted for by the linear relation with X . If $R = 1$ and the (x,y) pairs are plotted on a graph, they lie on a straight line. In the social sciences, $R \geq 0.8$ is considered a strong correlation. The values obtained here are much greater than that.

It is important to bear in mind that the correlation coefficient measures only the strength of the linear part of the relation. Correlation of course only suggests cause and effect; it does not prove it. If, however, the variables X and Y both increase in time but not linearly, then the observation that the relation between them is close to linear, as indicated by the very high correlation coefficients that were obtained, is stronger evidence in favour of a causal relationship.

The correlation coefficients, their squares and the p -values for the various incidences, prevalence and deaths due to diseases are summarised in Tables 3 and 4.

Table 3. Pearson's coefficients between disease and glyphosate applications (N=21 encompassing 1990-2010), except autism (N=16; autism data only available for 1995-2010).

Disease	Coefficient, <i>R</i>	<i>R</i> ² × 100	Probability, <i>p</i>
Thyroid cancer (incidence)	0.988	97.6	≤7.6E-9
Liver cancer (incidence)	0.960	92.1	≤4.6E-8
Bladder cancer (deaths)	0.981	96.2	≤4.7E-9
Pancreatic cancer (incidence)	0.918	84.2	≤4.6E-7
Kidney cancer (incidence)	0.973	94.8	≤2.0E-8
Myeloid leukaemia (deaths)	0.878	77.1	≤1.5E-6
Lipoprotein metabolism (deaths)	0.973	94.8	≤7.9E-9
Hypertension (deaths)	0.923	85.2	≤1.6E-7
Stroke (deaths)	0.925	85.5	≤1.5E-7
Obesity	0.962	92.5	≤1.7E-8
Diabetes (prevalence)	0.971	94.3	≤9.2E-9
Diabetes (incidence)	0.935	87.4	≤8.3E-8
ESRD (deaths)	0.975	95.0	≤7.2E-9
Renal failure (deaths)	0.978	95.6	≤6.0E-9
Autism (prevalence)	0.989	97.9	≤3.6E-7
Alzheimer's (deaths)	0.917	84.1	≤2.2E-7
Parkinson's (deaths)	0.875	76.6	≤1.6E-6
Dementia (deaths)	0.994	98.8	≤1.8E-9
Multiple sclerosis (deaths)	0.828	68.5	≤1.1E-5
Intestinal infection (deaths)	0.974	94.8	≤7.6E-9
Inflammatory bowel	0.938	88.0	≤7.1E-8

Table 4. Pearson's coefficients between disease and the percentage of US corn and soy crops that are GE (N=15 encompassing 1996-2010; GE crops were first planted in 1995).

Disease	Coefficient, <i>R</i>	<i>R</i> ² × 100	Probability, <i>p</i>
Thyroid cancer (incidence)	0.938	87.9	≤2.2E-5
Liver cancer (incidence)	0.911	82.9	≤5.4E-5
Bladder cancer (incidence)	0.945	89.3	≤7.1E-6
Pancreatic cancer (incidence)	0.841	70.7	≤4.0E-4
Kidney cancer (incidence)	0.940	88.4	≤2.0E-5
Myeloid leukaemia (deaths)	0.889	79.0	≤5.4E-5
Lipoprotein metabolism (deaths)	0.955	91.2	≤4.7E-6
Hypertension (deaths)	0.961	92.3	≤3.7E-6
Stroke (deaths)	0.983	96.6	≤1.4E-6
Obesity	0.962	92.5	≤3.5E-6
Diabetes (prevalence)	0.983	96.6	≤5.1E-7
Diabetes (incidence)	0.955	91.2	≤2.0E-6
ESRD (deaths)	0.958	91.7	≤4.2E-6
Renal failure (deaths)	0.967	93.6	≤2.7E-6
Alzheimer's (deaths)	0.937	87.9	≤9.6E-6
Parkinson's (deaths)	0.952	90.6	≤5.4E-6
Multiple sclerosis (deaths)	0.876	76.7	≤8.0E-5
Hepatitis C (hospital diagnoses)	0.946	89.4	≤6.9E-6

There are four diseases in Table 3 that are not in Table 4 because we did not plot the percentage of GE crops for autism, dementia, inflammatory bowel and intestinal infection. We plotted only glyphosate applications against inflammatory bowel and intestinal infection because the information from Samsel & Seneff (2013b) indicated that glyphosate causes intestinal problems by killing beneficial bacteria in the intestines. We plotted only glyphosate applications against autism and dementia because the correlation coefficients were already 0.989 and 0.994 respectively.

There is one disease (hepatitis C) in Table 4 that is not included in Table 3 because we did not plot hepatitis against glyphosate applications. This is because, according to Ho (2009; 2013), viral diseases may be activated by the CaMV promoter used in GE crops. We plotted the hepatitis C against the percentage of GE soy crops planted in the US because soy was more quickly adopted, is currently 98% of the total US soy crops, and is ubiquitous in packaged food in the US.

Table 5 provides a summary of which diseases have the highest correlation. All of these have a very strong correlation coefficient with very high significance (very low probability that the correlation is random). The highest correlations were found for senile dementia, autism, bladder and thyroid cancer with glyphosate applications and stroke and diabetes prevalence with %GE crops planted. In most cases, diseases that had correlation coefficients of less than 0.95 with glyphosate applications had greater than 0.95 with %GE crops planted and vice-versa. Some had correlation coefficients exceeding 0.95 for both GE crops and glyphosate applications: obesity, lipoprotein metabolism disorder, ESRD, renal failure, prevalence of diabetes as well as kidney and bladder cancers.

Table 5. Summary of correlation coefficients, showing the number of diseases with *R* in the various ranges for glyphosate applications and for %GE crops planted.

<i>R</i> -value range	Correlation with glyphosate		Correlation with %GE crops planted	
	No.	Disease	No.	Disease
$R > 0.98$ $R^2 \times 100 > 96\%$	4	Thyroid, autism, dementia, & bladder	2	Stroke, diabetes (prevalence)
$0.97 < R < 0.98$ $94\% < R^2 \times 100 < 96\%$	6	ESRD, diabetes (prevalence), lipoprotein metabolism, intestinal, kidney & renal		
$0.95 < R < 0.97$ $90\% < R^2 \times 100 < 94\%$	2	Obesity, liver	7	Parkinson's, hypertension, diabetes (incidence), obesity, lipoprotein metabolism, ESRD, renal
$0.90 < R < 0.95$ $81\% < R^2 \times 100 < 90\%$	6	Diabetes (incidence), inflammatory bowel, hypertension, stroke, Alzheimer's, pancreatic	7	Liver, bladder, kidney thyroid, pancreatic, Alzheimer's, hepatitis
$0.86 < R < 0.9$ $74\% < R^2 \times 100 < 81\%$	2	Parkinson's, myeloid leukaemia	2	Myeloid leukaemia, multiple sclerosis
Correlation ≥ 0.90	18		16	

Interpretation of results

Some of the plots show a significant linear rise that began prior to 1990. Others show a peak in the 1980s, then a decline followed by another rise in the 1990s. Clearly, there are multiple factors involved. Though the data for glyphosate are only available beginning in 1990, glyphosate was first introduced in the marketplace in 1974. Other known endocrine disruptors are: BPA (bisphenol-A) and phthalates (both in plastics), dioxins (by-product of smelting, paper bleaching, manufacture of herbicides and pesticides), hexane (cooking oil extraction), atrazine, and polychlorinated biphenyls (PCBs - used in electrical equipment, coatings, inks, adhesives, flame-retardants, and paints) (Kavlock, 1996).

The population of the US is bombarded with a veritable cocktail of chemicals daily in addition to GE food and glyphosate (Reuben, 2010). These include food preservatives (BHA & BHT), water contaminants (chlorine & fluoride), heavy metals, food additives (aspartame, monosodium glutamate, carrageenan) and food colouring, to name a few. The US President's Cancer Panel reported that a study by the CDC found many toxic chemicals in the blood and urine of most Americans that they tested, and the Environmental Working Group found up to 232 xenobiotic chemicals in the placental cord blood of newborns in the US (Reuben, 2010). The people have been exposed to an increasing background level of chemicals and other toxins for over 70 years, yet few, if any, have increased at the rate of glyphosate and GE crops.

According to Samsel & Seneff (2013a), glyphosate disrupts the ability of animals, including humans, to detoxify xenobiotics. This means that exposures to the numerous chemicals in food and the environment, such as endocrine disrupting chemicals and carcinogens, could be causing levels of damage that would not occur if the body were able to detoxify them. The accumulation of toxins as the result of low levels of poisoning over a long period of time leads to a high body burden or toxic load. Every person is unique and the ability of the body, or the path it takes to detoxify, is both genetic and acquired (Anderson, 2012). If the body burden becomes overwhelming, it could be that only a small amount of additional stress will induce the breakdown of the system in whatever way that manifests according to individual predisposition.

If we know that a causal factor exists, that is A causes B, then we would expect a high degree of correlation between the two data sets for A and B. The inverse is not true, i.e. because there is a high degree of correlation between A and B it is not necessarily the case that A causes B or vice-versa.

However, we have data for 22 diseases, all with a high degree of correlation and very high significance. It seems highly unlikely that all of these can be random coincidence. Ruling out coincidence, we are left with these three options:

1. There is a direct cause and effect relationship
2. The relationship may be caused by a third variable
3. The relationship may be caused by complex interactions of several variables

In 1965, Austin Bradford Hill addressed the problem of deducing causation when observations reveal an association beyond what can be considered random chance (Hill, 1965). Hill proposed nine conditions that should be considered as an aid in determining causation. These are the well-known Hill's Criteria. Since we have not performed an experiment, and we do not have information on dose/response, it would be difficult to go through and say this criterion is true and this one is not for all of the diseases as a whole. However, we quote from the American Academy of Environmental Medicine's position paper on genetically modified (GM) foods: "[S]everal animal studies indicate serious health risks associated with GM food consumption including infertility, immune dysregulation, accelerated aging, dysregulation of genes associated with cholesterol synthesis, insulin regulation, cell signaling, and protein formation, and changes in the liver, kidney, spleen and gastrointestinal system." "There is more than a casual association between GM foods and adverse health effects. There is causation as defined by Hill's Criteria in the areas of strength of association, consistency, specificity, biological gradient, and biological plausibility" (Dean & Armstrong, 2009, online). The document goes on to explain in detail why and how each of these criteria are met based on published research.

Conclusions

These data show very strong and highly significant correlations between the increasing use of glyphosate, GE crop growth and the increase in a multitude of diseases. Many of the graphs show sudden increases in the rates of diseases in the mid-1990s that coincide with the commercial production of GE crops. The large increase in glyphosate use in the US is mostly due to the increase in glyphosate-resistant GE crops.

The probabilities in the graphs and tables show that it is highly unlikely that the correlations are a coincidence. The strength of the correlations shows that there is a very strong probability that they are linked somehow. The number of graphs with similar data trends also indicates a strong probability that there is a link. Although correlation does not necessarily mean causation, when correlation coefficients of over 0.95 (with *p*-value significance levels less than 0.00001) are calculated for a list of diseases that can be directly linked to glyphosate, via its known biological effects, it would be imprudent not to consider causation as a plausible explanation.

We do not imply that all of these diseases have a single cause as there are many toxic substances and pathogens that can contribute to chronic disease. However, no toxic substance has increased in ubiquity in the last 20 years as glyphosate has. The disruption by glyphosate of the detoxification pathways in the human body can intensify the effect of other toxic chemicals. The disruption of the Cytochrome P450 pathways by glyphosate could account for it causing numerous diseases (Samsel & Seneff, 2013a). The Cytochrome P450 enzymes are the superfamily of enzymes that are responsible for around 75% of the reactions involved in drug metabolism and the oxidation of organic molecules (Guengerich, 2008). Another critical issue is that glyphosate is an endocrine disruptor and it has been argued that there are no safe levels of endocrine disruptors (Vandenberg et al., 2012; Bergman et al., 2013). This would imply that the current permitted residue levels in food could be causing multiple

health problems that have been documented in the scientific literature to be caused by endocrine disrupting chemicals.

The findings reported by Kruger et al. (2014) that there is no significant difference in glyphosate residues detected in the urine, tissue and organs of cows is evidence that glyphosate bio-accumulates in our bodies. The research showing that Roundup and glyphosate cause oxidative stress resulting in changes to cell functions, necrosis in cells and neurotoxic effects in brain, kidney hepatic, testis and Sertoli cells needs to be considered as a possible causative agent in a range of diseases (Cattani et al., 2014; de Liz Oliveira Cavalli et al., 2013; Lushchak et al., 2009; El-Shenawy, 2009).

The prevalence of certain diseases is likely to rise simply due to better treatments available, allowing people to live longer with the diseases. All of the graphs, save three (inflammatory bowel, hepatitis & autism), are age-adjusted. The age-adjustment would partially account for many of the people living longer with chronic diseases, and consequently this group of people would not be a significant reason for the dramatic increase in diseases found in most of the graphs.

An increase in surveillance of a particular disease can artificially increase the prevalence of the disease because it merely increases the known number of cases, when the actual number has not changed. Surveillance of certain diseases has been boosted over recent decades, and may have artificially increased the prevalence of some of these diseases. The increased surveillance would initially find more cases and subsequently show an increase; however this would be expected to capture approximately the same percentage every year and would thus level off fairly quickly. If the actual prevalence of diseases was not increasing, the graphs would show a new stasis that would remain fairly level reflecting the extra cases found by the surveillance. The rates of disease prevalence are steadily increasing, so increased surveillance cannot be a significant reason for the dramatic and continuing rise in many of the diseases shown in the graphs since the 1990s. Any increase in surveillance could only account for part of the increase shown in the graphs.

In reviewing the toxicity of chemicals based on the latest peer reviewed science, the US President's Cancer Panel report was critical about the current testing methodologies and the lack of action taken by regulatory authorities (Reuben, 2010). According to the report, the regulatory approach in the US is reactionary rather than precautionary. Instead of taking preventive action when uncertainty exists about the potential harm a chemical or other environmental contaminant may cause, a hazard must be incontrovertibly demonstrated before action is initiated. Instead of requiring industry to prove the safety of their devices or chemical products, the public bears the burden of proving that a given environmental exposure is harmful.

The current testing methodologies, length of feed trials of GE crops, and the parameters measured are insufficient to evaluate the health problems that may be caused by diets consisting of GE food (Seralini et al., 2011). The lack of proper testing protocols means that there is insufficient data to show that the increase in GE crops and glyphosate is not linked to the increase in diseases. The data presented in this paper highlight the need for independent scientific research to be conducted, especially in the areas of the endocrine disruption, cancer precursor, oxidative stress, gut microbiome and the Cytochrome P450 pathways. It is our hope that, in addition to more basic research in the form of toxicology and carcinogenic studies, epidemiology studies will be undertaken by experts in each of these disease categories.

Conflicts of Interest

The authors declare no conflicts of interest.

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Investigating the context of purchase choices to further understanding of switching behaviour

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Abstract

The organic food market continues to grow yet market share remains low. The majority of consumers in this market tend to switch between organic and conventional food products rather than being heavy users of organic branded products. The purpose of this research is to present a deeper investigation of the factors that can lead to the purchase or non-purchase of organic food in order to gain a better understanding of this switching behaviour. A qualitative grounded theory approach was utilised involving in-depth interviews with 21 participants in Australia. These were primary shoppers who switch between organic and conventional food. An emergent conceptual framework was developed from the data which identifies factors that influence whether or not organic food is bought. This framework includes three layers: consumer context; choice of retail outlet; and point-of-purchase. Depending on the specific situation, these factors influence buyers to different extents and hence their collective impact determines whether that individual purchases organic food on a given shopping event. The framework can be used by organic food marketers as a checklist to developing an understanding of their consumers and a basis for developing strategy.

Keywords: consumer behaviour, food marketing, organic food.

Introduction

The aim of this research is to explore and provide insight into why, despite the significant and continuous growth in the organic food market, overall market share remains very low. The basis for this low share is the 'switching' behaviour that occurs in this market: the majority of consumers tend to 'switch' between organic and conventional food rather than consuming a diet consisting mostly of organic food. To date, this switching behaviour has not been satisfactorily explained. Existing research and marketing frameworks such as segmentation, attitudes, beliefs and motivations go some way to providing insight, but fail to provide a cohesive explanation. This paper briefly highlights current marketing knowledge in this arena before offering grounded theory as a method of building upon consumer decision making processes (which include choice of retail outlet and point-of-purchase factors). The outcome is an emergent conceptual framework of organic switching behaviour which includes consumer context. This framework is based around three layers (consumer context; choice of retail outlet and point-of-purchase) that lead to either purchase or non-purchase of organic food at a given shopping event.

The organic food market

The growth in the global organic food market has continued despite challenging economic conditions. Consumer interest in organic food is reflected in global sales of USD 62.9 billion in 2012 which is an increase of USD 3.8 billion from 2010 and a growth of 170% since 2002 (Willer et al., 2013). In developed countries, the majority of consumers buy at least some organic food. Reports indicate that this may be as high as 90% in the United Kingdom (Soil Association, 2009) through to 70% in the United States (Demerit, 2009) and 65% in Australia (Monk et al., 2012). However, market share remains small (Aertsens et al., 2011). In Australia it is estimated to be between .8 and 1.2% (Monk et al., 2012) and around 4% in the United States (Organic Trade Association, 2011).

Existing consumer behaviour frameworks

Existing marketing and consumer behaviour frameworks such as segmentation, attitudes and motivations offer some assistance in facilitating our understanding of switching behaviour in the organic food market. Each are located in the literature and their limitations in relation to assisting us to understand low market share of organic food and switching behaviour are discussed below.

