Kelp extracts as biostimulants: An investigation of when and why they work

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DOCTOR OF PHILOSOPHY

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Table of contents

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Preface and structure of this thesis

This thesis describes a series of experiments designed to explore aspects of the benefits of kelp (brown algae; class Phaeophyceae) extracts as biostimulants for plant growth and development. The mechanisms for claimed benefits of kelp in horticulture, viticulture and agriculture are not well defined.

The topic is introduced with a literature review (Chapter 1) discussing research into the use of kelp extracts in agriculture until 2019. The review reflects the state of knowledge of the use of kelp in agriculture at the time of the commencement of my candidature. It provides an outline of the evolution and qualities of kelp and a comparison between the structure and composition of kelp and green plants. Defence mechanisms of plants and algae are discussed. The species of kelps used in agriculture and the various methods of extraction of the commercially available kelp products are reviewed. The various modes of action of kelp extracts in agriculture that have been proposed are presented and the molecules such as phytohormones and carbohydrates that have been proposed by researchers as being responsible for kelp bioactivity in green plants are discussed.

The overall purpose of the research carried out in this project was to improve understanding of the mechanisms responsible for the response of terrestrial plants to soil and foliar application of kelp extracts, identify the circumstances under which kelp extract application produces a growth and/or yield benefit and explore any observed differences between different kelp products. The research component of this thesis is organised in a "thesis by publication" style, with Chapter 1 (Literature Review) acting as a general introduction, and Chapters 2-4 each containing specific introductions relevant to those sections.

Chapter 2 describes four field experiments conducted to investigate the potential benefit of kelp extract treatment to broccoli growing on slaking soil on the grounds of the Waite Campus of the University of Adelaide. Broccoli was chosen for these experiments because it is suited to the Mediterranean climate of Adelaide and prior research has shown that broccoli responds to kelp under Australian conditions. These four field experiments are similar in design and each explores a different aspect of kelp treatment. In order to limit repetition, aspects of Materials and Methods common to each of the four experiments are presented at the outset.

The first field broccoli experiment (Experiment 1; Section 2.1) was a comparison between five commercially available kelp products, applied as either soil drench or foliar application and differing in either the kelp species extracted or the method of extraction or both. This experiment was to have been the centrepiece of the broccoli field experiments, but no statistically significant response to any of the kelp extract treatments was found. While this is an important result in itself, it should be interpreted as providing a lack of evidence of an effect of kelp rather than evidence of a lack of an effect of kelp. Several "side-experiments" were established in the same season on the same site, and these provided further important information, and further refined conditions under which kelp had no significant response effect and others in which a response to kelp was found.

The second and third experiments (Experiment 2 (Section 2.2) and Experiment 3 (Section 2.3)) were designed to explore interactions between applied kelp extracts and macro-nutrients. In Experiment 2, an extract from the kelp species *Ascophyllum nodosum* was applied to broccoli in conjunction with varying levels of phosphorus (P). While the response to P was significant, no interaction between the kelp and P was detected. In Experiment 3, two commercially available kelp products, extracted from *A. nodosum* by different means, and two forms of nitrogen (N) fertiliser were

studied to observe potential interactions between kelp and N on the production of broccoli. No response to either kelp extract was detected.

In Experiment 4 (Section 2.4), kelp extract was compared with commercially produced alginate as a soil treatment prior to planting broccoli seedlings. Alginates are extracted from the cell wall of brown algae. They have been shown to have properties consistent with being beneficial to soil structure and this field study was undertaken to determine the importance of kelp extract and/or alginate on the development of broccoli growing in soil susceptible to slaking. Although established in the same season as Experiments 1-3, this experiment was established approximately one month later, when ambient air temperatures were much cooler. Furthermore, a higher rate of P than for Experiments 1 and 3 was adopted because of observations from Experiment 2 where there was early response of broccoli plants to higher rates of P. In contrast to Experiments 1-3, significant treatment effects were found for Experiment 4, with both the kelp extract and the alginate positively stimulating broccoli production. These results, and the results of the other experiments are summarised at the conclusion of Chapter 2 (Section 2.5).

Chapter 3 describes a glasshouse pot experiment with broccoli, designed to follow on from Experiment 2 in the field. In this experiment, kelp was applied with a range of P fertilizer at a range of rates. Variation within treatments in the field was high and appeared to be associated with localised waterlogging experienced across the field experiments. It was felt that more uniformity and hence less within-treatment variation would be experienced in a glasshouse pot experiment. In the glasshouse, variation in growth parameters of broccoli within treatments was found to be very low when compared with variation in the field, but plant responses were very different to those observed in the field. There was no perceived response to P. Consideration is given as to what

might be done differently in order for a repeat of the glasshouse experiment to resemble the field more closely. Pot size and temperatures were considered important.

Chapter 4 describes a glasshouse experiment investigating the efficacy of the five commercially available kelp extractions on tomato production. There is much evidence that tomato plants respond to foliar and soil kelp applications but I have found no comparisons between the activity of these products on tomatoes in the literature. While some significant treatment effects were found, this experiment was compromised by the limitations imposed by the Covid-19 epidemic. It had been intended to look for interaction between kelp and mycorrhizal fungi, but this aspect of the experiment was omitted owing to inaccessibility to facilities when required. The kelp comparison experiment was terminated prematurely, due to the uncertainty that the pandemic was causing. Tomatoes were harvested at early fruiting and yields recorded. Results for all five of the kelp extracts gave a higher tomato yield at harvest than the untreated plants, but only the results for Kelp D (fermented *Ascophyllum nodosum* extract) were statistically significant. The mechanism for the plant response to kelp extract treatment was considered to be very different to that for the broccoli experiment.

Chapter 5 provides overall project conclusions based on the results from the field experiments with broccoli and the results from the glasshouse experiment with tomatoes. I have speculated as to the mechanisms for the plant responses to kelp where they have occurred. The response of broccoli to treatment in Experiment 4 is consistent with the activity of the alginate component of kelp in the soil, while the observed response of tomatoes in the greenhouse is consistent with stimulation of plant hormone production by the kelp extract. These two experiments with statistically significant responses to kelp application thus appear to have been the result of very different response

mechanisms. The results of these experiments highlight the need for further research to identify what triggers such responses.

After the conclusion, there are four brief appendices. The first was a simple experiment with wheat treated with a kelp extract at different rates. No benefits of the kelp applications were found in this experiment. Appendices 2 and 3 describe two experiments designed to study the effects of each of the five commercial kelps on seed germination and seedling emergence. The kelp applications were found to be of no benefit in these experiments. The fourth appendix describes the initial stages of a metabolomic study of broccoli curds retained from Experiment 4. There are (anecdotal) claims of improved food quality from kelp treatments and this second experiment was an ambitious exploratory study hoping to find evidence of differences in molecular composition between curds of treated and untreated broccoli. The work was suspended due to the constraints of Covid-19.

The outcome and implications of this work, including suggested future research, are discussed in the conclusion.

Preparations for Journal submission and conference attendance

Anderson GD, Smernik RJ, Cavagnaro TR (in review). No Help from Kelp, While Phosphorus Promotes Early Maturity of Broccoli on a Slaking Soil. Currently being prepared for submission to the Journal of Horticultural Science and Biotechnology.

Anderson GD, Smernik RJ, Cavagnaro TR (in review). Alginate alone explains kelp stimulus to broccoli growing in a slaking soil. Currently being prepared for submission to the Journal of Horticultural Science and Biotechnology.

Anderson GD, Smernik RJ, Cavagnaro TR (2021) Co-application of Seaweed extract and phosphate fertiliser to broccoli in the field: the biggest benefit is P application reducing time to maturity, In: "Soils: investing in our future". Proceedings of the 2021 Joint SSA and NZSSS National Soil Science Conference, Cairns, Qld, 27 June to 2 July 2021.

Chapter 1

Literature review:

Responses of terrestrial plants to the application of extracts of brown algae

Brown algae off the coast of King Is.

1.0 Literature review

Responses of terrestrial plants to the application of extracts of brown algae

1.1 Introduction

Seaweeds have many uses. They are harvested throughout the world as a food source, for a range of industrial and pharmaceutical applications and as fertilisers or plant biostimulants. Seaweeds are natural, edible, biodegradable, non-calorific, and have no GMO issues. The global production of seaweed biomass for soil and plant application is in excess of 550,000 tonnes per year (Arioli et al, 2015). This amounts to less than 1% of the overall seaweed industry (Craigie, 2011). Blunden (1991) stated "there is a sufficient body of information available to show that the use of seaweed extracts is beneficial, even though the reasons for the benefits are not fully understood". The more that we learn about utilising seaweeds, the greater the potential to use seaweed extracts to improve upon traditional plant husbandry techniques (Anderson, 2009; Crouch and van Staden, 1993).

Seaweeds have been used in agriculture and horticulture for a very long time, particularly in cold coastal regions in the northern hemisphere. Their use dates back to pre-Roman times. Records from the coastal regions of Iceland, Norway, Great Britain and France show kelp (brown algae) and seagrasses have long been used as soil conditioners for cropping and supplements for stock fodder (Guiry, 1989). With the introduction of commercial seaweed products in the twentieth century, the use of seaweed biostimulants was no longer restricted to coastal regions and has become accessible to the broad farming community. In the early 1900s, seaweeds were sold as dried pulverised meal and applied as soil conditioner (Milton, 1952). Commercial seaweed concentrate

preparations for agriculture were first introduced in 1949. A concentrate from the brown algae, *Ascophyllum nodosum,* was produced in the United Kingdom by a British biochemist Dr Reginald Milton. His extraction method was based upon a hot pressurised alkaline process that he patented, and which subsequently formed the basis of the process that he employed for extracting his product, named Maxicrop®. Milton realised at this time that the diluted rates of applied extracts contained insufficient levels of plant nutrients to have value as a fertiliser. He suggested that the observed responses to applied kelp extracts were due to the cell wall polysaccharides of the algae. He believed the responses were due largely to the strongly polar alginates and fucoidan, present in the brown algae, improving crumb structure and aeration of the soils, and that this stimulated the soil microorganisms and plant root systems, which ultimately improved plant growth (Craigie, 2011).

Scientific research into the use of kelp biostimulants has identified potential active components of the concentrates (Blunden, 1991), but evidence of how these components act to produce the documented beneficial results is inconclusive. Extensive research into the use of "seaweed fertilisers" was conducted in the United States, at the Clemson University, by Dr T L Senn and his team between 1959 and 1975 (Senn, 1987). Valuable research has been conducted over more than four decades from the mid-1970s in the United Kingdom by Dr Gerald Blunden and his team at the University of Portsmouth.

A wide range of beneficial effects have been reported from the use of liquid seaweed extracts in virtually all cropping situations. Recorded responses include increased crop yields, improved nutrient uptake, increased resistance to insect, fungal and nematode attack, increased shelf life of produce, and increased resistance to salinity and frost (Senn et al, 1961; Abetz, 1980; Blunden et al,

1992; Verkleij, 1992). Blunden (1977) highlighted increased resistance to some pests such as red spider mite and aphids and reductions in fruit losses during storage. Improved fruit quality, and fruit longevity are common observations for a range of crops treated with liquid kelp extracts, including peaches**,** vegetable crops and soybeans (Fryer, 1982; Blunden, 1991).

Seaweed extracts are now widely accepted in horticulture and viticulture, but the evidence of the impact of seaweed extracts continues to be inconclusive. Arioli et al (2015) state that the basis for the benefits of seaweed biostimulants is complex and poorly understood, and that the benefits of seaweed extracts have not been extensively reviewed in the context of Australia. Evidenced based data are essential for future Australian agriculture to develop effective strategies for the use of liquid seaweed extracts.

1.2 Brown algae

All commercially available seaweed conditioners or biostimulants for agriculture have been extracted from brown algae, commonly known as kelp. Brown algae grow abundantly worldwide in cold-current oceans and there are numerous species that have been used in agriculture (Blunden, 1991). These species are also harvested for industrial and medical uses, primarily the extraction of phycocolloids, including alginates and fucoidan (McHugh, 2003).

Marine algae are classified according to their pigmentation into brown (Phaeophyta), red (Rodophyta), and green (Chlorophyta) algae (Chan et al, 2006). It has been estimated that there are about 2,000 species of Phaeophyta (Hurd et al, 2014). The main species used in agriculture are *A. nodosum*, *Ecklonia maxima*, *Saragassum* spp. (Khan et al, 2009) and *Durvillaea potatorum*. In

Australia, Bull Kelp (*D. potatorum*) from Tasmania is used for the production of some commercially available products (Kelp Industries P/L *http://www.kelpind.com.au,* 2019). Imported *A. nodosum* is also utilised for locally produced products (Arioli et al, 2015). *Ecklonia maxima* is used for the production of the South African product, Kelpak66®. Numerous products are produced from the brown kelps *A. nodosum, Fucus serratus* and various *Laminaria* spp., all found in the northern hemisphere. Products derived from *A. nodosum* are the most researched (Ugarte et al, 2006).

Ascophyllum spp. are found in cold waters on the intertidal shores of Atlantic Canada and northern Europe. They grow in the eulittoral zone, the lower tidal region, forming distinct bands of dark brown, branched individuals 1-4 metres long. *A. nodosum* prefers somewhat sheltered areas and disappears where there is strong wave action. *Durvillaea* spp. are found only in the southern hemisphere, and grow best in rough water, near the top of the sublittoral zone, below the littoral zone and thus permanently covered in water, on rocky shores and offshore reefs. The algae grow best where the temperature does not rise above 15°C. Plants of 5 metres in length are not uncommon, but 2-3 metres is more usual. *Ecklonia* spp. are found in both northern and southern hemispheres, in warm temperate waters, usually on rocky substrates of the upper sublittoral zone (McHugh, 2003).

Understanding the physiology and evolution of brown algae is important in identifying how the algal extracts can provide a benefit to terrestrial plants. The polysaccharides synthesised by brown algae, and the algal defence mechanisms are thought to have a bearing upon how the algal extracts interact with plants. Brown algae are the largest and most complex of the algae. Species colour varies from dark brown to olive green, depending upon the proportion of brown pigment (fucoxanthin) to green pigment (chlorophyll). Sizes vary from small filamentous epiphytes

(*Ectocarpus* spp.) to complex giant kelps such as *Laminaria*. Species of the genera *Fucus* and *Ascophyllum* attach to rocky shores via a “holdfast”, while *Sargassum* spp. float freely in the ocean. Freshwater species are rare. Communities of large brown seaweed have been referred to as “forests of the sea”. They are primary producers providing habitat and food to other protoctista, marine mammals and microbes. These forests are responsible for a significant proportion of the world’s CO_2 reduction via photosynthesis (Hurd et al, 2014). The most complex multicellular brown algae have specialised tissues and organs that resemble those in plants. However, morphological and DNA evidence indicates that the similarities evolved independently in the algae and plant lineages (Campbell et al, 2008). Brown algae are simpler than plants, and lack the many distinct organs found in land plants. They are non-vascular and are *not* classified as plants. The algal body is referred to as the thallus, but unlike the body of a plant, a thallus lacks true roots, stems and leaves. The thallus consists of a root like holdfast which anchors the alga to rocks, and a stem like stipe which supports leaf like blades. The blades provide most of the alga’s photosynthetic surface. The stipes of brown algae may be as long as 60 metres (Hurd et al, 2014).

Phaeophyta have a complex evolution. They have aspects which make them distinct from animals and fungi and they have aspects which make them distinguishable from red and green algae and plants. Algae constitute a polyphyletic group (Nabors, 2004) since they do not include a common ancestor. The evolution is analogous not homologous. For example, although their plastids seem to have a single origin, from cyanobacteria, (Keeling, 2004), they were acquired in different ways. Green algae have primary chloroplasts derived from endosymbiotic cyanobacteria, which are often wrongly referred to as "blue-green algae" (Nabors, 2004). Brown algae have secondary chloroplasts derived from an endosymbiotic red alga (Palmer et al, 2004).

Brown algae have evolved a unique cell wall which differs from other algae and terrestrial plants, because it is not only composed of cellulose, but of anionic polysaccharides (alginates) and often the sulphated oligosaccharide fucoidan. Brown algae are the only eukaryotes that produce alginate. McHugh (2003) states that this cell wall structure in brown algae has evolved because they must survive tidal movements and ocean currents. Acquisition of the alginate pathway allowed kelps to develop large multicellular structures, with flexible cell walls. Consequently, brown seaweeds that grow in more turbulent conditions usually have a higher alginate content than those from calmer waters. The walls also contain high concentrations of phlorotannins, which act as defence substances against herbivores, micro-organisms and ultraviolet radiation.

The alginate pathway is unique to Phaeophyta and two bacterial genera, *Pseudomonas* and *Azotobacter* (Hay et al, 2010). Michel et al (2010) present data that shows a complex evolutionary history for the main components of brown algal cell walls. Cellulose synthesis was inherited from the red alga endosymbiont, whereas the terminal steps for alginate biosynthesis were acquired by horizontal gene transfer from an Actinobacterium. This horizontal gene transfer event also contributed genes for hemicellulose biosynthesis. In contrast, the production of sulfated fucans has evolved via an ancestral pathway, lost from terrestrial plants, but conserved with animals (Michel et al, 2010).

Although green terrestrial plants are much more complex than brown algae, they share similarities. In her review, Linda Graham cites ultrastructural, biochemical and molecular data to support the concept that all land plants (embryophytes) are monophyletically derived from a single common ancestral form related to the green algal class Charophyceae (Graham, 1996), and have evolved in parallel to brown algae. Adaption to the terrestrial environment resulted in many changes,

including cell wall structure. Less available sulphur resulted in loss of sulfated fucans in the plant matrix polysaccharides of the cell wall. All multicellular marine algae feature sulfated polysaccharides as major cell wall components. Ulvans and sulfated galactans feature in green algae (Lahaye and Robic, 2007; Farias et al, 2008), sulfated galactans are present in red algae and sulfated fucans occur in brown algae (Kloareg and Quatrano, 1988). Land based plants developed lignins and stronger cellulose fibres.

The defence mechanisms of brown algae are similar to defence mechanisms in terrestrial plants and animals with regard to responses to microorganisms. This commonality may be relevant to the biostimulatory effects of kelp extracts on plants. Animals and vascular plants are known to defend themselves against pathogens with innate receptors mediating their resistance. Defence compounds produced by brown kelp include phlorotannins which affect flavour of the kelp and oxidants which act on bacteria threatening the algae (Weinberger, 2007).

Microorganisms form biofilms on the surfaces of macroalgae and the macroalgae interact with these organisms. Küpper et al (2002) demonstrated the response of algae in the presence of potential pathogens on the surface of macroalgae. The researchers used specific enzyme inhibitors to block the defence of the algae against these microorganisms and the algae subsequently decomposed rapidly. Many of the microorganisms that are associated with apparently healthy macroalgae have the enzymatic capacity to disintegrate tissues of their host. Vascular plants and metazoans typically respond to predators with reactive oxygen species such as superoxide ions, hydrogen peroxide or hydroxyl radical.

Alginate is the functional analogue of agar and pectin in kelp and oligomeric degradation products of alginate have been shown to elicit an oxidative burst in kelp sporophytes (Küpper et al, 2001). The development of the alginate pathway led to a wealth of new molecular combinations available to Phaeophyta, as well as a basis for recognising “non-self”, following pathogen attack.

Thomas et al (2014) studied brown algae and their systemic defence responses relating to evolution. They found that the kelp *Laminaria digitata* elicited a systemic reaction including an oxidative response with increasing haloperoxydase activities and a stronger resistance against herbivores. Based on experiments with pharmacological inhibitors, the liberation of free fatty acids is proposed to play a key role in systemic signalling, reminiscent of what is known to occur in land plants. Macro-algae also produce oxylipids which are derived from C18 and C20 fatty acids and several studies indicate regulatory roles for at least some of these compounds in algal defence (Potin et al, 2002; Pohnert, 2004).

Flöthe et al (2014) studied the dynamics of inducible anti-herbivore traits in brown algae in response to grazing by the isopod *Idotea baltica*. Genes involved in lipid and carbohydrate metabolism, which decrease palatability, were found to be stimulated in response. At the same time photosynthesis was observed to be down regulated. This suggests herbivore induced re-allocation of resources. In another study, α-amylase from mollusc saliva perceived by *A. nodosum* caused the production of phlorotannins.

1.3 Kelp extraction

There are numerous processes used to convert brown algae to an algal extract. Extracts can be made by processes using water under high pressure, alkali or acid hydrolysis, alcohols, microwaves, CO_2, or by physically disrupting the seaweed through milling at low temperature to give a micronized suspension of fine particles (Hervé and Rouillier, 1977; Chatzissavvidis and Therios, 2014; Hervé and Percehais, 1983; Stirk and van Staden, 2006; Arioli et al, 2015). Kelp extract products vary between 10 and 20% solids suspended in solution.

Some concentrates are prepared by an aqueous alkali extraction technique (Arioli et al, 2015). Other company confidential processes include fermentation to disrupt the cell wall (Anderson, 2009). Another employs a cell burst process where milled kelp particles are passed from a high-pressure chamber to a low-pressure chamber to cause the cell walls to burst and thus resulting in a liquid concentrate (Stirk and van Staden, 1997).