Segmentation

Segmentation is a marketing tool aimed at dividing the market for a product or service into homogenous segments of consumers with similar characteristics, needs and behaviours (Kotler et al., 2012). These segments can then be used as a basis for developing targeted marketing communication. In the organic food market, the organic consumer has been examined in detail through the lens of segmentation (see, for example, Fotopoulos and Krystallis, 2002; Lockie et al., 2002; Storstad and Bjørkhaug, 2003; Lea and Worsley, 2005) with the majority of research using demographics as a basis (Henryks and Pearson, 2011). However, with the exception of segmentation on the basis of gender, results are inconsistent and consequently demographic variables are not a very useful predictive tool of who is and who is not an organic consumer. As Zepeda et al. (2006, p.392) pointed out "... focus group study confirms that only looking at gender, income, education, and family/household size may yield contradictory results because people's motivations are complex". It is for this reason that we need to look elsewhere in order to understand the organic consumer.

Behavioural segmentation would be another way of attempting to understand consumption patterns in this market. Unfortunately, within the organic category, frequency of purchase is difficult to measure, and how it is measured varies in different studies (Magnusson et al., 2001). While studies consistently report that most consumers buy organic food occasionally, around 65% of the time (Monk et al., 2012), this can range from buying a large amount of organic food irregularly, to buying a small amount irregularly. In other words, although a sizeable percentage of consumers buy organic food, there are inadequate consistent data to enable meaningful interpretation. Although it alerts us to the critical fact that a significant number of consumers are occasional buyers of organic products, that is, they switch between purchasing organic and conventional products, it does not explain why this may be the case (Pearson et al., 2013). Factors other than behavioural segmentation need to be examined in order to understand this switching behaviour.

Although as mentioned, the majority of the literature concerns itself with demographics as a segmentation variable, some studies have examined other types of market segmentation (Fotopoulos and Krystallis, 2002; McEachern and McClean, 2002; Monk et al., 2012) including psychographic and lifestyle. Whilst the various segments were interesting and provided some insight into the consumer behaviour in this market, they too fail to explain 'switching' behaviour, which as previously identified is a key feature of the organic market. Consequently, in the organic food market, segmentation as a marketing tool, with its reliance on correlation between consumption behaviour and stable characteristics of consumers, offers little insight into the majority of consumers that switch between organic and conventional food. It therefore is not useful for determining how marketers could best target these consumers.

Attitudes, motivations and barriers to purchase

Understanding attitudes and associated motivations helps provide a rational framework for the reasons consumers may or may not buy organic food; however, in the case of switchers, attitudes do not explain the switching behaviour. For example, consumers can be positively disposed and give reasons as to why they do purchase organic food (taste, health and environment, social responsibility, quality and food safety (McEachern and McClean, 2002; Lockie, Halpin and Pearson, 2006; Pearson and Henryks, 2008; Pearson, Henryks and Jones, 2011 Nasir and Karakaya, 2013;)), yet still switch between organic and conventional food.

Amongst the substantial group of consumers that do buy organic occasionally (and presumably for those who do not buy it), there are three major barriers to purchasing more of it: price, availability, and appearance. Certified organic food carries a price premium relative to non-organic food. Any study that considers barriers to organic consumption mentions price (Lockie et al., 2002; McEachern and Willock, 2004; Shepherd et al. 2005). The major argument for lowering prices is that consumers claim that organic food is unaffordable; however, price as a barrier warrants closer attention. The average Australian spends just over \$A20 per week on fresh fruit and vegetables and over \$A80 on alcohol, soft drinks, takeaway food and confectionary (ABS, 2011). This suggests that it is not an affordability issue alone but one of choice for many consumers. An Australian study found that lower income was not a barrier to commitment to organic food purchasing behaviour (Newspoll, 2008) further suggesting it is choice, and not affordability, that is the issue for the majority of consumers. In addition, two studies that looked at co-op shoppers found that, despite low incomes, there was substantial willingness to spend more on organic food (Jolly et al., 1989; Goldman and Clancy, 1991).

Repeated studies found that although consumers complain about the price differential, when asked to elaborate as to what it actually is, they are either unclear (Chang and Zepeda, 2005) or incorrect

(Lampkin, 2002). Studies into (conventional) supermarket shopping have also found that “a sizeable percentage of consumers buy products without knowing their price... (which) does not necessarily imply that consumers do not care about price” (Grunert, 2007, p. 170). So, although price is often cited as a barrier for reasons of affordability, closer examination reveals a complex issue: consumers that value organic food pay for it whilst those who don't, complain.

The second most cited barrier to the purchase of organic food is availability. Organic food is not as easily available as conventional food and consumers often need to alter their shopping behaviour if they are seeking to purchase organic food. The same studies that cited price as a barrier also cite availability (Lockie et al., 2002; Zanolli and Naspetti, 2002; McEachern and Willock, 2004; Shepherd et al. 2005). The lack of easily available organic food results in it requiring more effort and being less convenient for some consumers. However, as Lyons, Lockie, and Lawrence (2001, p.204) pointed out, “unavailable” means “not easily available from the supermarkets where I shop”.

The third cited purchase barrier pertaining to organic food, specifically fresh fruit and vegetables, is appearance. Organic food has not always had the same appearance as conventional food because without chemicals to kill pests, organic food will sometimes contain bug holes and other blemishes. However this has been reduced over recent decades as many organic producers have been able to improve the appearance of their products. Consumers are accustomed to the appearance of perfect-looking fruit and vegetables, consequently the imperfect appearance of some organic produce is a potential barrier for a number of consumers (Thompson and Kidwell, 1998; Fearn, 2008). Others, however, see pest markings as sign that the food is organic (Henryks and Pearson 2010).

Price and availability are the two most commonly-cited barriers to the purchase of organic food: however, upon closer examination of these two factors it is possible that they are simply convenient, logical post hoc rationalisations provided by consumers when they are asked why they do not purchase organic food.

The question then arises: how are the attitudes, motivations and barriers discussed above related to organic switching behaviour? Why do they ‘kick in’ on some occasions and not on others? While purchase motivations pertaining to organic food may go some way to aiding us in understanding *why* some people buy organic food and others do not, they fail to shed any light on the switching behaviour that occurs in this market.

Gaps in our understanding

One of the marketing challenges with organic food is that it is difficult to define as a discrete category that consumers belong to and are loyal to as the majority of consumers ‘switch’ in and out of buying organic products. A consumer may one week buy organic bread and the next week conventional bread. Another consumer may consistently buy organic fruit and vegetables but other categories of organic food sporadically or not at all. Further, not all consumers buy in each organic category for the same reasons or motivations. McEachern and Willock (2004) considered consumer motivations for purchasing organic meat (animal welfare amongst others) which can potentially be very different to motivations for purchasing organic vegetables (for example, taste) (McEachern and Willock 2004). This presents a further marketing challenge.

Given the behaviour in question pertains to consumer ‘switching’ behaviour, the literature on switching has also been explored for relevance to this challenge. This body of literature predominantly examines service relationships (such as banks, insurance or telecommunications) where a binary relationship tends to exist – consumers change service providers rather than continually switch between brands (for example, Oyeniyi et al., 2010; de Matos et al., 2013; Kaur, Sharma, and Mahajan, 2012). This is not the case with organic food, where consumers continuously move between conventional and organic food, and no relevant insights were gained.

Despite a considerable body of literature exploring organic food buyer behaviour, we are unable to explain the fundamental question: why do so many consumers switch between organic and conventional food? Recent research by the authors has explored this question from two perspectives: the choice of retail outlet (Henryks and Pearson, 2011) and the point-of purchase (Henryks et al. 2013). The aim of this paper is to build upon these perspectives and provide a framework for addressing this question.

Methodology

Consumers that switch between organic and conventional food were the focus of this research as they comprise a large segment of the food market. As noted, the context in which this group of consumers bought (or did not buy) organic food was missing from existing research. Thus a grounded theory approach was chosen as it focuses on building theory from the data and is an inductive approach (Glaser, 1998). This approach allowed for participants' stories to emerge from the data, inform the emergent theory and be incorporated back into further interviews until saturation was reached and no new data emerged. The overall intention was to gain a deeper understanding of the complexities and contexts involved in the organic food buying process.

Selected participants for this study were the primary shopper for their household and, to ensure that they were *switchers*, needed to be purchasing at least 3 organic items per week but not the majority of their food as organic. This was to ensure that they were *switchers* and not 'heavy' or 'non-consumers' of organic food. Further, as the aim was to uncover a diverse range of perspectives in order to increase the chances of developing well-rounded theory, participants were chosen to enhance demographic diversity and a range of stages in the family lifecycle. This selection criterion is shown in the table below and served to provide diverse context for the stories and perspectives that emerged from the data. A modified snowball sampling technique was used whereby informants, known to the researcher, were asked for their assistance in putting potential participants in touch with the researcher (Minichiello et al. 2008).

Table 1. Summary of final sample used in the study.

Household demographics	Armidale participants (n= 8)	Canberra participants (n=13)
Couple with no children living at home	Henry (retired) Gabrielle (children have left home) Dorothy (children have left home) Conrad (never had children) Betty (about to have children)	
Couple with young children	Elizabeth (1 preschool and 2 school aged children).	Sam (2 primary school aged children).
Single person with older children	Anna (2 primary and 2 high school children)	Queenie (family with 1 child in primary and 1 in high school) Rose (family with high school children).
Single person living alone	Felicity (working full time)	Kate (working full time) Jenny working full time) Ursula (working full time)
Single person in a share household		Lexi (children have left home) Olivia (university student in a share student household) Isabelle (house sitting and sharing) Penny (working full time) Meena (working full time)
Single parent with part time responsibility for children		Natalie (2 primary school aged children)
Single parent with full time responsibility for children		Tanya (1 primary and 2 high school aged children)

Applying theoretical sampling, the collection of empirical information was deemed to be complete when no new information emerged from the participants. This saturation point was reached after 19 interviews. As a further check on whether or not the saturation point had been reached, two additional interviews were conducted, and neither yielded any new insights. The final sample consisted of 21 participants and pseudonyms were assigned to protect their identities (see Table 1).

Participants were from two Australian cities: Armidale in New South Wales (population 20,000) and Canberra in the Australian Capital Territory (population 350,000) in order to examine if there was a difference between the urban and rural perspectives. (With the exception Canberra participants having access to farmers' markets, no notable differences were found; however, participants are identified by their location in order to maintain consistency and transparency.) Both these cities supported a range of retail outlets that stocked organic food including supermarkets (small and large), health food stores and food co-operatives. Although, as mentioned, at the time of this research, only Canberra had established weekly farmers' markets.

A grounded theory method gives clear structure to data analysis. The 'bones' of data analysis consisted of two practices: coding and memos. Coding in the present study followed the guidelines set down by Charmaz (2006). In her approach, coding is a two stage process where initial open coding is followed by selective coding. Additionally, memos were composed and used for the duration of the project in order to aid reflexivity.

The Emergent Conceptual Framework

Based on the analysis of the data, a conceptual framework of switcher buyer behaviour was developed and is illustrated Figure 1. It represents consumer switching behaviour for a given shopping event. All three layers of the framework are interrelated and consequently result in many different stories. This conceptual framework builds upon previously published papers on two of the three layers: choice of retail outlet (Henryks and Pearson, 2011) and point-of-purchase factors (Henryks, Cooksey and Wright, 2013) and adds the final layer 'consumer context'. The resultant switcher buyer behaviour framework maps the various factors that can be considered when developing marketing strategy for an organic product.

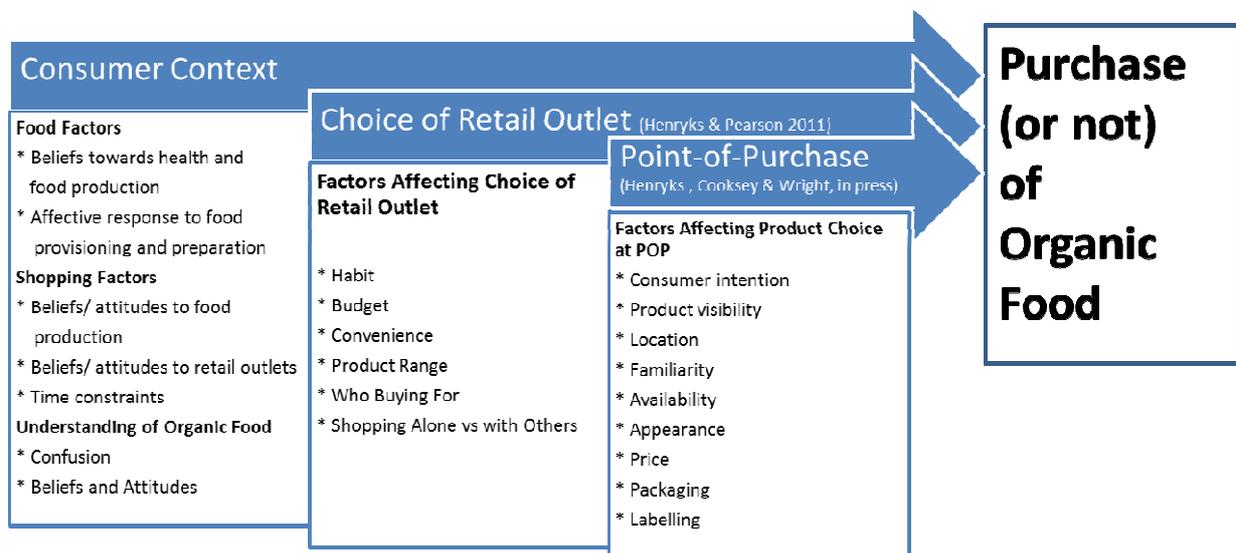


Figure 1. Switcher buyer behaviour conceptual framework.

Consumer context: factors influencing the purchase of organic food

Food shopping and food consumption are affected by the way consumers feel about food and the meaning that it has in their lives. The first layer of the switcher buyer behaviour conceptual framework consists of three contextual factors that can influence consumers' organic food choice: Food; Shopping, and Understanding of Organic Food. It is important to note that these various contextual factors pertaining to a shopping event can influence the other key components of the organic food buying framework. Consequently while each layer has been identified and separated from the overall buying process, it does not operate in isolation but as a synergistic part of the framework.

Food as factor of consumer context

Food as a contextual factor affects organic purchase decisions in two main arenas: beliefs towards health and food production, and affective responses to food provisioning and preparation. Health was intrinsically linked to food for all participants; however, the way in which it was linked meant different things to different people. For some it was as insurance policy:

I guess I have a personal belief that giving my kids healthy food is like our health insurance and their health insurance, if I can raise them up on a good healthy diet then I've set them up nutritionally hopefully for life and set them with good food habits. Tanya Canberra p.3

I think you can buy really cheap food and probably get sick.... I don't see the point in buying cheap, rubbish food. Sam Canberra p.4

For others it was about the importance of avoiding additives and chemicals found in conventional food:

I'm not putting food into my system that's pumped full of ... sprayed with chemicals and God knows what. And not knowing what effect various things have on my system... Felicity Armidale p.7

It seems to me that having no or less chemicals has got to be good for our systems I hate to think about all the stuff we're breathing in and that we're eating and consuming in all different manner of ways chemicals etc. Kate Canberra p.6

The second arena influencing consumer behaviour was the affective response to food provisioning and preparation. For some it was viewed as a source of stress and for others, pleasure. The perspective was dependent upon the time available and the context for the event. Those with children found it to be more of a chore, despite its perceived importance whereas those preparing for social gatherings or who had time found it to be a more pleasing experience.

It can be a bit of a chore if I'm rushed or during the week it's a bit of a hassle... sometimes when I have all the girls, there's three of them... sometimes it's a bit of a push. Natalie Canberra p.2

... and I like the experience of getting something that's really raw. I suppose cutting it, chopping it and putting things with it to make it really delicious. Olivia Canberra p.5

Beliefs towards food production also impact on Consumer Context across two of the Contextual Factors, Food and Shopping. The majority of participants were keen to have some sort of connection to the source of their food; this could be through knowledge of the production process or a relationship with the grower.

If I can have a sense of where the food comes from, that's important ... that there's the personal involvement in it. One would hope that if you know something about where your fruit and veg is coming from or if you can talk to the people that are selling it, who have grown it, that those people are also being looked after better. Felicity Armidale p.5

I really like it when apple season and I go to Mr C (orchardist) and can ask him..... about apple season.....it's a ritual and... I like the realness of it and that exchange of climate, the weather and cockatoos. Anna Armidale p.3

Animal and environmental concerns also dominate participants' beliefs about food production. Organic food was considered to be better for the environment as well as have superior standards of animal welfare.

...well I guess there's the whole political option... I feel like I'm feeding my kids right, and trying to use my money to support to buying food that's hopefully costing the planet less. Tanya Canberra p.7

what I resent (about supermarkets) – the over packaging, the waste, the non-pureness of processing... it's just the physical environment, the light, the air and not generating all that waste. Anna Armidale p.4

...these factory feed lot sort of things I find pretty horrific... I don't feel strongly enough that I'd want to be a vegetarian, but I do feel strongly enough that the animals should have a decent life before they get turned into food for us. Elizabeth Armidale p.7

Shopping as factor of consumer context

The second component of the context layer is the consumers' view of shopping. Food provisioning fulfils many needs for people from the basic need to eat, and feed the family for some, through to a more abstract health insurance policy. Three aspects of shopping impacting upon consumer choice are: beliefs and attitudes to food production, beliefs and attitudes to retail outlet, and time constraints.

Various food production concerns can impact upon attitudes towards and choice of retail outlet whether this is a local food co-op, farmer's market or supermarket.

I like food to be healthy to know where it's come from, that it's supporting other people's enterprise, that it's not damaging the environment, not sprayed and the social context of the co-op does matter... it is building connection between people... Anna Armidale p.4

Participant's views on retail outlets were influenced by the type of outlet. Those who shopped at the farmers' market felt that it provided a unique shopping experience that was not attainable in any other food retail outlet. The farmers' market experience comprised a combination of meeting social needs, high quality of fresh, healthy food and an extensive product range. Similar views were held about the food co-op; however, supermarkets were considered to be a 'necessary evil'. They provided a good range of products at affordable prices and were open at convenient times but had questionable practices.