The most widely used process involves heating the seaweed with alkaline sodium or potassium solutions. Anything that does not dissolve is removed by filtration. An aqueous alkali extraction technique results in a greater breakdown of the main alginate mass than dissolving in water (Blunden et al, 1992). However, the use of alkali to liquefy kelp components can generate a variety of compounds which are not present in the parent kelp (Craigie, 2011). Niemela and Sjostrom (1985) identified and quantified 8 to 10 mono-carboxylic acids which composed 10 to 14% of the starting mass of alginic acid. Lactic, formic and acetic acids were the principal mono-carboxylic acids formed (Craigie, 2011). Dicarboxylic acids, including various isomeric saccharinic, pentaric and tetaric acids with lesser amounts of malic, succinic and oxalic acids accounted for 17 - 42% of the

initial alginic acid mass with the higher concentrations produced with higher alkali concentration. Dilute alkali can convert 27% to 56% of purified alginic acid into a variety of products, some of which are known plant metabolites.

The nature and quantities of these reaction products depends upon the composition and chemical structure of the polymers originally in the seaweed as well as the processing conditions used. It therefore follows that the various commercial products are not equivalent in composition. The commercial seaweed extracts would thus be expected to exhibit differences in biological activity when applied to agricultural crops (Craigie, 2011).

The commercial extracts contain an array of compounds. Some are natural metabolites produced by the kelp, while others result from chemical processing but may still be biologically active either positively or negatively. Extracts rich in auxins can be produced by alkaline extraction under low pressure (Booth, 1969; Crampon et al, 2011). Fucoidan can be extracted by microwave assisted extraction (MAE) combined with water extraction under high pressure. Cytokinins can be extracted using chilled 70% ethanol, while extraction in 85% methanol leads to extracts rich in gibberellins.

Lotze and Hoffman (2016) suggest that the efficiency of the cell bursting pressure differential and centrifuge method as applied for Kelpak 66® production (Stirk and van Staden, 1997) can increase the concentrations of specific components such as alginate. This supports the findings of Craigie (2011). A product with a significantly higher alginic acid level may perform better than products with lower alginic acid levels when applied to soil.

Uchida and MiYoshi (2013) have reviewed algal fermentation. The focus of their review was the fermentation of brown algae with lactic acid bacteria with the possibility of obtaining products such as food diets and fertilizers from the algae. *Lactobacillus* species employed for alcohol fermentation include *Lactobacillus brevis, Lactobacillus casei* and *Lactobacillus plantarum*. The major components of brown algal tissue, alginate and fucoidan, are known to be unfavourable substrates for fermentation. However, seaweed can be used as a substrate for lactic acid and ethanol fermentation if the algal tissue is saccharified with a cellulase enzyme. Fermentations of the algal cell wall have typically involved lactic acid bacteria with or without yeast strains. Yeast strains alone have yielded unsatisfactory results. Salt concentration is important to control contaminant bacteria which will otherwise grow and spoil cultures.

The alkaline extraction process broadly involves the heating of the seaweed with alkaline sodium or potassium solutions. The reaction temperature may be elevated by pressurising the vessel as in the high-pressure process developed for Maxicrop®. Alternatively, the seaweed may be liquefied at ambient pressure as in the case of Acadian Sea Plant® extract. All such extracts are intensely coloured due to the high content of polyphenols and/or phlorotannin. The final product may either be dried or prepared in various liquid formats generally in the pH 7 to 10 range (Craigie, 2011).

Isolation of intact (polymeric) alginate requires gentler extraction conditions to avoid hydrolysis of the bonds linking the monomeric units (Draget 2009). In the algae, alginate occurs as insoluble entities crossed linked with multivalent ions found in sea water. The first step in alginate manufacturing is therefore to lower the pH with mineral acids to well below the pKa values of uronates in order to convert the alginate to alginic acid and release the cross-linking ions. Following extensive washing, the algae particle suspension is neutralised with an alkali such as sodium

carbonate in order to extract the water-soluble sodium alginate. This is concentrated by precipitation with other acid or calcium ions. Production of alginate is a very freshwater demanding process.

1.4 Molecules proposed to be responsible for the activity of algal biostimulants

There have been many modes of action of algal biostimulants proposed by researchers over the past eighty years. The efficacy of kelp as a biostimulant is likely to be due to many of the molecules in its composition. Proposed mechanisms for plant responses include mineral fertilisation, soil "conditioning" from the cell wall polysaccharides of the brown algae, response to plant hormones contained within the algae and plant defence responses induced by the cell wall polysaccharides. Several studies, including McHugh (2003), Blunden (1977) and Blunden and Gordon (1986) have concluded that the quantity of minerals applied to plants via seaweed extracts forms an insignificant proportion of the total requirements. The concentration of nutrients that these extracts contain is very small compared to normal plant requirements. Calvo et al (2014) has observed that the seaweed extracts are active as biostimulants at low concentrations being diluted at 1:12000 or more, suggesting that the effects observed are unlikely to be associated with a direct nutritional function. For any molecule to have an effect on agricultural production and fruit quality, it must be active at a very low concentration.

Many researchers have shown that brown algae contain plant hormones, and that the activity of the algal biostimulants is consistent with the activity of plant hormones. Another school of thought

is that the plant responses are more closely aligned to the influences of the polysaccharides, particularly the alginates (Michalak et al, 2017). Yusuf et al (2012) highlighted the complexities of seaweed extracts that make it difficult to ascribe the plant responses to a single growth stimulant.

Plant hormones

Research shows that in some cases plant responses to kelp application are consistent with stimulation by plant hormones, and that the kelp extract includes adequate concentrations of these compounds to elicit such a response.

Plant hormones, also known as phytohormones, are chemicals produced by plants that regulate their growth, development, reproductive processes, longevity and even death (Dilworth et al, 2017). Phytohormones, including abscisic acid, auxins, cytokinins, gibberellic acid and betaines, are also found in brown algae, where they have similar functions to those in plants (Blunden, 1991; Tarakhovskava et al, 2007). A range of plant growth hormones had been identified within marine algae prior to the development of commercial kelp extracts (Bradley, 1991).

Cytokinins

Cytokinin activity has been identified in kelp extracts, and some research shows plant responses to kelp extracts consistent with cytokinin stimulation. Cytokinins are a class of phytohormones that promote cell division, shoot and root morphogenesis, chloroplast maturation, cell enlargement, auxiliary bud release and senescence (Tarakhovskaya et al, 2007). Cytokinins are usually formed in the roots and then travel across the xylem to other parts of the plant such as fruits, seeds, and young leaves (Campbell et al, 2008).

Brain et al (1973) and Williams et al (1981) reported cytokinin-like activity in commercial kelp extracts using bioassay techniques. Considerable variation in activity was identified between different kelp products and within batches of the same product. Despite the variability, the activity was considered to be sufficient at the applied rates of the extracts to influence the physiology of the target plants (Williams et al, 1981). Blunden and co-workers compared a commercial kelp extract with kinetin, a synthetic cytokinin, and found significant yield increases with both the kinetin and the kelp extract for one of several potato varieties (Blunden and Wildgoose, 1977). Featonby-Smith and van Staden (1983) found tomato plants treated with a South African extract from *E. maxima*, produced a significant increase in root growth and a significant reduction in root knot nematode infestation. Finnie and van Staden (1985) found treatment with this same extract significantly increased the growth of *in vitro* cultured tomato roots. The effects were reproduced using 10^{-6} M of zeatin (a cytokinin). Indole-3-acetic acid (IAA), gibberellic acid and abscisic acid had no stimulatory effect. Featonby-Smith and van Staden (1987) reported that the beneficial effects of the kelp extract on peanuts (*Arachis hypgaea*) could be reproduced using the synthetic cytokinin benzyladenine. They identified several cytokinins, including *cis*- and *trans*-zeatin riboside, *trans*-zeatin, dihydrozeatin and N-adenosine, in Kelpak 66® using high performance liquid chromatography.

Cytokinins present in an extract from *D. potatorum* were isolated and quantified by Tay et al (1985) using gas chromatography/mass spectrometry stable isotope dilution. However, in this case they concluded that the levels of cytokinins were not sufficient to produce the beneficial effects reported.

Betaines

Blunden and Gordon (1986) found discrepancies in the importance of cytokinins from kelp extracts in the contribution to plant production. Wheeler (1973) had shown that glycinebetaine had similar activity to cytokinins. Blunden et al (1984) had hypothesised the existence of compounds that mimic cytokinins, and Blunden and Gordon (1986) suggested that quaternary ammonium compounds known as betaines may explain the conflicting results. Blunden et al (1985) found aminobutyric acid betaine, aminovaleric acid and laminine in *A. nodosum* before and after the extraction process. Betaine was not lost during extraction. Betaines are important in chlorophyll-retention, controlling plant cell osmosis, frost resistance and protecting the plant against environmental stress and are described as protective cytoplasmic osmolytes. They are usually associated with plants able to withstand extremes in temperature, salinity or osmotic imbalance (Rathinasabapathi et al, 1994).

Robinson and Jones (1986) demonstrated that glycinebetaine accumulated in the chloroplasts of salt stressed spinach, acting as a cytoplasmic solute. Grumet and Hanson (1986) reported similar findings for stressed barley. McDonnell and Wyn Jones (1988) reported accumulation of glycinebetaine in salt stressed wheat, and in unstressed wheat during leaf expansion. Glycinebetaine has been shown to play an important role in frost resistance of potatoes (Blunden et al, 1996). Whapham et al (1993) demonstrated that increases in chlorophyll levels of tomato leaves and cucumber cotyledons using an alkaline extract from *A. nodosum* could be replicated using betaines, which delay the degradation of chlorophyll. A positive effect of seaweed extract application on chlorophyll content has been suggested by several reports. For example, application of a low concentration of *A. nodosum* extract to soil or on foliage of tomatoes produced leaves with higher chlorophyll content than those of untreated controls. This increase in chlorophyll content

was a result of a reduction in chlorophyll degradation, which might be caused in part by betaines in the seaweed extract. Quaternary ammonium molecules, such as betaines and proline, that buffer against major osmotic changes, have also been reported by Wani et al (2013) and Karabudak et al (2014). These osmo-protectants have an important role in plant stress and importantly have been observed to accumulate during increased stress tolerance (Calvo et al, 2014). Craigie (2011) also reports that betaines have been reported in several brown algae.

Auxins

Auxins are a class of phytohormones that act on the plant cell wall to promote leaf elongation, phloem differentiation, apical dominance, tropisms and initiation of root formation (Tarakhovskaya et al, 2007). These phytohormones have been identified within many groups of marine algae (Bradley, 1991). Indole-3-acetic acid (IAA) was identified within a product extracted from *A. nodosum* (Kingman and Moore, 1982). Sanderson et al (1987) quantified the concentration of IAA in this product using GC-MS.

Jeannin et al (1991) showed seaweed products promote root growth and development. The stimulatory effect was more pronounced when applied at an early growth stage in maize, and the response was consistent with that caused by an auxin. Crouch et al (1990) claim kelp extracts improve nutrient uptake by roots, and in the following year, Crouch and van Staden (1991) hypothesised that the root promoting quality of extract from *E. maxima* could be due to a range of auxins identified in the product. Crouch and van Staden (1992) showed that this extract reduced transplant shock in tomatoes by increasing root size and vigour. Biddington and Dearman (1983) and Finnie and van Staden (1985) also showed that root growth promoting activity was observed when kelp extracts were applied either to the roots or as a foliar spray. It is common practice in

horticulture to apply auxins exogenously to enhance rooting in cuttings. Crouch and van Staden (1993) observed that treating the cuttings of some flowering plants with the *E. maxima* extract elicited a similar response.

Gibberellins, abscisic acid and ethylene

Gibberellins, abscisic acid and ethylene have been isolated from numerous marine algae (Blunden and Wildgoose, 1977; Kingman and Moore, 1982; van den Driessche et al, 1988). Furthermore, gibberellin activity has been detected in several commercial kelp extracts (Williams et al, 1981; Boyer and Dougherty, 1988). Nelson and van Staden (1985) detected the precursor of ethylene, but not ethylene, in Kelpak 66®. Gibberellins are a class of phytohormones that promote stem elongation and initiation of seed germination. Abscisic acid controls the function of the stomata, inhibits growth and controls seed dormancy. Ethylene induces senescence and initiates defence responses (Tarakhovskaya et al, 2007).

Carbohydrates

The cell walls of brown algae include polymers in common with terrestrial plants, polymers in common with terrestrial animals, and polymers unique to Fucaceae. Like plants, brown algae contain cellulose, like animals the algae contain sulphated fucans, which have been lost to plants, but brown algae also synthesise alginates and other polymers not found in terrestrial plants (Connan et al, 2006; Khan et al, 2009). The focus of recent reviews and research (Craigie, 2011; Calvo et al, 2014) has been upon the carbohydrate content of kelp, specifically the polysaccharides of the cell wall. When Maxicrop® was first developed, Milton assumed that the cell wall

polysaccharides acted as a soil conditioner, improving the soil environment for plant growth (Craigie, 2011). Stephenson (1968) reported on the value of kelp extract as a chelating agent. Algal cell wall polysaccharides are rich in functional groups capable of binding micronutrient ions in a reversible process (Tuhy et al, 2015).

Khan et al (2009) reviewed the chemical components of seaweed known to affect plant growth, including the polysaccharides laminaran, fucoidan and alginate. Laminaran is a glucan and has been shown to stimulate natural defence responses in plants and is also involved in the induction of genes encoding various pathogenesis related proteins with antimicrobial properties (Fritig et al, 1998). Fucoidans consist primarily of sulfated fucose and have biological activities in mammalian systems. Alginate is a block copolymer unique to brown algae.

Alginic acid is an unbranched glycuronan composed of mannuronic acid (M) and guluroronic acids (G) in blocks along the polysaccharide chain (Stiger-Pouvreau et al, 2016). Differences in M/G ratio and block configuration account for differences in alginate properties and functionality, especially in gelling capability and gel strength. The M/G ratio varies with the species of kelp and with the environment. The carboxyl groups within the M and G units are easily ion-exchanged and react with cations. This results in changes in alginate properties and functionality, allowing many commercial applications. Divalent calcium ions crosslink the alginate polymers, while monovalent ions such as sodium cannot. Draget (2009) showed that alginate is the most abundant polysaccharide in brown algae comprising up to 40% of the dry matter. The alginate is located in the intracellular matrix as a gel, containing sodium, calcium, magnesium, strontium and barium ions (Haug, 1964), forming a structural component of the cell walls. This accumulation of alginate gives flexibility to seaweed and allows the seaweed to withstand tidal forces (McHugh, 2003).

Some researchers (Craigie, 2011; Calvo et al, 2014) question earlier assumptions that plant responses to kelp extract treatments are due to plant hormones and related low molecular weight organic compounds produced by the kelp. There is evidence that the larger molecules, the oligomers and polysaccharide elicitors in the extracts, can be biologically potent, and should be investigated further. Research suggests that larger molecules including unique polysaccharides and polyphenols may also be important as biostimulants, as allelochemicals and for enhancing resistance to stress (Stadnik and de Freitas, 2014; Klarzynski et al, 2000; Zhang et al, 2006; Rioux et al, 2007; González et al, 2013). Kelp extracts are being increasingly used in agriculture to induce plant resistance to abiotic and biotic stresses (Craigie, 2011). Evidence suggests that many algal polysaccharides can be beneficial to both plants and animals, by stimulating host defence mechanisms (Sharma et al, 2012; Craigie, 2011; Mercier et al, 2001; Subramanian et al, 2011; Vera et al, 2012). There is speculation that oligo-alginates and oligo-carageenans may interact with a plasma membrane receptor involved in signal transduction leading to simultaneous activation of plant growth and defence against pathogens (Kemmerling et al, 2011).

The gelling and chelating abilities of these polysaccharides, coupled with their hydrophilic properties, make these compounds important in food processing and in the agricultural and pharmaceutical industries (Cardozo et al, 2007). Milton (1962) was of the opinion that alginates and fucoidans from the kelp extract Maxicrop® were important in the efficacy of the product because of their beneficial effect on soil structure.

Extraction of alginate from algal material begins with treating the material with mineral acid, then bringing the alginic acid into solution by neutralisation with alkali such as sodium carbonate or

sodium hydroxide to form the water-soluble sodium alginate. It should be noted that this process may produce molecules not existing in the cell wall.

Other compounds of potential interest

Other compounds of potential interest have been identified in kelp extracts. Blunden et al (1985) reported aminobutyric acid, betaine, aminovaleric acid and laminine in *A. nodosum* before and after the extraction process. Laminine has been identified in Natrakelp® (Blunden and Duthie, unpublished data). Chatzissavvidis and Therios (2014) discuss kahydrin, a derivative of vitamin K1, enhancing nutrient uptake by the roots by influencing the efficiency of proton pumps. Exogenous application of vitamin K1 induced the secretion of H^+ ions into the apoplast and consequent acidification of the rhizosphere (Spinelli et al, 2009), which helped in the reduction of Fe^{3+} to soluble Fe^{2+}, and thus it became available to the plant. The polyamines putrescine (Put) and spermine (Spm) have been quantified in Kelpak 66®. Polyamines accumulate in plants in response to stress (Sudha and Ravishankar, 2002) and they may lessen the stress on plants brought on by nutrient deficiency. The increase in polyamine (e.g., Put) concentration might be a physiological adaptation to ionic stress for plants (Young and Galston, 1984).

Arioli et al (2015) discuss other molecules typically found in plants which are not characterized but might also contribute to the efficacy of various seaweed extracts. Genomic and cell biology bioassay studies have uncovered hundreds of plant genes that respond when plants are treated with kelp extract (Rayirath et al, 2009; Khan et al, 2011; Nair et al, 2012; Jannin et al, 2013). The mechanisms of action of these complex kelp products are only now being studied through the use

of techniques developed for molecular biology, metabolomics, and genomics (Craigie, 2011). The application of molecular genetics to investigate the responses of an intact organism to kelp extract permits the identification of specific genes or suites of genes that may regulate the organism. Organisms where the genome has been mapped are valuable biological tools being used with seaweed extract to investigate biological responses. As genomes of plants are completely, or nearly completely sequenced, it will be possible to look at the whole genome transcript of a plant to better understand the actions of seaweed biostimulants on plants (Khan et al, 2009). Jannin et al (2013) used microarray analysis to assess the effect of a brown algae extract on the expression of 31,500 genes in *Brassica nappus*. About 1000 known genes were differentially expressed and grouped into nine clusters representing major metabolic functions of plants. Of these the most affected by algal extract application were those involved in photosynthesis, cell metabolism, nitrogen metabolism, sulphur metabolism and responses to stress.

1.5 Reported Benefits of Kelp Biostimulants

As stated earlier, there are many reports describing the beneficial effects from the application of seaweed concentrates either as a foliar spray or as a soil drench.

Fruit quality

Blunden (1972) found that the enhanced chlorophyll development and stability combined with delayed fruit senescence, as observed in the Malpas Road trial (Anderson, 2009), resulted in increased sugar and carbohydrate levels in the crops that he studied. Norrie et al (2002) demonstrated improved yields and fruit quality in several replicated commercial field trials conducted on seedless table grapes. Treatments were applied at pre-bloom, post-bloom and sizing

stages. Treatments were in addition to a standard fertility management programme. Their results indicated a consistent increase in berry size (from 6.1 to 8.6%), weight (from 3.2 to 29%) and firmness (from 8.6 to 27.1%) for table grapes.

Khan et al (2009) reported on the effect of kelp biostimulants on flowering. For example, tomato seedlings treated with seaweed set more flowers earlier than control plants (Crouch and van Staden 1992). They found that seaweed extract increased the size of tomato plants during the vegetative stage, producing larger size fruits with superior quality.

Root stimulation

There are also many reports of beneficial effects of algae products on root growth and development. Algal biostimulants, in general, are capable of affecting root development by both improving lateral root formation (Atzmon and van Staden, 1994; Vernieri et al, 2005) and increasing total volume of the root system (Thompson, 2004; Slavik, 2005; Mancuso et al, 2006). Rayorath et al (2008) reported that the application of extract from *A. nodosum* at very low concentrations increases in *Arabidopsis thaliana* root length (up to 32%), while seaweed concentrate prepared from *E. maxima* was found to enhance root growth of tomato plants (Crouch and van Staden, 1992). Applications of kelp concentrate reduced transplant shock in seedlings of cabbage and tomato by increasing root size and vigour (Aldworth and van Staden, 1987; Crouch and van Staden, 1992). A simple autoclaved extract of the brown kelp *Rosenvigea intricata* plus fertilizer stimulated lateral root development, increased pigment content and the number of leaves and fruits of okra (*Abelmoschus esculentus*) plants (Thirumaran et al, 2009). Wheat plants treated with seaweed concentrate Kelpak 66® exhibited an increase in root/shoot dry mass ratio, indicating that the

components in the seaweed had a considerable effect on root development (Nelson and van Staden, 1986).

Environmental stress

Plants treated with kelp extracts can exhibit enhanced salt and freezing tolerance (Mancuso et al, 2006). Commercial formulations of *Ascophyllum* extracts improved freezing tolerance in grapes. Grapevines sprayed with Seasol® showed a reduction in leaf osmotic potential, a key indicator of osmotic tolerance (Wilson, 2001). Burchett et al (1998) found that Maxicrop® improved winter hardiness and frost resistance in winter barley. Khan et al (2009) claim that the beneficial anti-stress effects of seaweed biostimulants may be related to cytokinin activity. However, it has also been found that Kelpak 66® seems to mediate stress tolerance by enhancing potassium uptake (Khan et al, 2009). The anti-stress effects could also be partly elicited by bioactive chemicals other than cytokinin (Beckett and van Staden, 1989).