I really enjoy it (farmers' market), it's more than just shopping... it's relaxing and ...I'll get a coffee and have a look at what's there and try things they have for tasting. I don't just go there to shop. I like the atmosphere of it. Ursula Canberra p.2

What do you get from the co-op? – it (Armidale food co-op) has got those elements about being part of being a movement. I can't crack what it is but I can sense it but it feels like a bit like anarchy, (it) feels like it is being true to my value. I like the community, I like the purity... the simplicity, it takes away the clutter. I like the pureness. Anna Armidale p.4

Well the supermarket you basically aim to get in and out as quickly as you can. It's an entirely different experience because there's no love in a supermarket. Isabelle Canberra p. 5

Time is the final aspect of shopping context. Mothers in particular felt that if they had more time to shop, their shopping experience would be different. The competing demands on their time meant that shopping had to be fitted in amongst other chores and consequently it was not always possible to shop at their preferred outlets. Sam would have preferred to shop at the farmers' markets more often but was rarely free when they were open and Elizabeth's enjoyment of food shopping was marred by a lack of time.

If I had time to go to the markets (I'd enjoy shopping).... Sam Canberra p.7

It just depends on how rushed I am and how many other things I've got to do. Elizabeth Armidale p.3

Consumer understanding of organic food as factor of consumer context

Consumer understanding of organic food comprises the final element of the contextual layer and affected participants' organic food purchase behaviour to various degrees. This understanding encompasses the confusion that exists amongst consumers in relation to organic food and attitudes and beliefs towards organic food. By understanding 'what' consumers believe about organic products, it is therefore possible to address these beliefs in the marketing of organic products. The three main areas of belief held by participants about organic food (health, taste and the chemical free nature of organic food) were consistent with the literature previously discussed on consumer beliefs pertaining to organic food.

It's healthier and often it tastes better to me it tastes more like food used to taste when I was a kid when we used to grow the vegetables. Kate Canberra p.4

The critics say look, there's no difference between organic food and conventionally grown food, and of course there is. You know, either chemically or taste wise, there is, although in some cases it's not as easily discernible. Henry Armidale p.9

Despite the beliefs held about organic food, a great deal of confusion also existed. This arose from several sources including recognising organic food in retail outlets and how the food was actually grown. Jenny's words captured the general confusion about what constituted organic food:

I like the concept, I like not having all the pesticides I guess one of the problems is having a definition of what exactly is organic...Jenny Canberra p.9

Another area of concern which affects the purchase of organic food is that participants often assumed that food sold at the farmers' market was organic. Interestingly, when Ursula and Rose reflected upon the issue during their interview for this study, they realised that it may not always be the case.

...thinking about it now I realise that the farmers' market might not always be organic, even though it's locally produced and that sort of thing. It will depend on which stalls you choose from, which ones are organic and which are not. Ursula Canberra p.8

...when I think of it I just assumed it was all organic but I hadn't looked, to be honest, to see if it was labelled organic or not. Rose Canberra p.10

These three Consumer Context areas: food factors, shopping factors, and understanding of organic food, all impact and influence consumer choice and decision making at the next layer of the conceptual framework - the choice of retail outlet.

Choice of retail outlet

Choice of retail outlet has been explored in detail in previously published research (Henryks and Pearson, 2011) thus is only briefly described here in terms of implications that relate to the Switcher Buyer Behaviour Conceptual Framework (Figure 1). Choice of retail outlet can dictate whether or not an organic product is chosen in a given shopping event. Hence the choice of outlet can impact the final behavioural outcome of purchase or non purchase of organic food. In any given shopping event factors such as habit, budget, convenience, product range, who they were buying for, whether shopping alone or with others, all served to impact on decision making pertaining to the choice of retail outlet which in turn could impact upon the purchase (or not) of organic food.

Point-of-purchase factors influencing purchase of organic food

The point-of-purchase is the final hurdle in determining whether a consumer purchases (or does not purchase) organic food on a given shopping event. It has also been explored in detail in previously published research (Henryks, Cooksey and Wright, 2014) thus is only briefly described here in terms of implications that relate to the Switcher Buyer Behaviour Conceptual Framework (Figure 1). Point-of-purchase issues that influence purchase of organic food are: whether or not the consumer went into the outlet with the intention to purchase organic food; visibility; location; consumer familiarity; product availability; appearance; price; packaging; and labelling.

Discussion and implications

Organic food and its consumers is a much researched group (Pearson et al., 2011) yet continues to capture low market share. As noted, the behaviour of a significant group of organic consumers presents a challenge to the organic food industry as they continue to 'switch' between organic and conventional food. This paper has presented a conceptual framework which contributes to our understanding of these consumers by expanding the range of factors that can impact upon the purchase (or not) of organic food. This framework includes three layers: the Consumer Context (food, shopping and consumer understanding of organic food); the Choice of Retail Outlet; and Point-of-Purchase factors. Although previous research has considered many of these factors in isolation, they have not been previously been considered in a cohesive framework.

The Switcher Buyer Behaviour Conceptual Framework (Figure 1) identifies factors which heavily influence and contribute to explaining consumer switching buyer behaviour in the organic food market and hence enhance understanding that is gained from other approaches to understanding buyer behaviour, such as the decision process model of buyer behaviour (Kotler et al., 2012). For instance, a switcher may have chosen a retail outlet for a given shopping event based on their proximity to the outlet while attending a child's sporting match (convenience). This choice of retail outlet will in turn determine availability of organic produce (for example the closest green grocer may not carry an extensive range of organic produce) and consequently can lead to the non-purchase of organic food for that given shopping event. This example demonstrates the contextual sensitivities of the Switcher Buyer Behaviour Conceptual Framework in that it provides keys to understanding switching behaviour. Furthermore, this Framework is inherently dynamic. It seeks to characterise the web of factors that result in behavioural change.

The challenge remains that the importance of factors identified in the Framework can and do change from one shopping event to next. Hence the choices made by a consumer on each occasion can vary according to the priority afforded to each factor at that time (for example, convenience) or by factors outside their control (such as product availability and price).

Being branded as organic is what brings together the wide range of products in the organic food market. However, consumers are not presented with one brand. Rather it is complicated as the 'organic' brand comprises many sub brands and labelling is not always clear to consumers (Henryks et al., 2013). In the Australian market, consumers need to be familiar with seven certification logos if they are to be confident they are buying organic food as Australia is one of the few developed countries not to have a national certification mark. Not surprisingly, research shows that these labels are not easily identified and result in confusion (Newspoll, 2008; Henryks and Pearson 2010). Until the introduction of a national logo in Germany, the situation was similar to Australia where the large number of labels resulted in consumer confusion and uncertainty (Soyez et al. 2012).

In part, this confusion is also due to the credence nature of organic food (Darby and Karni, 1973; Grunert, 2002). That is, the 'organic' attribute of the food product is part of the production process and invisible to consumers at every stage of the buying and consumption process. Consequently, consumers rely on heuristics and information to ascertain that a product is organic and certification labelling is a significant component of this identification process. Thus the first step in strengthening the organic brand is ensuring consistency and clarity in labelling and communicating this to consumers. This example of stronger branding relates to every stage of the conceptual framework. For instance, in the Consumer Context layer, assisting consumers with a clearer understanding of organic food through strengthening their beliefs and clarifying confusion; at the Choice of Retail Outlet layer through ensuring that consumers are positively predisposed to choosing a retail outlet that stocks organic food; and finally in the Point-of-Purchase layer, increasing consumer intention to buy organic and promoting familiarity with organic logos and/or packaging.

The Switcher Buyer Behaviour Conceptual Framework discussed in this paper contains factors which impact and influence consumer behaviour in relation to organic food purchases. These factors can be used as a 'checklist' by organic food producers and marketers for gaining an understanding of their customers as well as be used to develop marketing and marketing communication strategies that focus on facilitating switchers choosing organic food over conventional more often.

The purpose of presenting results in the form of a framework is to provide the factors in a useful schematic; however, it should be noted that further research is required to investigate whether there is a hierarchy of importance, or a logical sequence, that buyers follow. As with many studies, the limitations of this research are opportunities for future research. One clear opportunity is to design a longitudinal study to explore the differences in attitudes and behaviours over a longer time period. Given the increasing interest in the environmental impacts of our food choices, important trends may be defining and changing consumer behaviour in this market. Another opportunity for further research emerges from the observations that some switchers may be in a state of transition, either gradually increasing their consumption to becoming dedicated organic food buyers, or on a path to exiting from purchasing any organic food. A further limitation is that the data was limited to participants' recollections, which may have been partially forgotten over time, or not be an accurate match with actual behaviour. For example, evidence suggests some participants may have inadvertently claimed to be purchasing organic food when they were buying conventional food and vice versa (Henryks et al. 2013).

An additional complication with researching organic food as a category is that there are numerous organic food products and consumers may hold and exhibit different beliefs, attitudes and behaviours towards different categories such as bread versus dairy or specific products such as honey versus jam. Further research could be designed to explore the framework through specific food categories to see if additional differences can be found. And finally, although the large number of contextual variables present in this Switcher Buyer Behaviour Conceptual Framework would pose a challenge for modelling, it is worth pursuing this as a direction in gaining further awareness and knowledge of the consumer behaviour that operates in the organic food arena.

This research has contributed to our understanding of switcher behaviour in the organic food market and of the associated attitude behaviour gap. It has provided insight into areas that require further research. This Switcher Buyer Behaviour Conceptual Framework has potential applications to other areas of consumer buyer theory where switching behaviour occurs. One such example is that of health where consumers consistently express their desire for a healthy diet and lifestyle but fail to

deliver upon this by making consistent behavioural change to achieve desired results. Consequently we believe the Switcher Buyer Behaviour Conceptual Framework provides much scope for further research in a variety of consumer choice contexts which are of concern to industry, government and the not-for-profit sector.

Acknowledgements

This paper is based on an earlier version *Attitude Behaviour Gaps: Investigating switching amongst organic consumers* presented at the Institute of Food Products Marketing Conference, Budapest, Hungary, 20-21 June 2013. Our thanks go to the conference participants for their thoughtful feedback. Thanks also to Associate Professor Melissa Lindeman for her insightful comments.

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Control of root-burrowing nematode (*Radopholus similis*) in banana using extracts of *Azadirachta indica* and *Allium sativum*

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Abstract

The root-burrowing nematode, *Radopholus similis* is considered to be the most destructive nematode associated with banana production worldwide. This nematode can reduce plant growth and yield by more than 50% and decrease the productive life of banana fields. *R. similis* control in the Windward Islands banana industry has been based on the application of synthetic nematicides, which are now prohibited due to human health and environment hazards. One possible alternative is the utilisation of plant extracts with known nematicidal effects such as *Azadirachta indica* and *Allium sativum*. The efficacy of these phytochemicals at managing *R. similis* were assessed and compared with a synthetic nematicide, ethoprophos (Mocap 15G) in two banana pot trials. There were five treatments (Control A, Control B, Neem X, Garland, and Mocap 15G) with 6 plants per treatment. The results of the root and soil extractions showed that all treatments caused a significant reduction in *R. similis* population density ($P = 0.05$), with Mocap 15G being the most effective. From the plant growth data it was observed that all treatments caused a significant increase in plant growth ($P = 0.05$). No significant difference in the pseudostem length and girth, as well as, the leaf number and area were observed between treatments. The root and corm weights also showed no significant differences between treatments. The efficacy of botanical nematicides and their effect on banana production in the Windward Islands are discussed.

Keywords: allicin, azadirachtin, botanical control, nematostat, Trinidad and Tobago.

Introduction

The banana industry has been a key source of employment and foreign revenue to the Windward Islands for over eighty years (NERA, 2004). The banana trade has provided a direct living for thousands of small-scale producers and has accounted for up to 50% of the Windward Islands' total export revenue with sales of 274,000 tonnes a year to a value of US\$147m. This industry, while blessed with a warm tropical climate, is threatened by tropical storms and a complex of fungi, insects and nematodes, the latter being one of the most important limiting factors. These factors have led to a major reduction in banana production and exports to a mere 99,000 tonnes a year at a value of US\$45m (Wiltshir, 2004). The root-burrowing nematode, *Radopholus similis* (Cobb 1893) Thorne 1949 is considered to be the most destructive nematode associated with banana production worldwide (Gowen et al., 2005). *Radopholus similis* feeds on the cortical cells of root and corm (rhizome) tissues causing cavities to develop, which evolves as root necrosis (Brooks, 2008). This in turn reduces growth and yield by more than 50%, lengthens the time to fruiting, and decreases the productive life of banana fields (Quénéhervé et al., 2006). Plant anchorage is also affected, which results in toppling or uprooting (Gowen et al., 2005) Effective *R. similis* control is therefore essential for the survival of the banana industry.

The uptake of organic principles of production has been slow in Trinidad and Tobago. Although 164 countries report certified organic agriculture statistics, and some report organic banana production, no organically managed land is reported by Trinidad and Tobago (Willer & Lernoud, 2014). In the past, in Trinidad and Tobago, *Radopholus similis* control has been based mainly on the use of synthetic nematicides such as, ethoprophos, oxamyl and aldicarb (Chabrier & Queneherve, 2003). These products are now prohibited due to their adverse impacts on human health and the environment (Nagaraju et al., 2010). Windward Islands banana farmers are also barred from using other synthetic nematicides due to Fair-trade arrangements (Isaac et al., 2007). Plant-based pest control agents have long been touted as alternatives to synthetic nematicides (Javed et al., 2006). Such phytochemicals reputedly pose little threat to the environment or to human health and their use is approved under

organic and Fair-trade arrangements (Amadioha, 2003). However, the adoption of such a management strategy has been met with scepticism by banana producers who question the efficacy and consistency of these phytochemicals (Villanueva 2005). In this study, phytochemicals derived from *Azadirachta indica* and *Allium sativum* extracts were assessed and compared with ethoprophos for their effectiveness at reducing *R. similis* population density in the roots and soil, and preventing banana growth and yield losses.

Materials and methods

Two pot trials were conducted throughout 2009 and 2010 at the University of the West Indies Field Station (UFS) situated in Valsayn, Trinidad (10° 39' 0" N, 61° 25' 0" W). The average monthly temperature and precipitation at UFS were 27.2°C and 1720 mm, respectively for the length of the study. The growing medium comprised of sterilised Fluventic Eutropept soil (River Estate Loam), with a cation exchange capacity of 4.8 cmol/kg and a pH of 6.5.

Experimental design

Each trial consisted of 30 banana plants (cv. Lacatan), planted in 50 L plastic-drums and spaced 2×2m apart. The drums were arranged in a completely randomised design with five treatments: Control A, Control B, Neem X[®] (Marketing Arm International), Garland[®] (OMEX Agriculture Ltd) and Mocap 15G[®] (Bayer Crop Science) and six replicates. Plants were grown for 1 month and then inoculated with 500 *R. similis* following the procedures of Speijer & De Waele (1997). One month after inoculation, nematicidal treatments were applied as a soil drench (1L) at the base of the pseudostem. The active ingredients, sources, application rates and frequencies of each treatment are given in Table 1. Agronomic management was done according to the protocols recommended in the Windward Islands for banana production, which included fertilisation, irrigation, pruning, propping, de-suckering, and pest and disease management (Paul et al., 1993).

Table 1. List of treatments with active ingredients and application rate and frequency

Treatments	Active Ingredients	Application rate	Application frequency
Control A	No <i>R. similis</i>	-	-
Control B	<i>R. similis</i> only	-	-
Neem X [®]	Azadirachtin (3000 ppm)	2.7ml/L	7 days intervals for 3 applications
Garland [®]	Allicin (<1 ppm)	2.16ml/L	7 days intervals for 3 applications
Mocap 15G [®]	Ethoprophos	15g/m ²	7 days intervals for 3 applications

Data collection

Radopholus similis population density

An estimation of the *R. similis* population density was done using extraction methodologies described by Southey (1986) for the roots, and Whitehead & Hemming (1965) for the soil. The blender nematode filter extraction method was used to extract nematodes from 15g of roots while the Whitehead tray method was modified to extract nematodes from 200ml of soil. The collected nematode sample was identified and counted in three 1ml aliquots out of a 10ml aqueous suspension using a stereoscopic microscope. All vermiform stages (juveniles and adults) were counted.

Root necrosis index and banana root and corm fresh weight

At the end of each trial the plants were excavated, their roots and corms cleaned to remove soil particles and their root necrosis index (RNI) and root and corm fresh weights (RCFW) determined. A modified Bridge & Gowen (1993) root necrosis index was used to determine root rotting on a 0 to 4 scale [0 = no damage; 1 = <25% of total root cortex with necrosis; 2 = 26–50% of the total root cortex with necrosis; 3 = 51–75% of total root cortex with necrosis; 4 = >75% of total root cortex with necrosis]. Fresh weight (kg) was determined using a Rebur pocket balance (Germany).

Plant growth

Throughout the study the following crop measurements were recorded weekly:

- (a) The pseudostem length (cm) measured from the point of the lowest leaf to the base of the pseudostem.
- (b) The pseudostem girth/circumference (cm) measured from a point at half the pseudostem length.
- (c) The total number of fully opened functional leaves (Fogain, 2000).
- (d) Leaf area (LA) predicted with the regression models: $LA = 0.0266 + (L \times W \times 0.7629)$ ($r = 0.98$), where L = leaf length and W = leaf width. The third leaf from the top of the plants was selected as the standard leaf for measurement (Potder & Pauer, 1990).

Data Analysis

Differences in nematode density, necrosis index, roots and corm fresh weight, and growth parameters were analysed with the use of analysis of variance (ANOVA). Pearson correlation was used to determine the strength of relationship between the *R. similis* density in the roots and the necrosis index. Prior to analyses, variables were tested for homogeneity of variances and normality, and data found to be non-homogenous were either $\log_{10}(X + 1)$ or square root transformed before statistical analysis. Non-transformed means were reported in Figures and Tables and only significant differences ($P \leq 0.05$) are discussed unless stated otherwise. All statistical analysis was performed using the statistical software Minitab® 16.1.1 (Minitab Inc).

Results and Discussion

Radopholus similis population density

All nematicides were successful at reducing *R. similis* population density in the root and soil. Plants treated with ethoprophos had a lower *R. similis* density than those treated with *Azadirachta indica* and *A. sativum* extracts (Figure 1). Several studies have reported on the notable effects of ethoprophos in *Musa* spp. which acts as a nematostat in low concentrations (Stirling & Pattison, 2008; Quénehervé, 2009; Radwan et al., 2012). However, to maintain its high effectiveness, frequent applications were required, which increases its negative effects on the environment and human health (Sipes & Schmitt, 1995).