Seaweed extracts have been shown to enhance plant defence against pests and diseases in the soil (Allen et al, 2001; Khan et al, 2009). Besides influencing the physiology and metabolism of plants, seaweed biostimulants promote plant health by affecting the rhizosphere microbial community. Khan et al (2009) discuss research where plants treated with seaweed extracts had a reduction in nematode infestation. Root knot nematode infestation in tomato was reduced in soil treated with commercial kelp extracts from *E. maxima*. Khan et al (2009) suggest seaweed extract may impart nematode resistance by altering the auxin/cytokinin ratio in the plant. Soil application of liquid seaweed extracts to cabbage stimulated the growth in activity of microbes that were antagonistic

to *Pythium ultimum*, a fungal pathogen causing damping off disease of seedlings (Dixon and Walsh, 2004). Seaweeds are a rich source of antioxidant polyphenols with bacterial properties (Zhang et al, 2006). The application of *A. nodosum* extract and humic acid to a grass increased antioxidant enzyme activity which in turn significantly decreased dollar spot disease.

Plants protect themselves against pathogen invasion by the perception of signal molecules (elicitors), such as oligo-polysaccharide and polysaccharide peptides, proteins and lipids that are often found in the cell wall of attacking pathogens (Boller, 1995; Côté et al, 1998). Polysaccharides, such as alginates, laminarins and sulfated fucans, present in brown algal extracts, include effective elicitors of plant defence against plant diseases (Khan et al, 2009). Laminarin has been shown to stimulate natural defence responses in plants and is involved in the induction of genes encoding various pathogenesis–related (PR) proteins with antimicrobial properties (Fritig et al, 1998; van Loon and van Strien, 1999). Specifically, laminarin has been shown to up-regulate the production of phenylalanine ammonia-lyase, caffeic acid O-methyl transferase, lipoxygenase and salicylic acid as defence responses in tobacco, and of antifungal compounds in alfalfa (Craigie, 2011 and references therein).

Arioli et al (2015) discuss the role of strigolactones present in kelp extracts as a plant stress regulator in drought, salinity and nutrient responses and the suppression of plant diseases in numerous situations under Australian conditions.

Biostimulants and soil

Algal polysaccharides are likely to have beneficial effects on soil health. In the Malpas Road trial (Anderson, 2009), differences in shoot length and bunch numbers appeared following soil

treatment with a fermented extract from the kelp *D. potatorum*. The benefits to the vines at this stage may be related to soil health as well as the ability of the vines to take up nutrients from the soil. Khan et al (2009) claim that alginates and fucoidans are responsible for enhancing soil health by improving moisture holding capacity and by promoting the growth of beneficial soil microbes. The gelling and chelating properties of alginates and their hydrophilic nature give them important soil conditioning qualities (Blunden, 1991). When calcium is added to alginate, it forms strong insoluble gels. In the soil, the alginate chains are broken into smaller chains, still forming gels with calcium. Thus, the addition of brown seaweed extracts to the soil would be expected to improve aeration and soil structure.

Arioli et al (2015) discussed the treatment of broccoli (*Brassica oleracea*) with a kelp extract from both *D. potatorum* and *A. nodosum* combined. The extract was added to two contrasting soil types, a clay loam sodosol and a sandy podsol. In the sodosol soil, the extract significantly increased the leaf number, stem diameter and leaf area of the broccoli seedlings. The effect was less on the sandy podsol soil. The study demonstrated that the extract had the capacity to improve establishment of broccoli seedlings without increased nitrogen. In addition, the finding suggested that maybe the differences in cation exchange capacity, organic matter, and leaching properties contributed to the variation in growth response in the different soil types.

Calvo et al (2014) discussed the possibility of indirect root growth stimulation through enhancement of associated soil microorganisms by kelp extract. Root colonization and in vitro hyphal growth of mycorrhizal fungi were improved in the presence of extracts of brown algae (Kuwada et al, 1999). Alam et al (2013) showed that a kelp extract increased microbial diversity and activity in the rhizosphere of strawberry. Khan et al (2013) reported that kelp extract stimulated

alfalfa growth and root nodulation by improving the attachment of a mycorrhizal fungus to root hairs. Enhancement of root growth and nutrient and water uptake efficiency may also increase above ground plant growth and yield as well as resistance to abiotic and biotic stress (Khan et al, 2009).

The cell wall polysaccharides and derived oligosaccharides have also been shown to influence plant growth by enhancing carbon and nitrogen assimilation, basal metabolism, and cell division (González et al, 2013). Ishii et al (2000) observed that alginates from brown algae significantly stimulated hyphal growth and elongation of mycorrhizal fungi and triggered their infectivity on trifoliate orange seedlings. Kuwada et al (1999) showed that brown algal extracts improved root colonization by fungi on trifoliate orange. Numerous other studies show that alginate and other polysaccharides stimulate root growth directly and indirectly in association with microbes (Xu et al, 2003; Khan et al, 2012; González et al, 2013).

It has been long established that alginates are adsorbed by clays and stabilise clay suspensions (Zavorkhina and Ben'kovskii, 1958). Salts of alginic acid combine with the metal ions in the soil to form high molecular weight cross-linked polymers that absorb moisture well, retain soil moisture and improve crumb structure (Khan et al, 2009). In soil, chelated minerals are absorbed more rapidly by plant roots.

Improved soil structure results in better soil aeration and capillary activity of soil pores which in turn boosts soil microbial activities and stimulates the growth of the plant root system (Moore, 2004; Chatzissavvidis and Therios, 2014; Verkleij, 1992; Lattner et al, 2003).

Nutrient uptake

The reported synergy between kelp extracts and cations (Whapham et al, 1993, for example), suggests the observed plant responses to kelp extracts may be due in part to improved nutrient uptake through the chelating capacity of the extracts. A chelating agent facilitates the easy entry of ionic compounds into the plant. Mineral elements can be many times more plant-available in the chelated form. Sufficient quantities of essential nutrients in the soil is only one component of plant nutrition. The nutrients must also be in a form chemically available to the plant. The soil pH, composition, and the presence of other elements can affect the availability of nutrients as well.

In a study on potatoes, when kelp extract was added to soil, the availability of P increased (Eyras et al, 2008). This effect was attributed to the alginic acid sequestering cations, especially Al^{3+} and Fe^{3+}, that precipitate phosphates, increasing the availability of P (Lopez-Mosquera and Pazos, 1997). In line with these results, Papenfus et al (2013) experimented on okra seedlings and found that Kelpak66® was effective in relieving phosphorus (P) and potassium (K) deficiency. Crouch et al (1990) noted that in nutrient element deficient conditions, kelp extracts had no effect on ion uptake of lettuce, but at optimum nutrition levels of K, Mg and Ca, uptake increased notably. Application of seaweed extract on grapevine has been shown to increase N, P, K, Ca and Mg uptake under optimum nutrient element conditions (Turan and Köse, 2004).

It has been suggested that seaweed application to a soil can have effects similar to those of liming, i.e., increased pH, increased exchangeable Ca^{2+}, and reduced exchangeable Al^{3+} (Lopez-Mosquera and Pazos, 1997). Foliar sprays with seaweed extract (*E. maxima*) increased Ca^{2+} uptake by *Brassica oleracea* (Kotze and Joubert, 1980). In contrast, the presence of kahydrin and alginic acid in Actiwave® (*A. nodosum*) was claimed to contribute to a more efficient mobilisation and assimilation

of acid-soluble ions, such as Fe^{3+}, by acidifying the rhizosphere (Spinelli et al, 2009). Working with tomato plants, Eyras et al, (2008) attributed the increased production of treated plants to a combination of higher P availability through slight increases in the soil pH and increases in readily available K through improvement in soil physical conditions.

Experimenting with grapevines, Turan and Köse (2004) found that kelp extracts increased Cu, Fe, Mn and Zn uptake under optimum nutrient element conditions, while under limited nutrient levels in plant growth media, seaweed extracts improved only Cu uptake. In accordance with the above results, foliar applications of kelp extracts (*A. nodosum*) to olive trees increased Cu, Fe, Mn, Zn and B concentration in leaves (Tasioula-Margari et al, 2012).

There is a school of thought that the response from the kelp extract of increased protein levels in plants is due to organic molecules such as organic acids, methionine and polyamines in the extract increasing nutrient absorption in plants (Beckett et al, 1994) rather than the action of growth regulators such as auxins. The extraction process disrupts the cell wall molecules to produce organic acids which are thought to chelate the available nutrients, increasing their absorbance (Papenfus et al, 2013; Chatzissavvidis and Therios, 2014). Oligo-alginates derived from brown seaweed are reported to enhance nitrogen assimilation and basal metabolism in plants (Khan et al, 2011; Sarfaraz et al, 2011).

Protein content in wheat was found to increase by 15.6% and 13.1% when the plants were sprayed with extracts of *Kappaphycus alvarezii* and *Gracilaria edulis* (species of red algae), respectively (Papenfus et al, 2013). Similarly, Singh and Chandel (2005) reported protein content in wheat grain was increased by the application of an *A. nodosum* seaweed extract. They suggested the response

may be because of promotive effects on root proliferation and thus higher uptake of nutrients required in protein synthesis (N, P, and S).

1.6 Conclusions

The bulk of evidence from several decades of research is that both soil and foliar application of kelp extracts can promote plant growth and fruit quality. When applied to fruit, vegetable, cereal and flower crops, kelp extracts have been shown to offer benefits that include higher yields, frost resistance, increased uptake of soil nutrients, promotion of beneficial soil fungi, increased resistance to some pests, improved seed germination, and increased shelf-life of fruit. Numerous mechanisms for the observed plant responses have been proposed. The focus of much of the research has been identifying molecules and pathways responsible but not upon the conditions required for these mechanisms to be activated. Often no mechanism is identified, or is not obvious or is debatable. Not all studies report a positive effect of kelp on plant growth and there is also a potential for positive bias in that studies where no effect is found are less likely to be published. There is a gap between our knowledge of kelp and some of the "hype" that surrounds its adaptation.

Research to date has been focussed on only a few of the many commercially available kelp extracts, but notably these have been derived from several different kelp species using a range of extraction processes. While it has been noted that the different extraction processes result in variation in breakdown products (Craigie, 2011; Stirk et al, 2014), suggesting that the different commercial

extracts should realistically be expected to exhibit differences in biological activity, such differences are rarely explored by the scientific community. In general, individual research groups have focused on developing knowledge of specific products, not upon making comparisons between products.

The benefits of kelp products are focussed upon improving plant productivity. Given the evidence for the alginates in kelp products forming bonds with metal ions in the soil and thus improving soil structure (e.g., Blunden, 1991; Tuhy et al, 2015), these products may also play an important role in soil management. The importance of sustaining and rejuvenating the health of Australian soils is well recognised in the Australian Government National Soils Strategy (DAWE, 2021). The value of kelp products in the renovation of Australian soils should be researched.

For the potential for use of kelp extracts to be realised, there is a need to identify optimum applications and correct situations for their use. There is currently limited accumulated knowledge of kelp research under Australian conditions. Arioli et al (2015) have discussed developments in Australian research since liquid kelp extracts were introduced into Australian agriculture in the 1970s and emphasise the need for extensive research to identify strategies for kelp application in diverse situations.