Plants treated with *Azadirachta indica* had a high *R. similis* density in the soil (570) but a lower density in roots (275). This disparity was due to the anti-feedant properties of azadirachtin, the phytochemical found in *Azadirachta indica* (Sidhu, 2003). Rehman et al., (2009) suggested that azadirachtin can induce nematostasis, a process that inhibits nematodes from invading plants without directly killing them. The phytochemicals found in *Allium sativum* (allicin) demonstrated a similar but less effective anti-feedant effect and was inconsistent at reducing *R. similis* density in the roots. This inconsistency may be due to the life cycle of *R. similis*, which can be completed in the root, without any stage in the soil. Thus preventing exposure to the allicin applied in the soil (Araya, 2003).

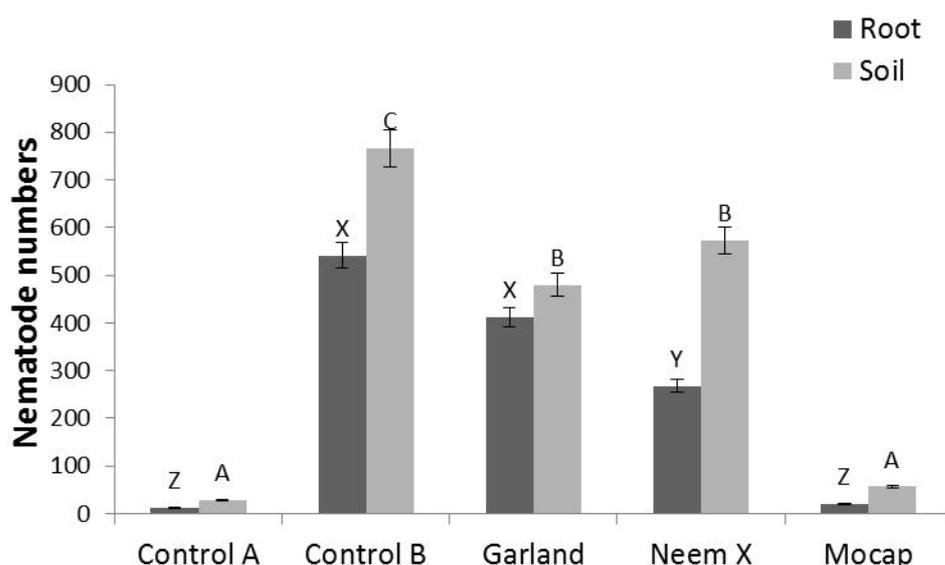


Figure 1. Effects of nematicidal treatments on *R. similis* density in the roots and soil of banana plants. Values are the average of 6 replicates. Bars with the same letter are not significantly different ($P > 0.05$).

Endo-parasitic nematodes, such as *R. similis* were expected to be more prevalent in the roots than the soil. This study showed a deviating trend which can be attributed to the nematostatic activities of the treatments along with the inefficiencies of Southey's (1986) extraction technique at recovering *R. similis* from the roots. This is a major deficiency in Southey's technique since endo-parasitic nematodes were unlikely to migrate from healthy/necrosis free root tissues to be extracted by this technique. Therefore, the estimated nematode density in the roots may have been lower than the actual nematode density.

Root necrosis index

The differences in the RNI between treatments were statistically significant ($P < 0.05$) in both trials, with Control A having the lowest index value and Control B the highest (Table 2). The Pearson correlation between *R. similis* density and the root necrosis were positive in both trials (Trial 1: $R^2 = 0.574$; Trial 2: $R^2 = 0.190$). Therefore, increases in the *R. similis* density in the roots will result in an increase in the root necrosis. The strength of the relationship was however inconsistent and the weak correlation in Trial 2 supports the assumption that highly necrotised root tissue may have a lower *R. similis* density. Dosselaere (2003) indicated that nematodes may move out of resource scarce necrotic banana root tissue and invaded healthy tissue. Plants treated with *Azadirachta indica* had the lowest root necrosis index in both trials due to the nematostatic activities of azadirachtin, which inhibits nematodes invasion into the roots. *Allium sativum* had similar but less effective nematostatic activities, while the efficacy of ethoprophos was inconsistent.

Table 2. Mean roots necrosis index values and root and corm fresh weight (kg).

Treatments	Root necrosis index		Root and corm weight (kg)	
	Trial 1 (SD)	Trial 2 (SD)	Trial 1 (SD)	Trial 2 (SD)
Control A	1.33 (0.52)	1.83 (0.75)	2.2 a (0.75)	2.1 a (1.11)
Control B	2.67 (0.75)	2.17 (0.75)	1.4 b (0.58)	1.6 b (0.80)
Garland	2.17 (0.82)	1.83 (0.98)	1.6 b (0.60)	2.2 a (1.24)
Neem-X	1.83 (0.75)	1.67 (0.82)	1.8 ab (0.71)	2.1 a (0.56)
Mocap	1.83 (0.98)	2.17 (0.98)	2.0 a (0.33)	2.1 a (1.14)

Banana root and corm fresh weight

All treatments were effective at maintaining healthy root and corm. Ethoprophos performed consistently in both trials and recorded the highest RCFW among the treatments. The anti-feedant effect of azadirachtin and allacin were also consistent and comparable to ethoprophos. *Radopholus similis* population densities in the roots and corm tissues had a direct effect on its RCFW. Moens et al. (2003) found a linear reduction of root weight when *R. similis* was inoculated at increasing densities. Marin et al. (1999) and Sarah (2000) also found decreases in root weight, ranging from 8-80%, several weeks after inoculation with *R. similis*. The data obtained in this study were similar to those reported in the literature, as Control A had the highest sum of RCFW while Control B had the lowest (Table 2). Therefore, RCFW shows some potential for used as a rapid technique for estimating *R. similis* density (Moens et al 2003). However, depending solely on RCFW to determine the implementation of nematode control strategies may not be advisable, as root and corm weight may vary due to factors other than nematode density, such as the banana weevil borer (*Cosmopolites sordidus*) and plant nutrient (Sarah, 2000).

Plant growth

The banana growth data confirmed that the phytochemical treatments were comparable to ethoprophos at preventing plant growth losses, as no significant differences were observed in both trials (Table 3). This may also be due to the ability of banana cv. Lacatan to withstand *R. similis* infestation. Therefore, plant sensitivity or tolerance must be considered since nematode tolerance has been identified in the *Musa* gene pool (Dochez et al., 2006) The selection of nematode tolerant banana cultivars can play a very important role in nematode management and is ranked as one of the ideotype requirements for the acceptance of banana hybrids (Tenkauan & Swennen, 2004). On the other hand, a tolerant but susceptible banana plant is of limited value, as nematode reproduction may increase the population density beyond the damage threshold (Cook & Starr 2006). Therefore, in the absence of true resistance, incorporating tolerant plants with phytochemicals may be an effective

strategy for preventing the *R. similis* density from crossing the plant damage threshold and causing yield losses.

Table 3. Effects of the treatments on the growth parameters of banana plant.

	Pseudostem		Leaf	
	Length (cm)	Girth (cm)	Numbers	Area (cm ²)
Control A	95 (0.52)	28.0 (0.75)	6.0 (0.75)	2,673 (1.11)
Control B	77 (0.75)	23.6 (0.75)	7.5 (0.58)	2,182 (0.80)
Garland	82 (0.82)	26.2 (0.98)	6.7 (0.60)	2,363 (1.24)
Neem-X	85 (0.75)	27.5 (0.82)	6.7 (0.71)	2,498 (0.56)
Mocap	92 (0.98)	28.5 (0.98)	6.5 (0.33)	2,691 (1.14)

Conclusions

The application of phytochemicals (azadirachtin and allicin) as alternatives to synthetic nematicides was effective and comparable to ethoprophos at preventing plant growth and yield losses. This will be beneficial to banana producers by decreasing the time for fruiting and increasing the productive life of the fields. Plants treated with azadirachtin also had a low root necrosis index which may result in fewer plants toppling and uprooting. However, the phytochemicals were less effective at reducing *R. similis* density in the soil which leaves the plants vulnerable to future infestations unless other nematode management strategies are adopted.

In the pursuit of more benign pest management and more consumer-acceptable solutions in banana production it would be appropriate for more research to be directed to organic research and some attention paid to the lessons learnt in other countries which are pursuing the expansion of their own organic banana production.

Acknowledgements

The authors thank the manager and staff of the UFS, for their kind cooperation and support in executing these trials. Thanks to the academic and technical staff of the Department of Food Production, the University of the West Indies, St. Augustine, Trinidad and Tobago for assistance.

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The impact of peat moss and sheep manure compost extracts on marigold (*Calendula officinalis* L.) growth and flowering

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Abstract

A nursery experiment was carried out during 2011 - 2012 at Najaf, Iraq, to determine the response of marigold plant to organic fertilisers and their extracts. A factorial experiment was conducted involving three factors (2 x 4 x 2) namely, type of compost (the extracts of peat moss and sheep manure), concentrations of foliar application (0%, 20%, 40% and 60%), and the mixed in soil and foliar application. The results showed that the type of organic fertiliser and the application method significantly affected vegetative growth (leaf number/plant, shoot dry weight, leaf chlorophyll content, and carbohydrate leaf content), and flowers parameters (length of the flower stem, flower number/plant, and flower diameter). This study showed that compared to the other fifteen treatment conditions, the application of extract of sheep manure applied at 40% concentration and as a foliar spray produced superior results on both vegetative growth and flower parameters.

Keywords: Organic fertiliser, peat moss, sheep manure, Iraq.

Introduction

Marigold (*Calendula officinalis* L.) is an annual, herbaceous plant. It belongs to the family Asteraceae. Roots are a white yellowish to light brown color with a length of about 20 cm and a thickness of 7 mm and carry many root hairs. Stems are long and strong, 50 cm in length. Leaves are simple, elongated, spoon-shaped, dark green in color and 20 cm in length (Muley et al. 2009). The original home of the plant is the basin of the Mediterranean and it grows wild in southern and central Europe and the Netherlands. Marigold is the third most important cut flower in the global market after roses and carnations. The flowers of the plant come in different colours. They are in high demand in the holiday season during Easter and Mother's Day (Biondo & Noland, 2000). Marigold flowers are a rich source of a natural yellow to orange dye, helenien (a dipalmitate ester of axanthophylls), which is in high demand by national and international companies, and this plant has been used for medicinal purposes (Ali & Hasan, 2013). The plant is also used as a spice and a tea (Četković, 2003; Isaac, 1994).

Flowering of marigold plants under short photo-periods, however there is a long flowering period. The plants can be used in various situations in the home garden and in landscaping. It is one of the best plants for rock gardens, borders, flower beds and balcony plantings (Golestani et al., 2013).

Organic farming is one of the practices to make the production system more sustainable without adverse effects on the natural resources and the environment (Kochakinezhad et al., 2014; Ram et al. 2014). It not only maintains soil fertility but also conserves soil moisture (Yadav et al., 2014). Many studies have demonstrated that organic residues can be used with outstanding results (e.g. Kononova, 1966; Tan, 1986). Organic fertilisers and their extracts enhance soil fertility via improved nutrient retention and cycling and also plays an essential role in growth and yield (Khalid & Shafei, 2005).

The use of organic matter plays an essential role in the growth and development of marigold plants (Elhindi, 2012). It positively affects vegetative growth parameters including plant height, stem diameter, fresh and dry shoot weight, as well as flower parameters, including flower number per plant, flower height, and flower size of the plant (Shadanpour, 2011), and it increases the availability and absorption of the essential nutrient elements, such as Fe^{2+} , Mg^{2+} and NH_4^+ cations, which are necessary for enzyme activation and chloroplast and chlorophyll formation (Elhindi, 2012).

Adding different organic fertilisers to the soil or to a plant as a foliar application resulted in increased growth and flowering characters of *Borago officinalis* plant (Ezz El-Din & Hendawy, 2010). Application

of organic fertilisers or their extracts also have positive effects on plant growth, dry matter yield and root development (Gharib et al., 2008; Ram et al., 2014). Addition of cow manure vermicompost at the 40% level resulted in high growth values of marigold plant (Rahbari, 2013). The purpose of this study is to determine the effects of the type of organic matter (peat moss and sheep manure), concentration of organic matter extractions, and the application method, on improving the growth, flowering and flower qualities of marigold plants.

Materials and methods

Plants were grown at a private nursery at Najaf, Iraq during 2011-12. The soil was silt loam in texture with a pH of 5.6 and an electrical conductivity of 2 dS/m. Seeds were planted in treated soil in 150mm diameter plastic pots, 200 mm deep. Each pot contained 1kg soil with one plant. The experiment was conducted using completely randomised design with three replicates per treatment, with three factors tested:

- * 2 types of organic matter (sheep manure and peat moss),
- * 4 concentrations of each extract (0%, 20%, 40% and 60%), and
- * 2 application methods (mixed with soil and spray).

Fertilisation with NPK fertiliser (10:10:17) at the level of 1 g/pot was applied for all treatments. At the end of the experiment on March 15, 2012, the following data were recorded:

- (1) number of leaves (leaves/plant),
- (2) dry weight of shoot (g),
- (3) total chlorophyll content in leaves (mg/100 g fresh weight) by acetone (Goodwin, 1976),
- (4) carbohydrates content in leaves (mg/ mg dry weight) were estimated according to Dubois (1956),
- (5) length of flower stem (cm),
- (6) number of flowers (flowers per plant),
- (7) flower diameter (mm), and
- (8) number of petals (petals per flower).

Pots were laid out in a factorial design according to Steel & Torrie (1980), and the data was analysed using analysis of variance with SAS software. Significant differences were accepted at $P < 0.05$ level of probability. Differences between means were determined using least significant differences (LSD).

Results and discussion

Number of leaves

The type of organic matter and the application method significantly ($P < 0.05$) affected the number of leaves per plant. The sheep manure increased the number of leaves by 36% over the control. Using the extract of sheep manure as a foliar application at the 40% level maximised the number of leaves to 76% over the control (Table 1). This increase could be due to the content of sheep manure of organic N and the immobilisation process rather than that the organic matter increased the availability of some nutrients in the soil (Sabey & Hat, 1975). According to Hocking & Steer (1982), nitrogen plays an important role in protein components and enzymes and organises hormone activity which is important in cell division and stimulates biological processes. This may account for the higher number of leaves in the plants that were treated by sheep manure.

Table 1. Effects of organic matter (OM) and application method with different concentrations on number of leaves per plant.

Type of OM	Concentration of application (%)	Application method		Average of concentration	Average of OM
		Soil mix	Spray		
Peat moss	0	23.67	21.00	21.92	25.54
	20	25.67	23.67		
	40	25.33	32.00	26.58	
	60	30.33	22.67		
Sheep manure	0	22.00	21.00	31.17	28.50
	20	26.33	30.67		
	40	30.33	37.00	28.42	
	60	36.00	24.67		
Average of application method		27.46	26.59		

LSD: OM = 1.3, concentration = 2.6, application method = 1.3, interaction = 3.7

Shoot dry weight

Table 2 shows the effect of organic matter on shoot dry weight (SDW). The application of sheep manure showed an increase ($P < 0.05$) of 14% over the peat moss treatment. Addition of the sheep manure at the 40% level showed the highest increase of 79% as compared to the control. Shoot DW was significantly ($P < 0.05$) affected by the interaction between organic matter, the application method and the concentration, which may be due to the humus in the sheep manure which carries a negative charge and associated cations and soil particles making fixed aggregations, reducing soil bulk density, enhancing soil structure and water-air relationship that will positively affect the activity of soil organisms leading to enhance root growth, which could result in better extraction of water and nutrients from the soil occupied by the roots (FAO, 1977).

Table 2. Effects of organic matter (OM) and application method with different concentrations on shoot dry weight (g).

Type of OM	Concentration of application (%)	Application method		Average of concentration	Average of OM
		Soil mix	Spray		
Peat moss	0	5.85	6.11	5.57	6.20
	20	6.19	5.29		
	40	7.00	6.87		
	60	7.01	5.28		
Sheep manure	0	5.97	4.36	8.02	7.09
	20	5.91	5.84		
	40	7.28	10.95		
	60	9.52	6.88		
Average of application method		6.84	6.45		
LSD: OM = 0.4, concentration = 1.3, application method = 0.4, interaction = 2.4					

Leaf chlorophyll content

The effect of the type of organic matter and the application method on chlorophyll content in leaves is presented in Table 3. The extract of sheep manure increased ($P < 0.05$) chlorophyll by 3% compared with the peat moss treatment. Chlorophyll content in leaves showed a significant ($P < 0.05$) interaction between organic matter, application method and concentration. Due to this interaction, marigold gave the highest Chlorophyll content in leaves with sheep manure applied sprayed at 40% (76.9 mg/100 g fresh weight). According to Elhindi (2012) organic matter includes essential nutrients for plant growth which has a positive effect for chlorophyll molecules and chloroplast formation. Organic acids and carbon dioxide have a role in enhancing the availability of some nutrients such as Mg which plays an important role in the formation of the chlorophyll molecule.

Table 3. Effects of organic matter (OM) and application method with different concentrations on total leaf chlorophyll content (mg/100 g fresh weight).

Type of OM	Concentration of application (%)	Application method		Average of concentration	Average of OM
		Soil mix	Spray		
Peat moss	0	57.01	56.06	57.11	62.14
	20	57.28	62.75		
	40	64.81	64.38		
	60	72.68	62.18		
Sheep manure	0	56.28	59.11	66.86	63.74
	20	57.59	63.04		
	40	61.35	76.91		
	60	73.87	61.77		
Average of application method		62.61	63.27		
LSD: OM = 1.0, concentration = 2.5, application method = 1.0, interaction = 3.9					

Leaf carbohydrate content

Organic matter increased ($P < 0.05$) leaf carbohydrate content (Table 4). Sheep manure gave the highest rate (6.08 mg/g dry weight). Leaf carbohydrate content was significantly ($P < 0.05$) affected by the interaction between organic matter, application method and concentration. The magnitude of this increase was maximised in sheep manure at 40% concentration level as foliar application. It is believed that the fulvic acid consists of carbohydrate and amino acids (Chen et al., 2002) thus have these materials ready for absorption via the leaf surface. This result is in accordance with the findings of Tisdale et al. (1985) who reported that the addition of humic extracts increases the production of carbohydrates.