Australian studies include the work of Mattner et al (2013), which showed that the response of broccoli (*B. oleracea* var. *Cymosa L*.) to soil applied kelp extract (a blend of *A. nodosum* and *D. potatorum*) varied with soil type. Other Australian work shows that kelp applications can benefit yield and quality of wine grapes in Australia (Anderson, 2009; Arioli et al, 2021). Mattner et al (2018) showed that a blend of *A. nodosum* and *D. potatorum* could stimulate root growth and yield of strawberries under Australian conditions.

There is much overseas knowledge of responses in tomatoes treated with kelp extracts (e.g. Crouch and van Staden, 1992; Whapman et al, 1993; Khan et al, 2009). With ABARE statistics showing the value of Australian tomato production to be $560 million in 2021 (Hort Innovation, 2022), there is a need for studies of the use of kelp products with tomatoes. Since this current study commenced in 2019, Hussain et al (2021) have reported an Australian study where tomatoes growing in pots in the glasshouse responded to kelp applications. This work showed responses of total plant increase including yield and quality, as well as an increase in soil microflora.

1.7 Project Aims

The literature review provides a general overview of the scientific knowledge of kelp at the time of commencement of candidature in 2019, and thus a platform from which to launch this research work. A need to acquire a knowledge of the use of kelp under Australian conditions and in Australian soils, has been identified so that effective strategies for the use of liquid seaweed extracts in Australian agriculture can be developed. There is strong evidence that kelp can benefit soil structure, and the management and improvement has been identified as critical to Australian food production going forward.

Because the commercially available seaweed extracts are derived from different sources and different extraction processes, with different breakdown products (Stirk et al, 2014), it is important to compare and understand the situations and conditions in which the different kelp extracts will be effective. There is a need to identify optimum applications and correct situations for the use of each of the commercial seaweed extracts. Defining the requirements for the many mechanisms by which the kelp products stimulate plant development is important in this understanding.

The aim of this work was to compare five commercial kelp products under field and glasshouse conditions and look for differences in biological activity. Two quite different plant species, broccoli (*Brassica oleracea*) and tomato (*Solanum lycopersicum*), were chosen for the study. Broccoli was chosen because it has been shown to respond to kelp application to the soil, and that the level of response can be dependent upon soil type. Tomato was chosen because international research shows that tomato production can be stimulated by kelp application (*A. nodosum* or *E. maxima*) and this is a high value crop in Australian horticulture. Plant responses throughout growth

development were to be studied. In addition to comparison of the kelp products, interactions between kelp extracts and nutrients were to be studied as well as responses of plants to kelp growing within a poorly structured soil.

1.8 References

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Chapter 2

Field experiments with broccoli

Fig 2.0.1 *Broccoli field experiment 1, May 2020. Treatments were applied to individual broccoli plants, with buffer plants in between and on the perimeter.*

2.0 Field experiments with broccoli

2.0.1 Introduction to broccoli field experiments

Kelp extracts have been applied to a range of crops with variable results. Some researchers have concluded they provide no observable benefit (e.g., Miers and Perry, 1986; Edmeades, 2002). Others, while acknowledging a lack of benefit in some situations, take heart in the studies that do report benefits and argue that more research is needed to understand when and why kelp extracts may be beneficial (e.g., Arioli et al, 2015; Abbott et al, 2018). The latter identify brassica crops as being amongst those for which benefits have been reported.

Broccoli (*Brassica oleracea*) is a cruciferous vegetable which is growing in popularity because of its reported health benefits, including lowering cancer risk and boosting the immune system (Sanlier and Guler Saban, 2018). Broccoli is grown commercially in all Australian states (Griffith, 2011). Victoria was the largest producer in 2009, with 50% of national production; production in the other states ranged from 1.5% in South Australia to 20% in Queensland in 2009. In 2021, Australia produced 80,264 tonnes of broccoli valued at AUD258.9 m at the farm gate. South Australia's share was 2%. Production In South Australia occurs between May and January.

Broccoli plants grow best on a well-drained loam, high in organic matter and with a pH 6.5 to 6.8 (ICL Speciality Fertilisers; Ilc-sf.com/uk-en/product-guide/vegetable-grower/broccoli/). They have a high demand for the macronutrients nitrogen (N), phosphorus (P) and potassium (K). They are shallow rooted, so they are sensitive to compacted soils, in which the roots have poor oxygen availability when the soil is wet and in which water penetration is limited. Because broccoli are

shallow rooted it is important that the crop is watered frequently (Hossain and Mohona, 2018). Hilton (2018) cites an approximate figure for water requirement for broccoli of 25 mm per week during the growing season. Henderson (2006) reports that to prevent water stress during plant establishment and head development, the total crop water requirement for broccoli under Australian conditions is between 2.5 and 3 ML ha^{-1} (equivalent to 250-300 mm) per growing season. Broccoli responds to a range of organic amendments (Shapla et al, 2014), as these improve soil structure, aeration, water holding capacity and microbial activity (Pare et al, 2000).

Mattner et al (2013) reported that the application of kelp extract stimulated the establishment and growth of broccoli (*B. oleracea var.* Cymosa L.) under controlled conditions in the glasshouse. In the field it was found that the response varied with soil type. On a clay-loam Sodosol, a soil drench of kelp extract significantly increased the leaf number, stem diameter and leaf area of broccoli seedlings by 6% to 10% at application rates from 2.5 to 25 L ha^{-1}. In a sandy Podosol, only the leaf area of broccoli was increased significantly (by 11%) following treatment with kelp applied and only at the highest rate. The difference in response for the different soils was attributed to differences in the cation exchange capacity, organic matter content and/or leaching properties of the different soils. The clay-loam soil may have a greater capacity to bind the kelp extract, while the sandy soil may not be able to retain the kelp. Response of broccoli to kelp extracts has been reported in open field production internationally. In Poland, Gajc-Wolska et al (2013) found that the application of a kelp extract to broccoli could increase the average weight and quality of broccoli curds. In Iraq, Manea and Abbas (2018) reported applications of seaweed extracts combined with rice residues resulted in increases in plant growth, head yield and quality in broccoli. In contrast, in Canada, Warman and Munro-Warman (1993) conducted field experiments with a range of vegetables, including brassicas, in loamy sand and sandy loam soils and none of the range of seaweed

amendments or kelp extracts tested in the experiment led to significant differences from the control for plant growth or crop yield.

This chapter describes a set of four related experiments conducted in 2020 on adjoining sections of one plot. These field experiments were conducted to investigate several aspects of kelp extract addition on broccoli growth and production under field conditions. In each experiment, broccoli (*B. oleracea* var. prophet) was grown under simulated commercial conditions throughout the winter and spring. The growing conditions were essentially the same for each experiment, other than the kelp extract and nutrient amendments applied and the timing of the experiments. Planting and harvest times were interleaved, but all experiments were carried out in one growing season. Broccoli was chosen as the experimental crop because clear evidence exists for positive response of broccoli to kelp extract (Mattner et al, 2013) and the suitability of broccoli to environmental conditions in the Adelaide region. While each experiment is presented with its own introduction and specific methods, common aspects are described here first.

The first field experiment (Experiment 1, hereafter) was designed to compare the efficacy of five commercially available kelp extracts on broccoli production. The kelp extracts differ in either the species of Phaeophyta used or the method of extraction or both. In the second and third experiments (Experiment 2, and Experiment 3, hereafter), selected commercially available kelp extracts were applied to broccoli in conjunction with varying levels of either phosphorus (Experiment 2) or nitrogen (Experiment 3) to observe whether the kelp interacted with the nutrition of broccoli. In the fourth experiment (Experiment 4, hereafter), kelp extract was compared with commercially produced alginate as a soil treatment prior to planting broccoli seedlings.

The rationale of the first experiment was that if there are differences in the activity of kelp extracts from different biological origins with different means of extraction, this experiment may show differences in broccoli response to treatments. Such a result would help in understanding the kelp activity. Experiment 2, with one kelp and varied levels of P, and Experiment 3, with two kelps and varied levels of N, were introduced because of evidence in the literature that interactions can occur between kelp and macronutrients (Craigie, 2011). There has been some conjecture as to the appropriate rate of P for broccoli production, so for the second experiment, P was studied across a wide range of application rates. The N experiment was established to study kelp interaction with two forms of N (urea and calcium nitrate), because the rate of N uptake varies between N forms. This experiment was established six days after the P experiment. The fourth experiment, the alginate experiment, was conceived because of the poor soil structure and the occurrence of waterlogging across the earlier experiments (see Fig 2.0.3). This field study was undertaken to determine the importance of alginates from the cell wall of brown algae on the development of broccoli growing in soil susceptible to slaking. Experiment 4 was established 29 days after the N experiment, when ambient air temperatures were much cooler. A higher rate of P was adopted in response to observations from Experiments 1 and 2. The broccoli plants in these experiments were visually responding to higher rates of P and there was no evidence of response to kelp. Delayed timing for this experiment meant the growing environment for this experiment differed appreciably from the other experiments.

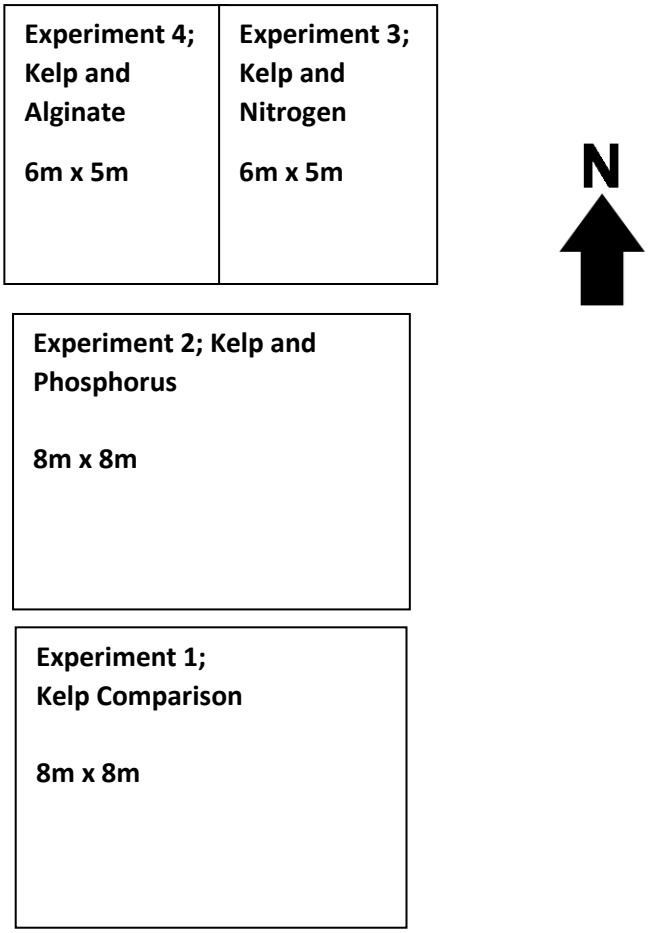

Fig 2.0.2 *Physical layout of the four broccoli experiments on the Waite Campus, University of Adelaide, 2020. Experiment 1 was planted out 5 May 2020; Experiment 2 planted out 7 May 2020; Experiment 3 planted out 11May 2020; Experiment 4 planted out 10 June 2020.*