Table 4. Effects of organic matter (OM) and application method with different concentrations on leaf carbohydrate content (mg/g dry weight).

Type of OM	Concentration of application (%)	Application method		Average of concentration	Average of OM
		Soil mix	Spray		
Peat moss	0	4.17	4.37	4.22	5.17
	20	4.63	5.57		
	40	5.40	4.67	5.27	
	60	7.03	5.53		
Sheep manure	0	3.90	4.43	6.19	6.08
	20	4.93	5.93		
	40	5.63	9.07	6.83	
60	8.13	6.63			
Average of application method		5.48	5.78		

LSD: OM = 0.3, concentration = 1.0, application method = 0.3, interaction = 1.8

Length of flower stem

Flower stems of the plants was significantly ($P < 0.05$) influenced by the type of organic matter and the application method (Table 5). Peat moss addition increased the flower stem by 30% as compared to sheep manure. This may be because the organic extracts include organic N and increase the nutrient availability. This result coincides with that of Sabey & Hart (1975) who showed that the organic extract increased the nutrient availability.

Table 5. Effects of organic matter (OM) and application method with different concentrations on length of flower stem (cm).

Type of OM	Concentration of application (%)	Application method		Average of concentration	Average of OM
		Soil mix	Spray		
Peat moss	0	14.93	16.90	15.64	23.27
	20	16.53	17.77		
	40	17.17	19.23	18.10	
	60	19.67	17.93		
Sheep manure	0	15.10	15.63	19.63	17.93
	20	16.07	16.03		
	40	18.70	23.43	19.01	
60	21.03	17.40			
Average of application method		17.40	18.04		

LSD: OM = 4, concentration = 5, application method = 4, interaction = 6

Number of flowers per plant

The type of organic matter and the application method had a significant effect on the number of flowers per plant. The highest number (14.7) of flowers was in the pots treated with sheep manure. The number of flowers exhibited the maximal response with sheep manure applied at 40% as a foliar spray (Table 6). Increasing the number of flowers as a result of applying organic extracts (Shadanpour, 2011) may be due to the significant impact of the nutrients in the organic extracts in stimulating growth regulators, including auxins and gibberellins, that play an important role in increasing the proportion of the pollination through the control of transport nutrients toward the flowers (Sergeant, 1965).

Table 6. Effects of organic matter (OM) and application method with different concentrations on the number of flowers per plant.

Type of OM	Concentration of application (%)	Application method		Average of concentration	Average of OM
		Soil mix	Spray		
Peat moss	0	7.33	11.00	10.50	11.92
	20	9.33	12.67		
	40	14.33	14.00	11.83	
	60	16.00	10.67		
Sheep manure	0	12.00	11.67	15.83	14.67
	20	12.33	13.00		
	40	14.67	20.33	15.00	
60	18.33	15.00			
Average of application method		13.04	13.54		

LSD: OM = 0.4, concentration = 1.0, application method = 0.4, interaction = 2.3

Flower diameter

Flower diameter (cm) was affected by the type of organic matter and the application method (Table 7). Marginal increase in flower diameter occurred with added sheep manure. Flower diameter of marigold plant showed an increase of 84% due to the interaction effect between organic matter, application method and concentration (maximum at 40% sheep manure foliar application), as compared to the control treatment (water spray). The organic extracts increase plant growth and enhance the flower characteristics.

Table 7. Effects of organic matter (OM) and application method with different concentrations on flower diameter (cm).

Type of OM	Concentration of application (%)	Application method		Average of concentration	Average of OM
		Soil mix	Spray		
Peat moss	0	2.40	2.40	2.37	3.10
	20	2.70	2.87		
	40	3.23	4.30	2.80	
	60	3.47	3.47		
Sheep manure	0	2.43	2.23	3.73	3.16
	20	2.40	3.23		
	40	3.27	4.10	3.63	
60	4.27	3.33			
Average of application method		3.02	3.24		

LSD: OM = 0.1, concentration = 0.8, application method = 0.1, interaction = 1.6

Number of petals per flower

Table 8 shows the positive impact of the type of organic matter and the application method on the number of petals per flower; the response to sheep manure and peat moss was not significantly different as a main effect ($P > 0.05$). There was a significant ($P < 0.05$) interaction between organic matter, application method and concentration (maximum at 40% sheep manure foliar application), in the number of petals per flower. The number of petals showed an increase of 74% (in the 40% sheep manure foliar application treatment) compared with the control.

Table 8. Effects of organic matter (OM) and application method with different concentrations on the number of petals per flower.

Type of OM	Concentration of application (%)	Application method		Average of concentration	Average of OM
		Soil mix	Spray		
Peat moss	0	23.33	23.33	22.33	27.08
	20	24.33	25.67		
	40	28.33	32.67	24.75	
	60	30.33	28.67		
Sheep manure	0	21.00	21.67	31.83	28.08
	20	24.00	25.00		
	40	28.67	37.67	31.42	
	60	36.33	30.33		
Average of application method		27.04	28.13		

LSD: OM = 1.0, concentration = 3.0, application method = 1.0, interaction = 3.7

Conclusion

The outcome from this experimental study is that it is reasonable to conclude that the application of organic extracts (both peat moss and sheep manure), increased the plant characteristics measured: number of leaves per plant, shoot dry weight, leaf chlorophyll content, carbohydrate leaf content, length of flower stem, number of flowers per plant, flower diameter and number of petals per flower. These increases were higher when using sheep manure extract (except length of flower stem) rather than peat moss. For the sixteen treatments (2 x 4 x 2) in this study, compared to the other treatments, the application of sheep manure extract at a concentration rate of 40% as a foliar spray resulted in superior growth values and flower parameters of marigold plants.

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Phosphorus-use efficiency, growth and yield of spelt wheat (*Triticum aestivum* ssp. *spelta*) compared with standard wheat (*T. aestivum* ssp. *vulgare*) in south-eastern Australia

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Abstract

Experiments were conducted in the glasshouse and the field to assess the phosphorus-use efficiency, yield, and yield components of several spelt wheat genotypes in comparison with standard bread wheats. Spelt genotypes had much lower grain yield than standard bread wheats, in both a well-watered glasshouse and three field situations. The reduction in yield was often as great as 60% and was largest in late-flowering spelt genotypes. Spelt genotypes responded to increasing amounts of applied phosphorus (P) fertiliser, adequately acquired P from soil, and some had higher total amounts in their tissues; however, these P reserves were not as efficiently converted into grain yield as standard bread wheat cultivars, primarily due to the growth of tall, unproductive tillers, and lower kernel number and kernel size. There was no evidence of spelt yielding better than common wheat under conditions of P-deficiency. There is great potential to breed improved spelt genotypes through relatively simple modification of yield components and phenology, but whether this can be achieved while maintaining the grain quality attributes valued highly by the organic industry remains to be seen. Breeding for improved spelt should target reduced height and tiller number, early flowering, and larger kernels.

Keywords: phosphorus-use efficiency, spelt, grain yield, tillering, wheat.

Introduction

Globally, there is increasing interest in using organic farming systems and maximizing grain production in low-input scenarios. Organic residues are being employed as fertilisers (Davari et al., 2012) and plant breeding is being used to develop new cultivars that are specifically adapted to organic and/or low-input systems (Wolfe et al., 2008, Vanaja et al., 2013). Old or 'neglected' crops are receiving more attention from growers and processors seeking a niche product, while consumers are seeking potential nutritional, sensory, and health benefits by eating food from more 'traditional' sources.

Consumer interest in spelt (*Triticum aestivum* ssp. *spelta*) grain is believed to be due to its rich flavour, described as sweet and nutty, agreeable texture in baked products, and good nutritional profile. The grain may have higher contents of protein and fibre (Abdel-Aal et al., 1995, Moudry and Dvoracek, 1999, Escarnot et al., 2012), minerals and P (phosphorus) (Ruibal-Mendieta et al., 2005), and β -carotene and retinol equivalent (Abdel-Aal et al., 2007), than standard bread wheat (*Triticum aestivum* ssp. *vulgare*). Spelt is used to make a wide range of consumer products (Neeson, 2011) but mainly leavened bread.

The vast majority of Australian spelt production is organic because the much higher price paid for the organic product offsets the reduced yield (Neeson et al., 2008). The reduced yield is as a result of three factors: 1) poorly-adapted genotypes (i.e. those not specifically bred for local conditions and/or low-input agriculture); 2) the inherently lower-yielding capacity of the organic, low-input system; and 3) spelt produces hulled grain which requires additional processing before flour milling resulting in some losses due to chipping and splitting (Neeson et al., 2008).

Commercially-available spelt in Australia is presently restricted to a few poorly-adapted genotypes. Evidence for poor adaptation is limited but it suggests that the grain yield of current genotypes,

including the predominant commercial genotype 'Kamarah' (a manual mixture of two spelt genotypes that originated from northern Spain), is low relative to standard bread wheats. No direct comparisons between spelt and common bread wheat in organic versus conventional systems have been published. It is known that wheat production under an organic system can yield from 14 to 44% less than under a high-input system (Mäder et al., 2007, Seufert et al., 2012). In our experience the current commercial spelt genotype in Australia, 'Kamarah', yielded 45% less than a common wheat comparator (unpublished data); however, spelt genotypes with only a 28% reduction were also observed (unpublished data).

A significant cost for all cereal production in Australia is P fertiliser, because most grain production is carried out on soils that are highly weathered and inherently low in available P, and the cost of P fertiliser is increasing. Therefore, more efficient utilisation of soil or applied P for grain production is desirable. Several publications have addressed this issue for standard wheat (Horst et al., 1993, Manske et al., 2000, Osborne and Rengel, 2002), but the P efficiency of spelt has not been reported. Current spelt production largely services the organic grain industry. A low P requirement (or a high P-use efficiency) in spelt would be of particular interest to this industry, because organically-managed broad-acre soils are often marginal for available P (Evans, 2005, Cornish, 2009), and such soils are difficult to improve in the short-term using the allowed inputs of rock phosphate or organic compost (Conyers and Moody, 2009, Evans and Condon, 2009). In marginal areas for cereal production, and under low input conditions, the performance of spelt has been claimed to be better than standard bread wheat (Ruegger and Winzeler, 1993), possibly due to better utilisation of the scarce nutrients in low input systems (Moudry and Dvoracek, 1999, Richardson et al., 2009).

Various definitions have been used to describe P efficiency. For example, Osborne and Rengel (2002) described three measures that focus on biological yield; one of these, shoot dry weight relative to shoot P uptake, also known as biological P efficiency, has merit from a climate-warming perspective describing P-use efficiency in relation to C capture via crop photosynthesis. Alternatively, Ortiz-Monasterio (2001) described P-use efficiency as grain yield per unit of applied P, which has merit from an economic perspective. In this study, we compared genotypes for: (i) efficiency of P uptake being the amount of P at flowering obtained by plants from a defined amount of applied P (%); (ii) shoot P efficiency ($\text{g mg}^{-1} \text{P}$) being the weight of shoot tissue dry mass produced at flowering per weight of P applied, and; (iii) grain yield P efficiency ($\text{g g}^{-1} \text{P}$) being the weight of grain produced per weight of P applied.

In this study, we compared growth and yield attributes of spelt (using genotypes with a range in maturity) with standard bread wheats, under different regimes of P availability in a glasshouse study and in the field. Our objectives were to quantify the P-use efficiency of spelt relative to standard bread wheat, identify superior yielding lines of spelt, and make recommendations regarding the need to adjust P fertiliser rates for spelt as compared to standard bread wheat.

Materials and methods

Glasshouse study

Spelt genotypes (*Triticum aestivum* ssp. *spelta*), nominally designated SP2, SP10, SP16, SP18, SP19, SP22, SP29, SP40 and SP41 were acquired from a local gene bank: AWCC (Australian Winter Cereals Collection, Industry & Investment NSW, Tamworth, NSW). Two genotypes reputed to be spelt, SP76 and SP77, were sourced from single plant selections taken within a commercial spelt crop. These genotypes, and three standard bread wheat (*Triticum aestivum*) cultivars differing in maturity, namely, Waagan, Gregory, and EGA-Wedgetail (hereafter just Wedgetail), were established in pots and treated with soluble, surface-applied inorganic P fertiliser (KH_2PO_4) at levels equivalent to 6.8 (low), 13.8 (moderately low), 20.3 (moderate), or 33.8 (high) kg P ha^{-1} . The P treatments were allocated to main plots within which the genotypes were randomised. Each genotype was duplicated so that destructive sampling could be performed, firstly for dry matter and P content at flowering and, secondly, for grain yield. Duplicates were adjacent paired pots. There were 3 replicated blocks of the treatments.

Pots were large enough to hold 2 kg of growth medium, which comprised a sand and peat mix (75% sand by volume). Nutrients other than P were supplied in ample amounts and comprised (g kg^{-1}): KNO_3 0.5, urea 0.158, K_2SO_4 0.174, CaCl_2 0.074, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.029, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.001, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.0011, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.0024, H_3BO_3 0.0008, $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ 0.0004, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.0002, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.01114. The nutrients were dissolved then irrigated into the growth medium.

Five seedlings were established in each pot and were adequately supplied with water through regular irrigation that varied in frequency from once per week to daily as plant biomass increased.

The experiment was sown on 10 May 2007. Days-to-flowering was recorded for each genotype. At flowering, all above-soil biomass was removed, tiller number per plant, was determined and then the material was dried at 80°C and weighed to obtain dry matter per pot (g pot^{-1}). The dried material was digested in Kjeldahl solution, the digest assayed for its P content using a Flame Ionisation Analyser (LACHAT) and these data were then used to estimate tissue P content (mg P pot^{-1}). 'P biological efficiency in dry matter at flowering' was derived from 'dry matter at flowering' divided by 'total P content of shoots at flowering'. 'Efficiency of P uptake at flowering' was calculated from 'total P content of shoots at flowering' divided by 'P applied'.

At maturity, grain was recovered using a small grain thresher, then de-hulled manually (if required), and weighed (grain yield, g pot^{-1}). Yield components (kernel number, and kernel weight) were also measured. Grain P content (%) was estimated using the same method as used for dry matter. The remainder of the shoot material at maturity was also dried and weighed, and added to seed weight to give total mature shoot weight (g pot^{-1}). Grain harvest index was calculated as 'grain yield' divided by 'total mature shoot weight'.

Field studies

Wagga Wagga 2008

Three of the higher-yielding spelt genotypes used in the glasshouse study (SP18, SP19, SP40) and the commercial spelt variety 'Kamarah' [non-registered cultivar name, also known as "Brown's Mixture" or "Booth's Mixture") and one standard bread wheat cultivar (Wedgetail) were established with four rates of P (0, 4.4, 8.9, 17.8 kg P ha^{-1}) applied as superphosphate, drilled at sowing. The soil was an Oxic Paleustalf (USDA, 1983) or Red Kandosol (Isbell, 1996), with an available P content (Olsen P) (Olsen et al., 1954) of 11 mg P kg^{-1} , located at Wagga Wagga (S 35° 01', E 147° 20'). Sowing rate was 45 kg ha^{-1} . The design consisted of main plots of P rate, split for genotype, with the P treatments randomised in 3 replicate blocks and genotypes randomised within P treatments. Plots were 10 m in length and comprised 8 rows spaced at 0.18 m. Prior to sowing, the soil was cultivated and treated with urea (127 kg N ha^{-1}) and gypsum (200 kg ha^{-1}), which were applied and incorporated with soil on 27 May 2008. Sowing occurred on 5 June 2008. Total rainfall in 2008, 413 mm, was below the long-term yearly average of 525 mm, and growing season rainfall (May to October), 164 mm, was well-below the cumulative long-term average for those months (291 mm).

Days-to-flowering, crop dry matter and P content at flowering, tiller number per plant (fertile and sterile), grain yield, yield components, and grain P content were recorded. Crop dry matter at flowering was estimated from five x 1 m sections of row per plot, and tiller number was determined on the plants from 1 m of row per plot. Grain yield was estimated from the machine-harvested plot yields. Dry matter and grain yield data were converted to a per hectare unit. For the spelt genotypes, manually dehulled grain was used to estimate grain yield. The cultivar Kamarah was not used in the glasshouse study because it is known to be a mixture of several different genotypes which would have led to deceptive plant-to-plant variation in pots (but not so in plots).

Condobolin 2008

Two of the higher-yielding spelt genotypes used in the glasshouse study (SP18, SP19), two standard bread wheats (Carinya, Ventura), and two barley cultivars (Buloke and Hindmarsh), were used. There were six rates of P (0, 4, 8, 12, 16, 24 kg P ha^{-1}) applied as double superphosphate, and the applied P was drilled with the seed at sowing. Information on the barley cultivars is not reported in this manuscript. The site location (S 33° 04', E 147° 14') was on a Calcic Rhodoxeralf (USDA, 1983) or Red Chromosol (Isbell, 1996) soil with an available P content (Colwell P) (Colwell, 1963) of 22 mg P kg^{-1} . The plots were sown on 23 June 2008. Seeding rate was 100 seeds m^{-2} except for SP18, which was only 80 seeds m^{-2} owing to limited seed supply (see Results). Sowing occurred on a cultivated fallow with considerable stored moisture following 140 mm of rain during January to May. Annual rainfall for 2008 was 346 mm compared to a long-term average of 456 mm and growing season rainfall (June-October) was 124 mm, compared to the long-term average of 231 mm. Each factorial combination of genotype and phosphorus rate was randomly allocated within each of three replicated blocks. Plots were 15 m in length and comprised 8 rows at 0.18 m spacing.

Plant establishment (number m^{-2}), dry matter (kg ha^{-1}), tiller number plant^{-1} , and plant P content were recorded at flowering time.; Dry matter (kg ha^{-1}), crop height (cm), grain yield (t ha^{-1}), harvest index (t t^{-1})

¹), 100 seed weight (g), seeds per ear, fertile ears (number m⁻²), and grain P content (%) were recorded on material harvested at crop maturity. Plant establishment was estimated from four random quadrats per plot, each quadrat was 0.17 m². Dry matter was estimated from two quadrats per plot, each quadrat was 0.35 m². Grain yield was the machine-harvested plot yield. For the spelt genotypes, a subsample was manually dehulled and used to estimate plot grain yield. Grain yield components were estimated from 50 ears per plot. Plant establishment, dry matter and yield data were converted to per hectare units.

Rutherglen 2008

This field site (S 36° 06', E 146° 31') was certified organic so the use of water soluble P fertiliser was disallowed. Soil type was a Brown Chromosol (Isbell, 1996) with an available P (Olsen P) of 14 mg P kg⁻¹. Four genotypes of spelt (SP18, SP19, SP40, Kamarah) and one standard bread wheat (Wedgetail) were sown with 6 phosphorus treatments (Nil, PL270, PL361, PL480, Guano250 and Guano500). PL is a mixture of ground reactive phosphate rock and finely-divided elemental sulphur (30% by weight) providing 23, 31, or 41 kg P ha⁻¹ for PL270, PL361 and PL480, respectively. The guano provided 33 kg P ha⁻¹ or 65 kg P ha⁻¹ for Guano250 and Guano500, respectively. These products were broadcast and then incorporated with soil on 4 December 2007. The trial was sown on 28 May. The design comprised randomised main plots of the P treatments split for genotypes randomised to the P treatments. The P treatments were randomised within each of 3 replicate blocks. Plot length was 15 m and comprised 6 rows spaced at 0.18 m. Dry matter at flowering was estimated from 4 x 1 m sections of row per plot, and grain yield was determined from the machine-harvested plot yields. These data were converted to per hectare units.

Statistical analysis

Data were analysed using REML (Genstat v. 10.2) to account for spatial effects. Days-to-flowering was used as a covariate in the analysis of all variables (except flowering itself). The covariate was used to increase the analysis precision for each trait by centering across differences in phenology. Differences between the REML-predicted treatment means ($P < 0.05$) were resolved by the least significant difference procedure (LSD) ($P < 0.05$). Fitted curves were calculated and drawn using SigmaPlot v8 software.

Results

While this study was underway, DNA analysis of the putative spelt genotypes was completed (data not presented), and it showed that SP76 and SP77 were not true spelt genotypes but probably unselected common bread wheat genotypes.

Glasshouse study

Genotypic differences between standard bread wheats and spelt

Mean days-to-flowering of spelt genotypes varied widely from 87 to 150 days. Early-, mid-, and late-flowering spelt groups were similar to the three bread wheat controls (Table 1). There was a group of three very late-flowering spelt genotypes that did not match a representative bread wheat cultivar.

Spelt tillered significantly more than the standard bread wheat cultivars (Table 1) except for SP40, and differences between spelt genotypes were also evident. At flowering, in Groups 2 and 3, the dry matter of spelt was greater than the three standard bread wheats (Table 1). Significant variation in dry matter within spelt genotypes was also evident, generally increasing as days-to-flowering increased. Late-flowering spelt genotypes were visually taller than the standard bread wheats.

The highest yielding genotypes (grain yield > 6 g pot⁻¹) were the standard bread wheats (Table 1); only SP40 was not statistically lower yielding than these standard wheats. With the exception of SP40, the lower yields of the spelt wheats were associated with lower mean kernel weights relative to the standard bread wheats (Table 1). Excluding data for the three 'very late flowering' spelt genotypes (SP2, SP22, SP29), higher tiller number was significantly correlated with lower grain yield ($R^2 = -0.66$). The three excluded spelt genotypes were high tillering but their grain yields fell below the general regression of grain yield on tiller number, suggesting that an additional factor further decreased the grain yield of these spelt wheats (see below). Higher tiller number was also correlated with reduced mean kernel weight (Figure 1).

Table 1. Average effect of genotype on growth parameters of spelt and standard bread wheat genotypes in a glasshouse experiment at Wagga Wagga. Values tabulated are means over all rates of applied phosphorus. Days-to-flowering was used as a covariate in the analysis of all variables (except days-to-flowering itself). The genotypes are shown grouped by their days-to-flowering category. Values followed by a common letter within a column are not significantly different ($P = 0.05$) using an LSD test.

Genotype	Group	Flowering category	Days-to-flowering	Tillers (number plant ⁻¹)	Dry matter at flowering (g pot ⁻¹)	Grain yield (g pot ⁻¹)	Kernel weight (mg seed ⁻¹)
Waagan	1	Early	82.6 a	6.0 a	12.0 a	6.78 f	41.9 cd
SP10	1	Early	87.8 b	9.2 bc	13.1 a	5.44 cde	34.8 b
Gregory	2	Mid	105.6 c	6.1 a	13.5 a	6.62 f	46.4 e
SP16	2	Mid	101.8 c	8.8 bc	17.1 b	4.51 bcd	35.6 b
SP18	2	Mid	102.8 c	8.9 bc	16.0 b	5.47 cde	37.6 b
SP19	2	Mid	103.8 c	8.8 bc	15.6 b	5.40 cde	42.7 d
Wedgetail	3	Late	119.8 d	6.2 a	16.9 b	6.73 f	44.6 de
SP40	3	Late	126.3 e	5.4 a	26.4 ef	5.77ef	54.3 f
SP41	3	Late	122.1 de	9.7 c	23.3 d	4.79 cde	35.0 b
SP76	3	Late	118.5 d	11.8 d	19.4 c	5.10 cde	37.7 b
SP77	3	Late	118.1 d	12.3 d	19.6 c	4.42 bc	38.0 b
SP2	4	Very late	144.8 fg	8.3 bc	24.6 de	3.66 ab	38.2 bc
SP22	4	Very late	149.4 g	8.0 b	27.8 f	2.89 a	30.4 a
SP29	4	Very late	143.4 f	8.2 b	25.0 de	3.45 ab	37.3 b
LSD (5%)			4.64	1.35	2.08	1.073	3.51

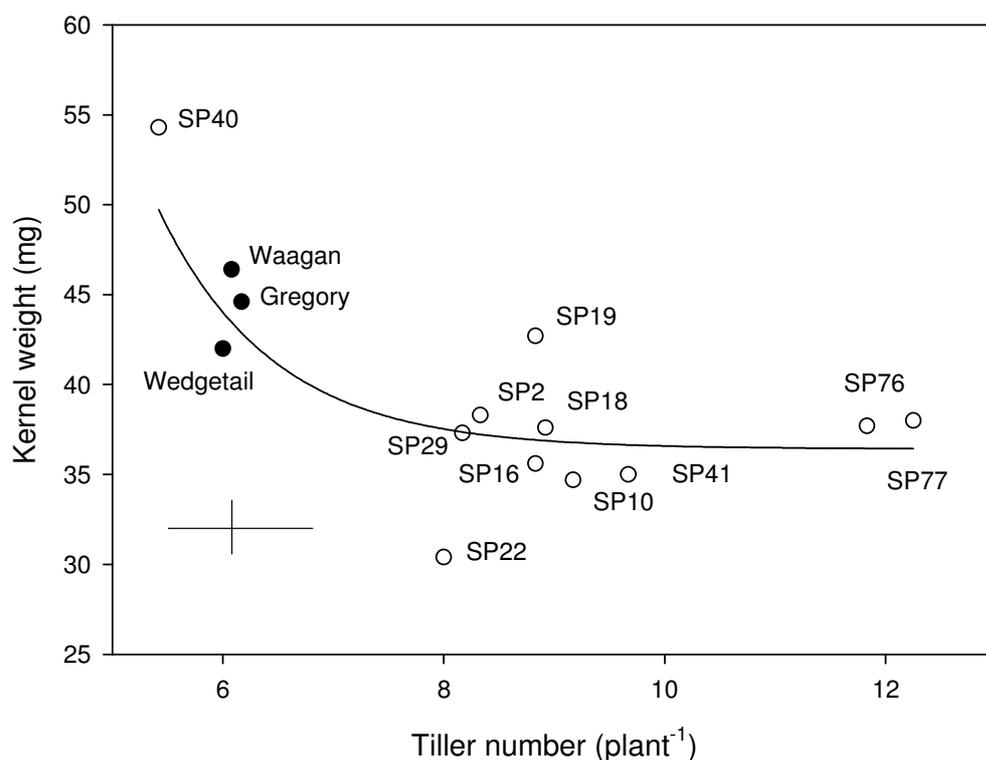


Figure 1. Relationship between mean kernel weight and tiller number . Values are means for genotypes over the applied P treatments. Filled symbols are the standard bread wheat genotypes. Bars indicate the LSD ($P = 0.05$). Kernel weight = $36.4 + 2475 \times (1 - e^{-0.964 \times \text{tiller number}})$, $R^2 = 0.66$.

With the exception of SP40, greater dry matter in spelt genotypes was also associated with lower grain yield (Figure 2), because of the negative association of greater dry matter with the other primary grain yield factor, kernel number (Figure 3). Spelt genotypes SP2, SP22 and SP29, and SP40 were particularly reduced for kernel number. Lower kernel number was not generally compensated for by higher kernel weight except with SP40, where lower number was partly offset by higher weight, which helped support the grain yield of this genotype (Table 1).

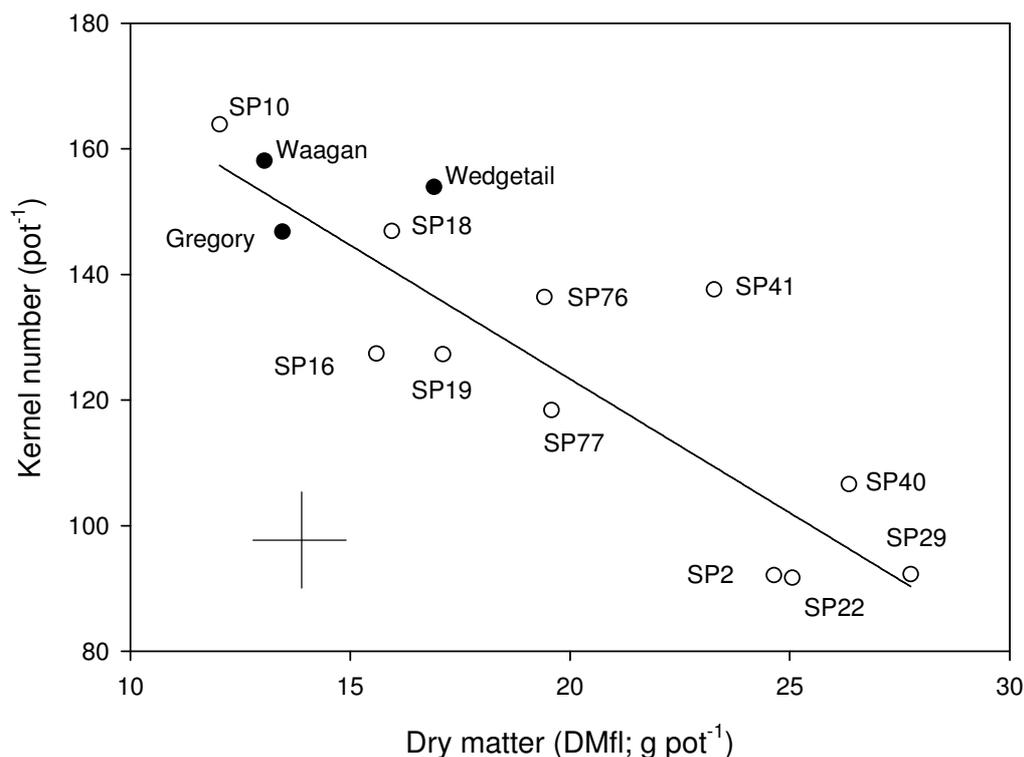


Figure 2. Relationship between kernel number per pot and the amount of plant dry matter present at flowering (DM_{fl}) for a glasshouse experiment in 2007. Values are means of genotypes over all applied P treatments. Filled symbols are the standard bread wheat cultivars. Bars indicate the LSD ($P = 0.05$). Kernel number = $214 - 4.47 \times$ dry matter at flowering, $R^2 = 0.78$.

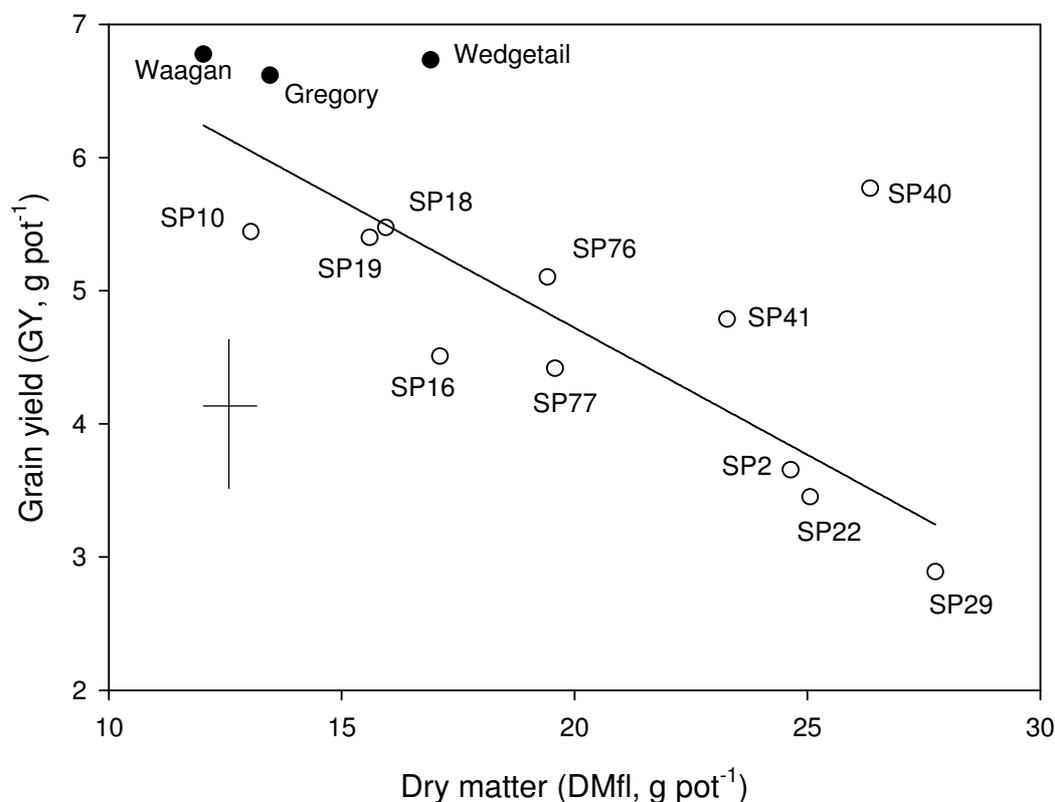


Figure 3. Relationship between grain yield (GY) and dry matter at flowering (DM_{fl}). Values are genotype means over applied P levels. Filled symbols are the standard bread wheat genotypes. Bars indicate the LSD ($P = 0.05$). Grain yield = $8.54 - 1.91 \times$ dry matter at flowering, $R^2 = 0.67$.

Across all genotypes, higher grain yield was positively associated with higher grain harvest index (grain harvest index = $0.06 \times$ grain yield $- 0.09$; $R^2 = 0.93$). The negative impact of a higher dry matter on kernel number and ultimately on grain yield (Figures 2 and 3) therefore resulted in a reduction in

grain harvest index in genotypes with higher total mature shoot dry matter (Figure 4). Particularly at the two highest P levels, the grain harvest index of the commercial bread wheats were much less affected than the spelts because the total mature shoot weight of the commercial wheats was less than that of the spelts (Figure 4). At the lowest P level, despite a similar dry matter in commercial wheats and some spelt genotypes the grain harvest index of the spelts was reduced.

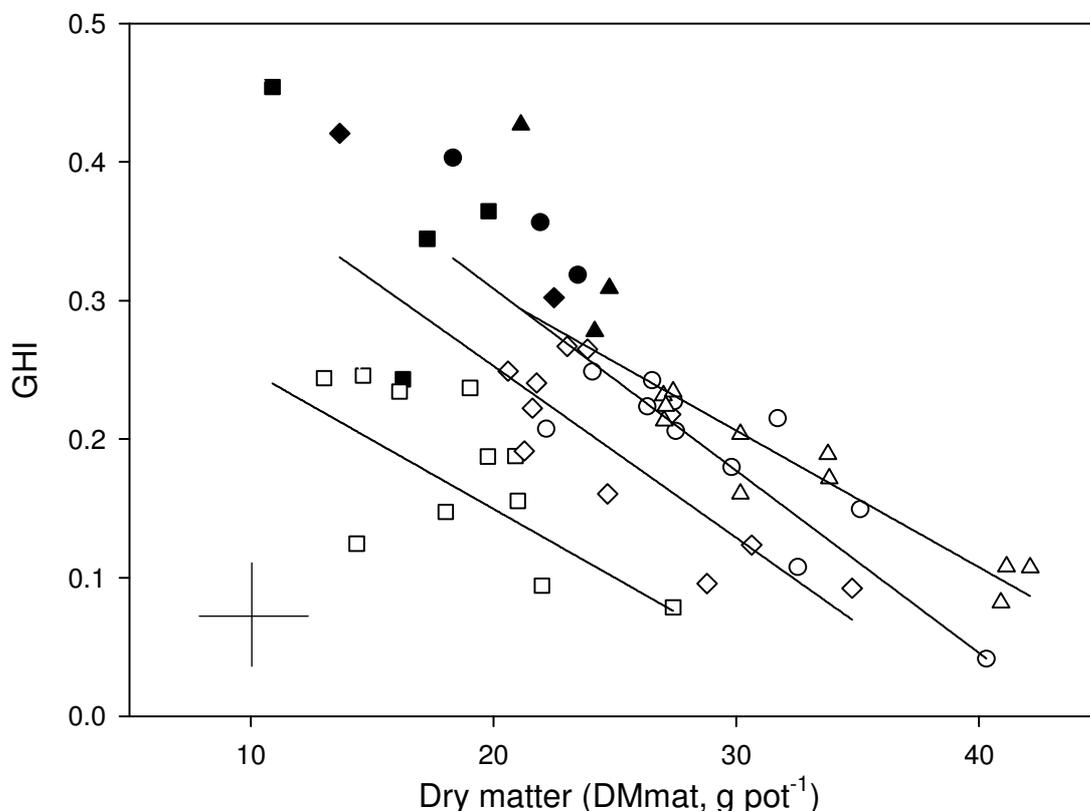


Figure 4. Relationship between total mature shoot dry matter (DM_{mat}) and grain harvest index (GHI). Values are means of genotype within each applied P treatment. Filled symbols are the standard bread wheat genotypes. Bars indicate the LSD ($P = 0.05$). Squares = 11.1 mg P per pot ($R^2 = 0.48$); diamonds = 23.1 mg P per pot ($R^2 = 0.71$); circles = 34.7 mg P per pot ($R^2 = 0.92$); and triangles = 57.6 mg P per pot ($R^2 = 0.89$).