Fig 2.0.3 *Waterlogging on slaking soil on the Waite campus, University of Adelaide, 2020; an unwanted source of variation. Facing north, the photo shows Experiment 1 in the foreground, then Experiment 2, with Experiment 3 and Experiment 4 yet to be planted. Broccoli growth was restricted where water lay for an extended time most notably in the front centre.*

2.0.2 Methods common to all broccoli field experiments

2.0.2.1 Site location

The experiments were conducted at the Waite Campus of the University of Adelaide, Urrbrae, South Australia (34.97° S, 138.63° E), from 27 April 2020 to 1 October 2020. The climate at this site is typically Mediterranean with a mean annual rainfall of 626 mm, which falls mainly between the austral autumn and spring. The soil at the Waite Campus is a Red Chromosol (Isbell 1996) with a fine sandy loam surface texture. This soil slakes readily, thus resulting in waterlogging when wet (Fig 2.0.3) and hard setting when dry. Soil pH($_{1:5\ in\ water}$) is 5.9 and there is no more than a negligible amount of calcium carbonate present. The soil at this site naturally has inadequate P for optimal growth of a range of crops, and phosphatic fertilisers are essential to maximise yield (Grace et al, 1995). The plot had been sown to faba beans in the previous season (2019). Plant available (Colwell) P analysis of the soil in 2020 prior to treatment showed there was 120 mg kg^{-1} available P, which is assumed to be a legacy of P application for cropping in previous seasons. A typical soil analysis for the site, taken 6th April, 2022, is presented in Table 2.0.1.

Daily rainfall and daily temperature data were obtained from the nearest Bureau of Meteorology observation site, station number 23105 (35.0S, 138.6E), located 3 km away. Rainfall was supplemented with irrigation (Fig 2.0.4) to ensure a minimum average water addition of approximately 25 mm per week (as recommended by Hilton, 2018) in order to provide the regular watering required by shallow rooted brassica plants. Daily maximum temperatures were cool throughout June, July and August, but conditions were unseasonably warm during September (Fig. 2.0.5, Fig. 2.0.6). These conditions accelerated maturation of the broccoli unharvested to this point in time. Broccoli development is influenced by temperature rather than photoperiod (Tan et al,

2000), and Salter et al (1984) found that plants grown during the cooler months have more uniform growth than those grown during warmer weather.

Table 2.0.1. *Soil analysis for the site, Waite Campus of the University of Adelaide, Urrbrae, South Australia. Analysed 6th April, 2022, by Australian Precision Ag Laboratory (APAL).*

Soil analysis WRI	Units	Result
pH 1:5 water	pH units	6.66
pH CaCl2 (following 4A1)	pH units	5.90
Organic Carbon (W&B)	% (40°C)	1.48
MIR - Aus Soil Texture		Silty loam
Nitrate - N (2M KCl)	mg/kg	14.8
Ammonium - N (2M KCl)	mg/kg	5.4
Colwell Phosphorus	mg/kg	109.6
PBI + Col P		55.8
DGT-P	µg/L	438.3
KCl Sulfur (S)	mg/kg	8.70
Calcium (Ca) - AmmAc	mg/kg	1367.5
Magnesium (Mg) - AmmAc	mg/kg	222.35
Potassium (K) - AmmAc	mg/kg	335.6
Sodium (Na) - AmmAc	mg/kg	68.8
Calcium (Ca) - AmmAc	cmol/kg	6.8
Magnesium (Mg) - AmmAc	cmol/kg	1.83
Potassium (K) - AmmAc	cmol/kg	0.86
Sodium (Na) - AmmAc	cmol/kg	0.30
Ca:Mg Ratio		3.77
K:Mg Ratio		0.47
ECR		11.9
Exchangeable acidity	cmol/kg	<0.02
Exchangeable aluminium	cmol/kg	<0.02
Exchangeable hydrogen	cmol/kg	<0.02
ECEC	cmol/kg	9.8
Calcium	%	69.6
Magnesium	%	18.5
Potassium	%	8.7
Sodium	%	3.1
Aluminium	%	0
Hydrogen	%	0
Salinity EC 1:5	dS/m	0.11
Ece	dS/m	1.03
TDS	mg/L	68.73
MIR - Clay	%	14.7
MIR - Sand (+20 micron)	%	49.1
MIR - Silt (2-20 micron)	%	36.2

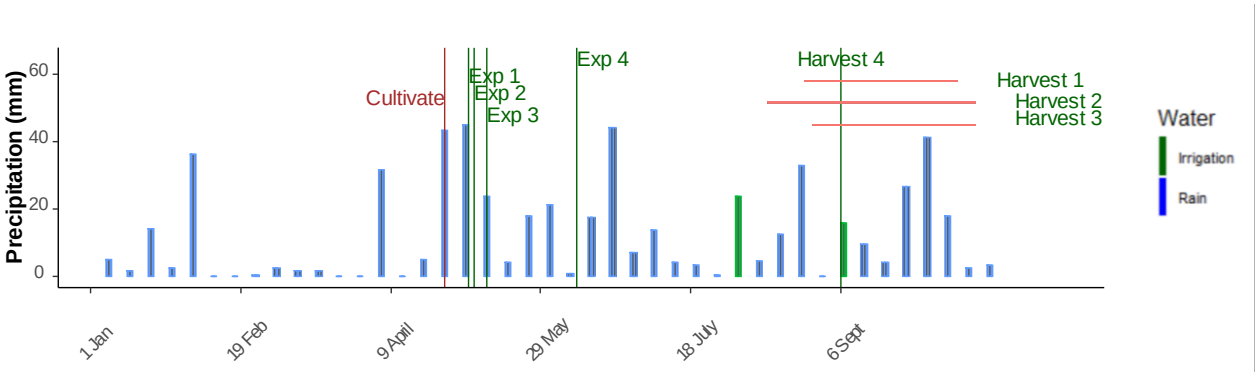

Fig 2.0.4 *Rainfall and supplementary irrigation at the field site. Bureau of Meteorology observation site, station number 23105 (35.0S, 138.6E). Timing of site cultivation, planting of seedlings for individual experiments and harvest dates for Experiments 1, 2, 3 and 4 are indicated. Experiments 1, 2 and 3 harvests were staggered, while harvest for Experiment 4 occurred on 6 September, 2020.*

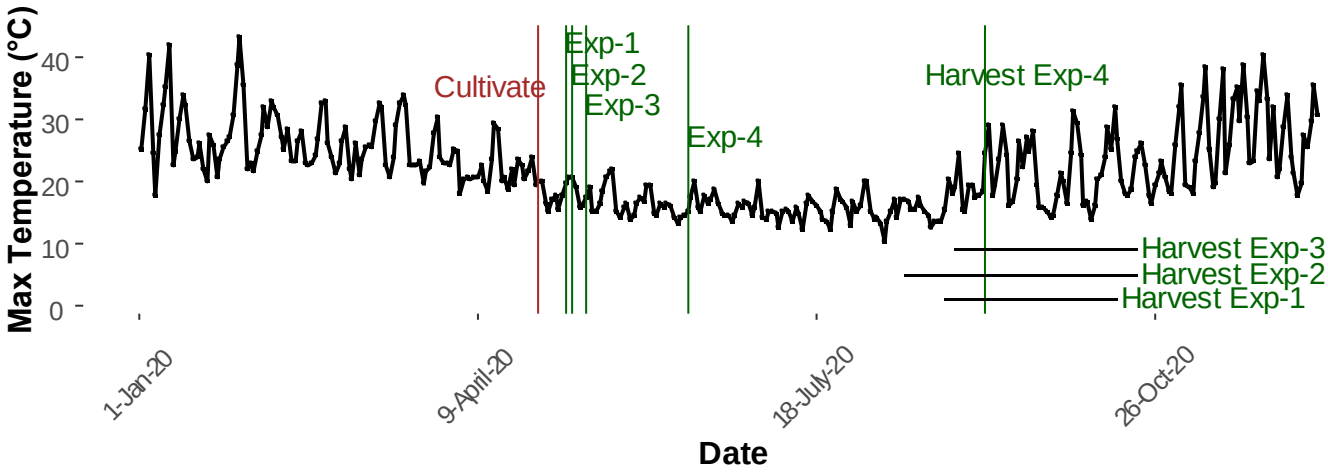

Fig 2.0.5 *Daily maximum temperature at the field site, Bureau of Meteorology observation site, station number 23105 (35.0S, 138.6E). Timing of site cultivation, planting of seedlings for individual experiments and harvest dates for Experiments 1,2,3 and 4 are indicated. Experiments 1, 2 and 3 harvests were staggered, while harvest for Experiment 4 occurred on 6 September, 2020.*

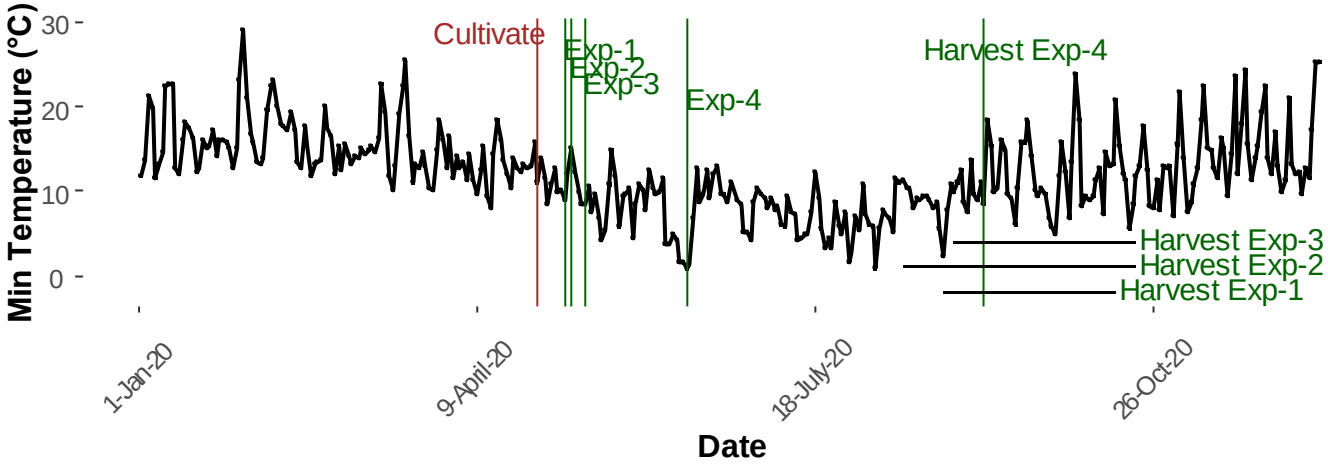

Fig 2.0.6 *Daily minimum temperature at the field site, Bureau of Meteorology observation site, station number 23105 (35.0S, 138.6E). Timing of site cultivation, planting of seedlings for individual experiments and harvest dates for Experiments 1,2,3 and 4 are indicated. Experiments 1, 2 and 3 harvests were staggered, while harvest for Experiment 4 occurred on 6 September, 2020.*

2.0.2.2 Site design

Broccoli seedlings were planted in a randomised block design for each experiment. The site was disc cultivated on 27 April 2020. Broccoli (*Brassica oleracea* cv Prophet) seedlings were obtained from Virginia Nursery, Virginia, SA. Plants were spaced 0.3 m apart in rows 0.5 m apart, in accordance with commercial practice (Hilton, 2018); this corresponds to an effective area of 0.15 m^2 per plant or a planting density of 67,000 broccoli plants per hectare. Seedlings were supplied in trays with individual cells of approximately 2 cm^3 volume. For each seedling, a cylindrical core of soil 10 cm deep by 2 cm diameter was removed from the planting site, to accommodate some of the applications of nutrients and kelp. For example, in Experiment 2 all P applications were added in this way, to accurately target the specific plant. The hole was backfilled with soil as the seedling was planted. Untreated "buffer" plants were alternated within the rows between treated plants to minimise "spill-over" effects. Buffer rows were planted around the perimeter of the plot. Snail bait was applied across the site according to label recommendations on 10 May 2020. A hoe was used for manual weed control. The site was monitored for insect pests, but no control was required throughout the growing period.