Influences of P nutrition

Plant total P content at flowering (mg P plant^{-1}) increased with higher rate of applied P: means = 6.8, 10.6, 15.0 and 26.1 ($P < 0.001$; LSD = 1.55), for the four levels of P applied, respectively. This occurred as a result of both greater dry matter at flowering: means = 13.0, 18.1, 21.7, and 23.4 ($P < 0.001$; LSD = 1.08), and increased tissue P concentration at flowering: means = 0.056%, 0.066%, 0.076% and 0.120% ($P < 0.001$; LSD = 0.0081), for the four P applied levels, respectively. However, within a P applied level there were no significant differences between genotypes for total P content at flowering or efficiency of P uptake at flowering. Consequently, because spelt genotypes generally produced more dry matter compared to the standard bread wheats, total P content of shoots at flowering was usually less in spelt (Table 2) due to a simple dilution effect (total shoot dry matter at flowering vs. total P content of shoots at flowering, $R^2 = -0.66, -0.67, -0.83, -0.89$ for the four P applied levels, respectively). P biological efficiency in dry matter at flowering of spelt was generally greater than for standard bread wheat cultivars (Table 2).

Grain yield increased with P applied: means = 3.68, 5.23, 5.89 and 6.18 ($P < 0.001$; LSD = 0.554), for the four P applied levels, respectively; explained by a significant increase in kernel number (data not shown) as the supply of P increased. The increase in kernel number was explained by a significant increase in tiller number as the P applied level increased: means = 6.5, 7.6, 8.7, and 10.1 ($P < 0.001$; LSD = 0.70), for the four P applied levels, respectively. However, there was no significant interaction between genotype and P applied for either grain yield or tiller number. Grain harvest index was increased as P applied increased (Figure 4).

Given that plant total P content at maturity was not different between genotypes grown at the same P applied level than the higher yielding standard bread wheats were more efficient in converting P applied into grain yield.

Table 2. Effect of genotype on shoot phosphorus (P) uptake and P biological efficiency at flowering in a glasshouse experiment at Wagga Wagga. Values shown are means pooled across P application rates. Days-to-flowering was used as a covariate in the analysis of both variables. The genotypes are shown grouped by their days-to-flowering category. Values followed by a common letter within a column are not significantly different.

Genotype	Group	Total P content of shoots at flowering (%)	P biological efficiency in dry matter at flowering (g mg P pot ⁻¹)
Waagan	1	0.13 d	0.88 a
SP10	1	0.11 d	1.03 abc
Gregory	2	0.12 d	0.94 ab
SP16	2	0.09 c	1.26 c
SP18	2	0.09 c	1.25 b
SP19	2	0.09 c	1.25 b
Wedgetail	3	0.09 c	1.21 b
SP40	3	0.06 a	1.81 d
SP41	3	0.06 a	1.84 d
SP76	3	0.07 b	1.57 cd
SP77	3	0.07 b	1.59 d
SP2	4	0.05 a	2.23 e
SP22	4	0.05 a	2.25 e
SP29	4	0.05 a	2.26 e
LSD (5%)		0.016	0.311

Across all treatments, grain yield was significantly ($R^2 = 0.53$) and positively associated with higher total P content of shoots at flowering (Figure 5). The 95% maximum grain yield level, calculated from the fitted regression equation was 7.89 g pot⁻¹. Using 1 standard error (= 0.825) below this value, as a basis for the minimum critical P concentration to achieve 95% of maximum grain yield, resulted in a threshold estimate of 0.12% total shoot P content at flowering. Only a few spelt treatment combinations achieved this critical value or greater. Therefore, the lower P content in spelt dry matter, compared with standard bread wheat cultivars (Table 2), was a further factor acting against high grain yield in the spelt genotypes.

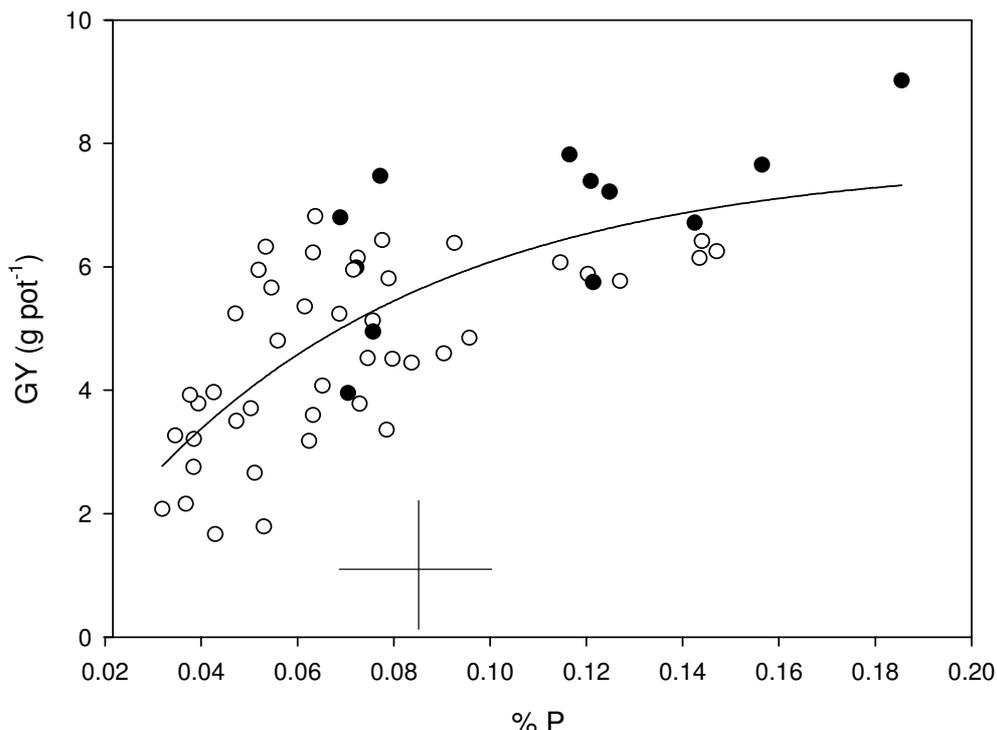


Figure 5. Relationship between grain yield (GY) and total shoot P concentration (%P) at flowering. Values are treatment means; filled symbols indicate the standard bread wheat genotypes. Bars are LSD ($P = 0.05$). Grain yield = $8.31 \times (1 - e^{-16.08 \times \%P}) - 0.56$. $R^2 = 0.53$.

Grain yields for each of the genotypes at each P applied level are given in Table 3. The grain yield of Gregory and Wedgetail was maximal at the moderately low P level. As compared to these standard bread wheats, the grain yields of SP18 (Group 2), SP40 and SP76 (Group 3), and SP29 (Group 4) were also maximised at the moderately-low P level. These spelt genotypes, therefore, may have a similar fertiliser P requirement for maximal grain yield as some bread wheats, albeit with a lower grain yield potential. Other spelt genotypes, namely SP16, SP19, SP41, SP77 and SP2, had a higher P requirement for maximal grain yield than their comparative bread wheat (Table 3). The grain yield of Waagan may not be maximised at the highest applied P level used here: only SP16 and SP22 were similar in that regard.

When the supply of P was low (mimicking a low P input cropping system) the grain yield of spelt wheats did not differ significantly from the standard bread wheat cultivars (Table 3). However, the apparently larger grain yield of Wedgetail, relative to spelt genotypes with similar days-to-flowering, is consistent with its higher total shoot P concentration (0.07%) as compared to the spelt genotypes (ranging from 0.03% to 0.05%). In this range of total shoot P concentration, grain yield reduces rapidly as P concentration declines (Figure 5).

Table 3. Grain yields (g pot^{-1}) of a range of genotypes of standard bread wheat and spelt in response to four applied P levels in a glasshouse experiment at Wagga Wagga in 2007. Values in bold indicate the statistically significant maximum grain yield for each genotype when compared to the yield at the next lowest P applied level ($P = 0.05$).

Genotype	Group	Applied P level (equivalent kg P ha^{-1})			
		6.8 (low)	13.8 (moderately low)	20.3 (moderate)	33.8 (high)
Waagan	1	4.95	5.75	7.39	9.02
SP10	1	4.51	5.13	5.88	6.25
Gregory	2	3.78	7.22	7.82	7.65
SP16	2	3.60	4.07	4.60	5.77
SP18	2	3.18	6.15	6.43	6.14
SP19	2	3.96	5.24	5.99	6.42
Wedgetail	3	5.95	6.80	7.47	6.71
SP40	3	3.92	5.95	6.82	6.39
SP41	3	3.71	3.97	5.66	5.81
SP76	3	2.66	6.32	5.36	6.07
SP77	3	1.79	4.80	6.23	4.85
SP2	4	3.26	2.76	5.24	3.36
SP22	4	2.16	3.21	1.67	4.52
SP29	4	2.08	3.78	3.50	4.44

Grain P content (data not shown) was estimated only at the two highest P applied rates. Averaged over these P rates, the grain P content of the standard bread wheat cultivars was significantly less than for some of the spelt genotypes (SP16, SP18, SP40 and SP41), which was partly a dilution effect resulting from the higher grain yield of the standard bread wheats (grain yield = $-17.4 \times$ grain P content + 11.6; $R^2 = 0.49$). However, the absolute P content of the grain of the standard bread wheat cultivars, particularly Wedgetail and Waagan, exceeded that of spelts ($P < 0.01$). Furthermore, the total amount of grain P relative to the total amount of P in dry matter at flowering ranged from 0.72 to 0.82 for the standard bread wheat cultivars, exceeding the range for the spelt genotypes: 0.46 to 0.70 (the highest value for spelt occurred with SP19). Across all genotypes and cultivars, grain P content was positively correlated with grain yield ($R^2 = 0.91$).

Field studies

Wagga Wagga 2008

Flowering of SP18, SP19 and Wedgetail occurred over a similar period (165-172 days after sowing), but before SP40 (165-176 days) and well before Kamarah (182-185 days). Higher P applied reduced the number of days for crops to reach flowering. Therefore, in contrast to the glasshouse study, the days-to-flowering of SP18 and SP19 in the field were similar to Wedgetail.

The site was P-responsive because P applied increased total shoot dry matter at flowering ($P < 0.001$) from 2001 kg ha^{-1} to 3245 kg ha^{-1} between the nil and highest levels of P applied. However, there

were no significant differences in the rate of increase in total shoot dry matter at flowering in response to increased P applied between the genotypes. Averaged over P applied levels, though, Kamarah produced significantly ($P < 0.001$) less, and SP18 significantly more, dry matter than the other genotypes; therefore, the P efficiency of uptake of SP18 was greater than the standard bread wheat.

The total amount of P in the shoots of crops at flowering depended significantly on the genotype and the P applied level ($P = 0.02$, Figure 6). SP18 and SP40 accumulated more P, more rapidly than SP19, Kamarah or Wedgetail. The superior P response of SP18 and SP40 occurred as a result of significantly ($P < 0.001$) greater shoot dry matter at flowering compared with SP19 and Wedgetail. Kamarah had the highest total shoot P at flowering but also the least dry matter. The significant genotype \times P applied interaction for total P at flowering seen here in the field differed from the findings in the glasshouse study, where there was no such interaction.

Tiller number was increased at the highest P applied level ($P = 0.07$), though only in genotypes Kamarah and SP18 ($P = 0.06$), and only by 1 tiller plant⁻¹ compared to tillering at nil P applied. Averaged over P levels, Kamarah produced significantly ($P < 0.001$) more viable tillers (8.0 plant⁻¹) than all other genotypes, and Wedgetail the least (2.5 plant⁻¹), with SP18 and SP40 having a similar number (4.6 and 4.9 plant⁻¹, respectively) and significantly more than SP19 (3.6 plant⁻¹). Abortion of tillers was high in Kamarah (70%) and SP40 (53%) compared with a mean of 20% across the other genotypes.

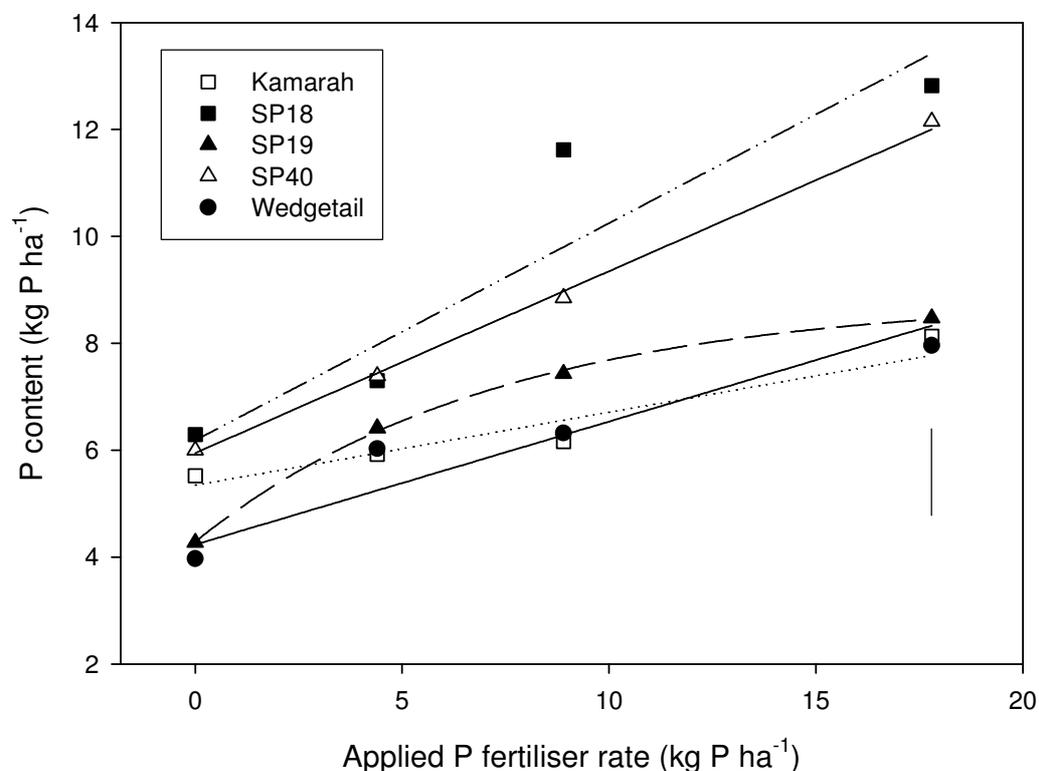


Figure 6. Influence of P applied fertiliser level on the total shoot P content of the dry matter at flowering of standard wheat and spelt genotypes in an experiment at Wagga Wagga in 2008.

The vertical bar is the LSD ($P = 0.05$; genotype \times P_{app}). Regression equations:

Wedgetail Total shoot P content at flowering = $4.2 + 0.23 \times \text{P applied}$, $R^2 = 0.91$,
 SP40 Total shoot P content at flowering = $5.9 + 0.34 \times \text{P applied}$, $R^2 = 0.99$,
 SP19 Total shoot P content at flowering = $4.3 + 4.6 \times (1 - e^{-0.14 \times \text{P applied}})$, $R^2 = 0.99$,
 SP18 Total shoot P content at flowering = $6.46 + 0.39 \times \text{P applied}$, $R^2 = 0.87$, and
 Kamarah Total shoot P content at flowering = $5.29 + 0.15 \times \text{P applied}$, $R^2 = 0.93$.

Grain was low ($< 0.4 \text{ t ha}^{-1}$) and determined only by main factors of P applied ($P = 0.02$) and genotype ($P < 0.001$). Increasing P applied significantly increased grain yield ($P < 0.001$), total shoot P at flowering ($P < 0.001$) and total P content of grain at maturity ($P < 0.001$), but these responses were maximal at only the second P applied level, 4.4 kg P ha^{-1} . Therefore, under these field conditions at Wagga Wagga in 2007, all genotypes had a similar requirement for P applied to achieve maximal grain yield. Averaged across P applied levels, grain yield increased in the following order: Kamarah $<$ SP40 = SP18 $<$ SP19 $<$ Wedgetail; and was strongly, and inversely, related to tiller number (Figure 7). Thus, P efficiency in grain yield was greatest in the standard bread wheat, but also differed between

the spelt genotypes, being greatest for SP19. Grain P concentration did not vary between genotypes, but total grain P increased with grain yield ($R^2 = 0.98$).

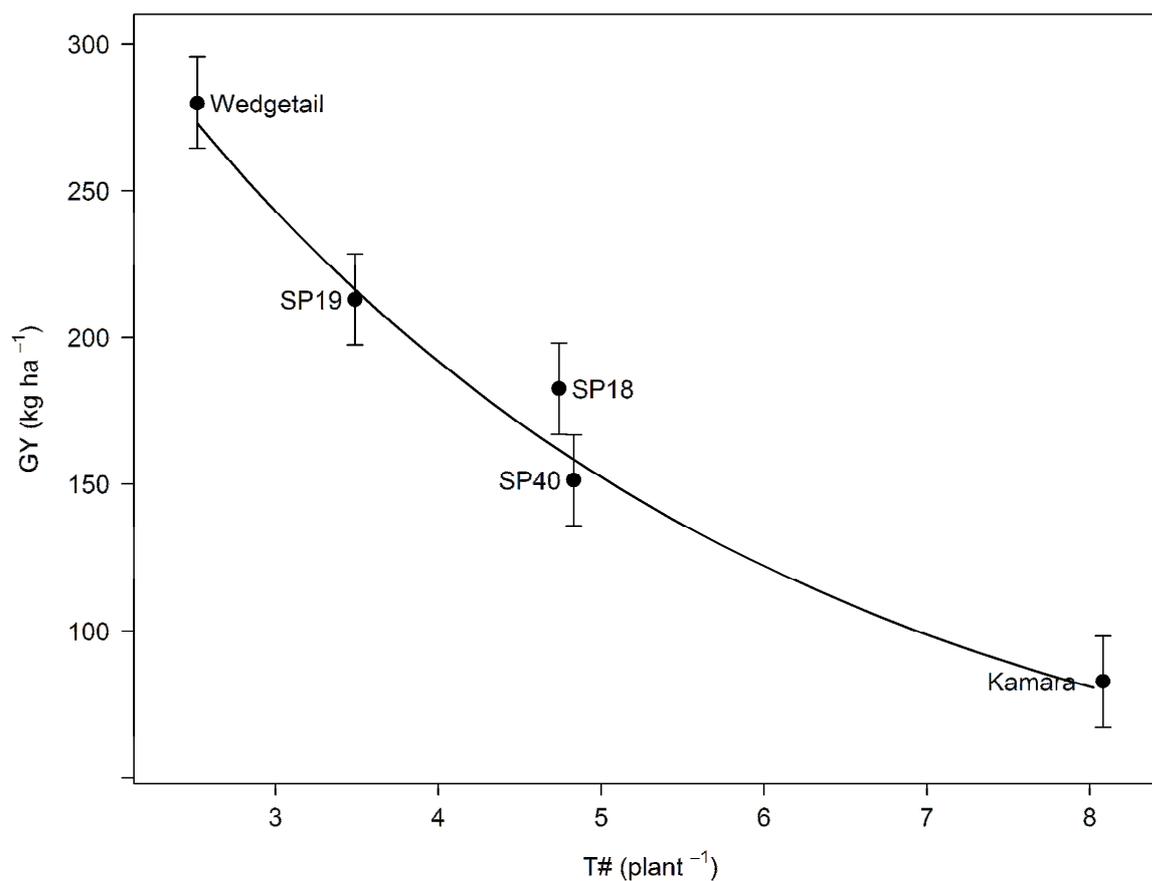


Figure 7. Relationship between grain yield (GY) and tiller number per plant (T#) for five genotypes grown in the field experiment at Wagga Wagga, 2008. Values are means over all applied P levels. Error bars are standard errors for difference of means. Fitted line is: $GY = 21.4 + (488 \times e^{-0.263 \times T\#})$, $R^2 = 0.97$.

Condobolin 2008

Across the range of P applied treatments, the number of plants established in the standard bread wheat genotypes (Carinya and Ventura) significantly exceeded the number in the spelt genotypes (Table 4), and significantly better establishment occurred with SP19 compared to SP18, despite the fact that SP19 was sown at a lower rate, due to limits in seed supply (SP18 sown at 59 kg ha⁻¹; SP19 at 47 kg ha⁻¹).

The site was responsive to P applied with average crop dry matter increasing significantly ($P < 0.001$) from 2177 kg ha⁻¹ to 4880 kg ha⁻¹ as P applied increased from 0 to 24 kg P ha⁻¹, with a plateau at 12 kg P ha⁻¹. Total shoot P content at flowering (kg P ha⁻¹), estimated for Carinya, SP18 and SP19, increased linearly over the range of P applied levels (Figure 8), and although it depended on both P applied and genotype ($P = 0.02$), the data for SP18, the genotype mainly responsible for the interaction, were less reliable as these values were based on only two replicates, because of a shortage of seed of this line. Average total shoot P content at flowering was sustained at 0.11% as total shoot dry matter at flowering increased to the plateau, before increasing to 0.15% at the highest P applied rate.

Averaged over P applied levels, tiller number was significantly higher in the spelt genotypes (Table 4), and crop height (cm) was significantly greater for SP18, compared with the standard bread wheats. Grain yield and grain harvest index of the spelt genotypes were significantly inferior to the standard bread wheats (Table 4). The higher grain yield of the bread wheats did not result from a higher mean kernel weight, or from a higher number of ears per unit area, but from a higher number of seeds per ear (Table 4). Notably, seeds per ear was strongly and inversely related to tiller number ($R^2 = 0.94$, at the genotype level).

Table 4. Characteristics of two standard bread wheat and two spelt genotypes in a field experiment at Condobolin in 2008. Values are the means over all P applied fertiliser treatments. Values with a common letter, within a column, are not significantly different ($P = 0.05$) using a simple LSD test.

Genotype	Plants m ⁻²	Tillers plant ⁻¹	Mature crop height (cm)	Shoot dry matter at flowering (t ha ⁻¹)	Grain Yield (t ha ⁻¹)	Grain harvest Index	100 Seed weight (g ⁻¹)	Seeds ear ⁻¹	Ears m ⁻²
Carinya	84.2 c	3.05 a	468 a	3.70 b	1.09 c	0.46 c	3.07 c	25.5 c	190 b
Ventura	81.2 c	2.54 a	570 c	3.87 b	0.98 b	0.45 c	2.73 a	27.8 d	145 a
SP18	46.5 a	5.64 c	681 d	3.20 a	0.61 a	0.23 a	2.90 b	13.3 a	165 ab
SP19	68.7 b	4.00 b	520 b	3.55 ab	0.70 a	0.32 b	3.49 d	17.8 b	187 b
LSD (5%)	8.42	0.829	12.0	0.37	0.101	0.017	0.098	1.68	23.4

The initial grain yield response to P applied was greater for the standard bread wheats compared to the spelt genotypes ($P < 0.001$; Figure 8). The reduced response of SP18 and SP19 may be caused by a more rapid but ultimately unproductive tillering response to P applied in these genotypes. Thus, the rate of change in tillers plant⁻¹ per unit of applied P was 0.26 for SP18 ($R^2 = 0.80$) with a predicted maximum of 9.0 plant⁻¹ at 24 kg P ha⁻¹, as compared with 0.08 ($R^2 = 0.67$) and a corresponding maximum equal to 4.1 plant⁻¹ for Carinya; with SP19 intermediate to these [initial rate of change = 0.24 ($R^2 = 0.89$), with a plateau of 4.6 plant⁻¹ occurring at 12 kg P ha⁻¹]. Except for Ventura, no grain yield plateau was reached in response to higher P applied levels (Figure 8). Relative to Ventura, maximum grain yield with SP18, SP19 and Carinya required a greater amount of applied P. In general, P efficiency in grain yield for the standard bread wheat cultivars was superior to the spelt genotypes.

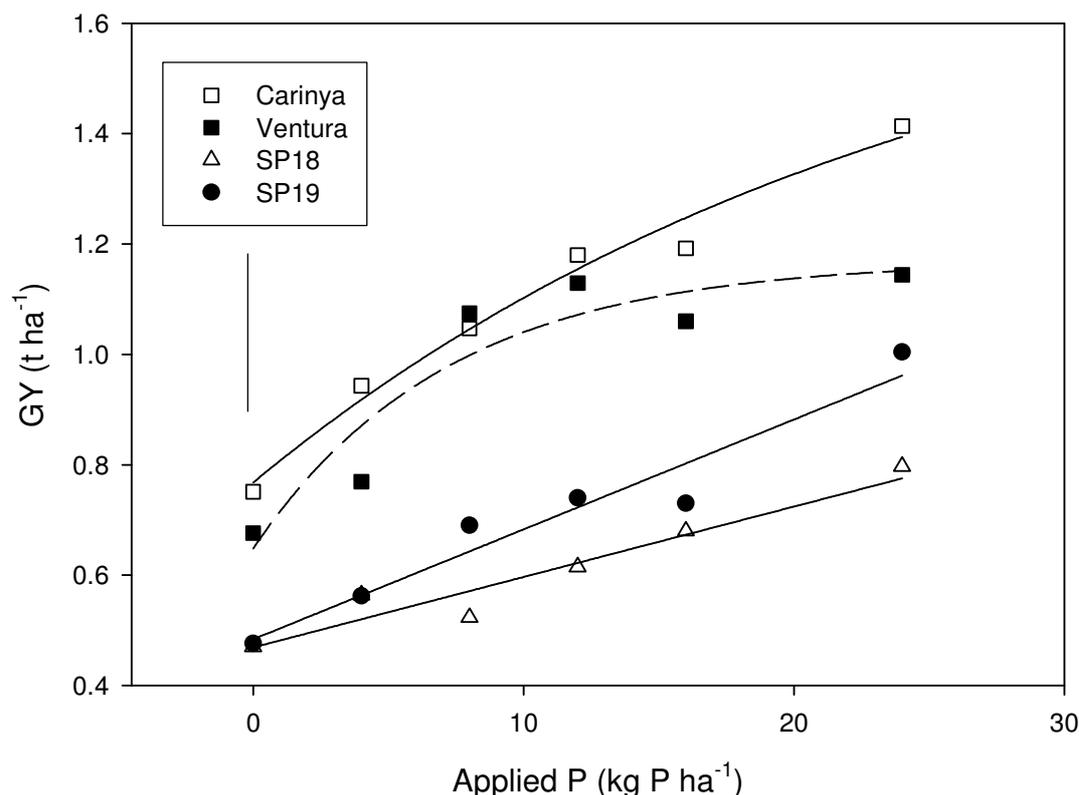


Figure 8. Influence of applied P on the grain yield (GY) of two standard wheats and two spelt genotypes in a field experiment at Condobolin in 2008. Vertical bar is the LSD ($P = 0.05$) for the genotype x applied P interaction ($P < 0.001$). Regression equations:
Carinya Grain yield = $0.77 + 1.02 \times (1 - e^{-0.04 \times P \text{ applied}})$, $R^2 = 0.98$,
Ventura Grain yield = $0.52 + 0.52 \times (1 - e^{-0.139 \times P \text{ applied}})$, $R^2 = 0.88$,
SP18 Grain yield = $0.47 + 0.013 \times P \text{ applied}$, $R^2 = 0.98$, and
SP19 Grain yield = $0.48 + 0.02 \times P \text{ applied}$, $R^2 = 0.94$.

Rutherglen 2008

Flowering of SP18, SP19, SP40 and Wedgetail occurred around 19 October 2008, and flowering of Kamarah around 11 November. At this field site there was no effect of the P applied treatments on

either total shoot dry matter at flowering or grain yield, nor any interaction between genotype and P applied. However, averaged over P treatments, the total shoot dry matter at flowering of SP19 and SP40 was similar and significantly ($P = 0.002$) greater than for Wedgetail and Kamarah, and SP19 significantly greater than SP18. Also averaged over the P applied treatments, the low grain yield (which was very low due to drought conditions) varied significantly ($P < 0.001$) between genotypes: Kamarah (347 kg ha^{-1}) < SP40 (490 kg ha^{-1}) < SP18 (669 kg ha^{-1}) < SP19 (918 kg ha^{-1}) = Wedgetail (1001 kg ha^{-1}). There was no relationship between total shoot dry matter at flowering and grain yield. The growing season (June-October) rainfall at the Rutherglen site was only 182 mm; compared to a long-term average of 292 mm. The year (2008) was a significant drought with annual rainfall of only 429 mm compared to the long-term mean of 588 mm.

Discussion

After this study was completed, a report on genetic diversity in spelt germplasm using DNA markers (Raman et al., 2009) concluded that the genotypes SP18 and SP19 contained alleles that suggested that these genotypes were derived from crosses between spp. *spelta* and common bread wheat. This is partly evident here in the intermediate response of these genotypes compared to the extremes of the commercial wheat cultivars (e.g. Wedgetail) and the "pure" spelts (e.g. Kamara) (Figures 1, 2, 3 and 7).

Spelt did not show the grain yield potential of the highly-developed and highly-selected standard bread wheat cultivars, either under well-watered glasshouse conditions or under field conditions. In the glasshouse, the average maximum grain yield of spelt (excluding the three late-flowering genotypes: SP2, SP22, SP29) was 77% of the average maximum grain yield of the standard bread wheats, with a range from 56% to 84%. In the field, at Wagga Wagga, the spelt averaged 65% (range of 54-76%) of the grain yield of Wedgetail; at Condobolin, 60% (range of 55-64%) of Carinya; and at Rutherglen, 60% (range of 34-92%) of Wedgetail. Where Kamarah was included in the field experiments, its grain yield was < 55% of Wedgetail, supporting the anecdotal evidence that current commercial crops of spelt yield substantially less than commercially-grown bread wheat. The range of alternative spelt genotypes used in this study also yielded significantly less than standard bread wheats. Furthermore, the P applied requirement to achieve the maximum grain yield of spelt was no less than for bread wheats, suggesting that an economic return from spelt similar to standard bread wheat can only be achieved through the higher market value per tonne of spelt grain.

There was no evidence from our glasshouse or field trials that, in P-deficient soil (low input cropping systems), spelt was capable of higher grain yield than standard bread wheats, indeed in most cases its P uptake and utilisation efficiencies were also lower than bread wheat

The field experiments showed that Kamarah yielded significantly lower than alternative spelt genotypes. Thus, over a range of soil P fertility conditions, the grain yield of Kamarah was only 38% and 39% of the grain yield of SP19 at Rutherglen and Wagga Wagga, respectively. These data suggest that greater economic returns could be achieved through commercialisation of the highest-yielding spelt genotype, SP19, provided that the grain quality attributes valued by the organic spelt industry can be maintained. The long days-to-flowering of Kamarah meant that it was poorly adapted; the plant is also poorly-structured (high tiller number and tall) preventing it from yielding well, at least under moisture-stressed conditions. The glasshouse data also suggested that the minimal P applied requirement for maximal grain yield among the spelt genotypes may differ, so that breeding and selection within spelt may optimise P efficiency for grain yield.

The higher tiller number, total dry matter at flowering, and dry matter at maturity of the spelt genotypes may have value in an organic system for increased competitiveness against weeds, for grazing, or for the production of forage (hay or silage). However, these characteristics were not conducive to high grain yield. Under glasshouse conditions some growth attributes of spelt were negatively correlated to grain yield; including greater dry matter at flowering and greater tiller number. The yield component that appeared to be most adversely influenced by higher dry matter at flowering was kernel number per plant. This yield component is reported to have a major influence on the grain yield of standard wheat (Guitard and Newman, 1961, Shah et al., 1994) as it is a key component of overall sink size. The yield component most adversely influenced by greater tiller number was mean kernel weight. The general conclusion from the glasshouse and field observations reported here is that the greater propensity for tillering and higher dry matter production in spelt render it less able to yield at an equivalent level to standard bread wheats.

Genetic advance in the grain yield of standard winter and spring wheats has shown a correlation with an increase in grain harvest index often through the use of dwarfing genes (Austin et al., 1980, Domnez et al., 2001, Shearman et al., 2005). A reduction in tiller number or plant height in spelt may provide a pathway for increasing its grain harvest index by enabling an increase in mean kernel weight and/or kernel number.

In our glasshouse experiment, greater biomass was not only associated with lower kernel number, but also with a lower total P content at flowering. According to several authors (Boatwright and Viets, 1966, Chapman and Keay, 1971, Batten et al., 1986) the P acquired by wheat up to flowering is usually sufficient for maximal grain yield, because P stored in leaf and stem tissue is re-mobilised to support grain production (Batten et al., 1986). However, at a given level of P applied, the consistency in plant total P between genotypes varying in grain yield, suggested that total P *per se* could not explain grain yield variation. Total shoot P content at flowering was greater in the higher-yielding standard bread wheats than in spelt wheats grown at a fixed level of P applied. Others (Rashid et al., 2005) have reported a strong correlation between total shoot P at flowering and relative grain yield in spring wheat, with a critical concentration of 0.13%; similar to the suggested lower limit (0.12%) estimated here. Tissue that has a higher P concentration, such as that present in the standard bread wheats, may contain a greater fraction of P stored in more readily mobilised compounds which can be used to meet the requirements of grain production. The fact that the mean kernel weight of the standard bread wheat cultivars was significantly superior to nearly all spelt genotypes, and kernel number was lower, suggested that resources for seed growth were in better supply in the standard bread wheat cultivars. As well as carbon (C) (Grafius, 1972), these resources may include mobile P and N that are transported from leaf and stem material to grain (Chapman and Keay, 1971).

Simply enriching plant tissue with P is not likely to be sufficient to maximise the grain yield potential of spelt wheat. In the glasshouse study, applying more P fertiliser increased the tissue concentration of SP18 and SP19 to an adequate maximum value of 0.14 %P, yet these genotypes still did not produce grain yield comparable to the standard bread wheats. A significant amount of P was 'wasted' on unnecessary height and unproductive tillers. Despite the significant grain yield differences between standard bread wheats and spelt genotypes in the field at Wagga Wagga and Condobolin, tissue P concentration was similar or greater in the spelt genotypes; and all values exceeded 0.12%. At Wagga Wagga, the significantly higher tissue P concentration in SP18 and SP40 compared to Wedgetail and SP19 occurred in parallel with greater dry matter, which suggests that these spelt genotypes may have greater ability to acquire P from soil, at least under dry soil conditions. This may be due to better root system architecture or functioning (Richardson et al., 2009) and warrants further investigation. From this work it is apparent that tissue P concentration is unlikely to be responsible for determining grain yield differences between spelt and bread wheat, which seems more probably caused by morphological and physiological differences, related to tillering, height, ear size, and seed size.

Acknowledgements

The authors acknowledge financial and infrastructure support provided by: the Rural Industries Research and Development Corporation, NSW Department of Primary Industries, and the Department of Environment and Primary Industries Victoria. The authors have no competing interests to declare.

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