2.0.2.3 Commercial Kelp Extracts

Five commercial kelp extracts (not named for confidentiality reasons) were available for testing:

Kelp A. Extracted from the species *E. maxima*, using a cold cell burst extraction process. For vegetables, a fortnightly foliar application rate of 2 L ha^{-1} commencing at the 3 to 4 leaf stage is recommended. There is no indication that higher rates will be detrimental. There was no specific recommendation for soil application at the time of the experiment.

Kelp B. Extracted from the species *A. nodosum*, then dried and granulated. There is no specific recommendation for vegetables. There is a general recommendation for application to plants of 0.3 to 1 kg ha^{-1}, and no indication that higher rates will be detrimental. There was no specific recommendation for soil application at the time of the experiment.

Kelp C. Extracted from the species *A. nodosum*, using a hot caustic extraction process. For vegetables, it is recommended to apply a total of 10 to 18 L ha^{-1} as soil or foliar applications throughout the season. There is no indication that higher rates will be detrimental.

Kelp D. Extracted from the species *A. nodosum*, using a fermentation process. Applications of 6 to 10 L ha^{-1} are recommended. Soil application is recommended at 20 L ha^{-1} . There is no indication that higher rates will be detrimental.

Kelp E. Extracted from a blend of *Durvillaea* species and *A. nodosum*, using a caustic extraction process. Foliar applications of 6 to 10 L ha^{-1} are recommended. There is no indication that higher

rates will be detrimental. There was no specific recommendation for soil application at the time of the experiment.

All five extracts (A – E) were used in Experiment 1, Kelp B in Experiment 2, Kelps C and D in Experiment 3 and Kelp E in Experiment 4.

2.0.2.4 Monitoring plant development

Data were collected for leaf area index (LAI), days to curd emergence (CE), curd diameter (CD) (throughout plant development), days to harvest (HD), fresh plant weight at harvest (FW), fresh curd weight at harvest (FCW) and curd quality.

Leaf Area Index (**LAI).** The leaf area index (LAI) is a dimensionless quantity used in this research to characterise plant canopies. It expresses either the leaf area, or in this case, leaf canopy area, per unit ground or trunk surface area of a plant and is commonly used as an indicator of the growth rate of a plant (Lui and Pattey, 2010). It should be noted that the term LAI has different meanings in different parts of the plant literature. Barclay (1998) identified five different definitions and the one used here corresponds to fifth of these "projected area of inclined leaves, but counting overlapping areas only once".

Treated plants were photographed on 20 June 2020 (Fig 2.0.7) with a Canon 400D digital camera mounted on a steel frame to position the camera 1 m above the soil surface. The area of the leaf canopy was determined using ImageJ, a public domain Java image processing program that measures leaf area in digital images. Leaf Area Index was calculated as area of the plant canopy

divided by area per individual plant (0.15 m^2); at this stage of growth there was no overlap of canopies of adjacent plants.

Fig 2.0.7 *Broccoli seedling after 45 days from planting(Exp 1). Image used in calculation of LAI approximation.*

Curd emergence. Plants were monitored throughout the growing period to register timing of curd emergence, defined as when the curd first appeared from the heart of the plant. The crop was inspected twice weekly (Tuesday and Friday), and the date when the curd was first visible was recorded. Curd emergence was adopted as a non-destructive approximation of curd initiation. Curd initiation is important, because it is the stage in plant development when plant energy is redirected from vegetative development towards reproductive development. It is a time of high nutrient demand by the plant (Poethig, 2013; Thakur et al, 2018; Tan et al, 2000).

Harvest. A protocol for timing of harvest of individual plants was adopted from commercial criteria. Broccoli curds reached their optimum stage of maturity for harvest at different dates, so the decision was made to harvest at the optimal condition as opposed to identical times. There was

little variation in timing in Experiment 4, so all broccoli plants in this experiment were harvested simultaneously. A visual grading system was introduced (Fig 2.0.8). The crop was inspected twice weekly (Tuesday and Friday) and plants were harvested when they reached the criteria. Harvest date was recorded for each plant. Plants were harvested when the curd diameter was 12 cm or greater, unless curd quality deteriorated to C grade before the curd achieved the desired 12 cm diameter. These inferior plants were harvested before the quality deteriorated further. For uniformity, the curd was cut from all plants at 6 cm below the lowest floret; this complies with local criteria for broccoli sold in supermarkets (*pers. comm.*). Fresh weight of the plant and curd was determined at harvest. Curds were dissected in the laboratory, and a portion of each curd dried at 60^{o}C for 72 h to determine curd dry weight.

A grade: Fresh, compact head, tight florets.

B grade: Compact, but with florets emerging from the head.

C grade: More open appearance

D grade: Very open and uneven

Fig 2.0.8 *Visual grading protocol for harvesting of broccoli, a subjective system was devised along commercial lines.*

2.0.2.5 Statistical analyses

All statistical analyses were performed using R statistical software, version 4.0.2 (R Core Team, 2019). Data for parametric tests were analysed using two-way analysis of variance (ANOVA). Pairwise comparisons were made using the Tukey HSD test to compare individual treatments. Curd quality was analysed using the non-parametric Wilcoxin signed rank test.

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2.1 Broccoli Field Experiment 1. Comparing five commercially available kelp products

2.1.1 Introduction

Seaweed biostimulants used in agriculture have all been extracted from brown seaweed species, otherwise known as kelp (*Phaeophyta*). It is estimated that there are about 2,000 species of *Phaeophyta* (Hurd et al, 2014) and only a small number of these are used to produce biostimulants. *Phaeophyta* grow in the shallows of cold-current oceans. *Ascophyllum nodosum*, *Ecklonia maxima*, *Macrocystis pyrifera* and *Durvillea* spp, including *D. potatorum* are the most frequently used in the production of bio-stimulants for plant production (Khan et al, 2009). *Ascophyllum nodosum* is found in cold northern hemisphere waters; *E. maxima* grows off the coast of South Africa and *D. potatorum* is found in shallow, fast flowing waters off Tasmania. There are numerous proprietary extraction processes used to convert brown algae to an algal extract. The most common processes involve alkali hydrolysis at high temperature. Other processes include, but are not limited to, fermentation of the kelp, alcoholic extraction of the kelp, acid hydrolysis under a range of conditions and cell rupturing under low pressure and cold conditions. These processes have been detailed in the literature review (Chapter 1). With such a range of kelp species, growing environments and extraction processes, the chemical composition, and hence the biological activity, of the various commercially available kelp extracts would be expected to vary considerably (Battacharyya et al, 2015).

This experiment was designed to compare the effect of the different commercial kelp extracts on broccoli development. Any observed differences in their activity may shed some

light on the mechanisms for kelp activity on plants. Foliar and soil applications were compared.

2.1.2 Materials and Methods

Broccoli seedlings were planted out on 5 May 2022, 20 days from receiving the seedlings, following the traditional season break. Soil temperatures were not measured but ambient air temperatures during April were generally below 25°C (Fig 2.0.5 and Fig 2.0.6). Broccoli prefer soil temperature between 18 and 24°C (Hilton, 2018).

Site design

The broccoli seedlings were planted in a split plot randomised block design with soil and foliar applied treatments for each of the five kelp products described in Section 2.0.2.3 (Kelps A, B, C, D, and E). There were 12 replications for each treatment. For each seedling, a cylindrical core of soil 10 cm deep by 2 cm diameter was removed from the planting site and all soil applied kelp applications were added into this core to accurately target the specific plant. The hole was backfilled with soil as the broccoli seedling was planted. Phosphorus was surface broadcast in the form of mono-ammonium phosphate (MAP, N 10%: P 22%: K 0%) prior to planting the broccoli seedlings; MAP pellets were applied via a manual hand-held spinner to distribute pellets evenly at a rate of 75 kg ha^{-1} across the site. This is equivalent to 16.5 kg ha^{-1} P or 0.25 g P per plant. In addition to the 7.5 kg ha^{-1} N added as a component of the MAP addition, MAP, N was applied post planting along the mid row furrow between each row as a 50:50 blend of urea and calcium nitrate in solution. A total rate of 140 kg N ha^{-1}, inclusive of addition in MAP, was applied across the site over a period of 8 weeks, as per industry recommendation (Pers. comm. Platinum Ag Services, Virginia).

It was decided to apply all five kelp extracts at the same rate. This rate exceeded label recommendations for all kelps, but there is no indication in the literature or in company recommendations for any of these products that the rate should have a negative impact upon target plants. For the soil treatments, liquid kelp extracts were added as a soil drench at a rate of 20 L ha^{-1} (0.30 ml per plant) and powdered kelp at a rate of 2 kg ha^{-1}. The liquid kelps were applied in a 1% solution. The powdered kelp was applied in a 1 g L^{-1} solution (0.1% solution). Foliar treatments were applied via a hand-held sprayer, initially on a weekly basis for the first five applications and then on a fortnightly basis for the next four applications. The liquid kelps were applied in a 1% solution at a rate of 12.5 L ha^{-1}. The powdered kelp was applied in a 1 g L^{-1} solution (0.1% solution) at a rate of 1.25 kg ha^{-1}.

2.1.3 Results

Responses in plant growth:

Leaf Area Index (LAI) at 45 days from planting of broccoli

There were no significant differences (P>0.05) in LAI at 45 days from planting (Fig 2.1.1), when the broccoli plants were still in the vegetative growth stage. None of the kelp treatments had significantly different leaf area to that of the control.

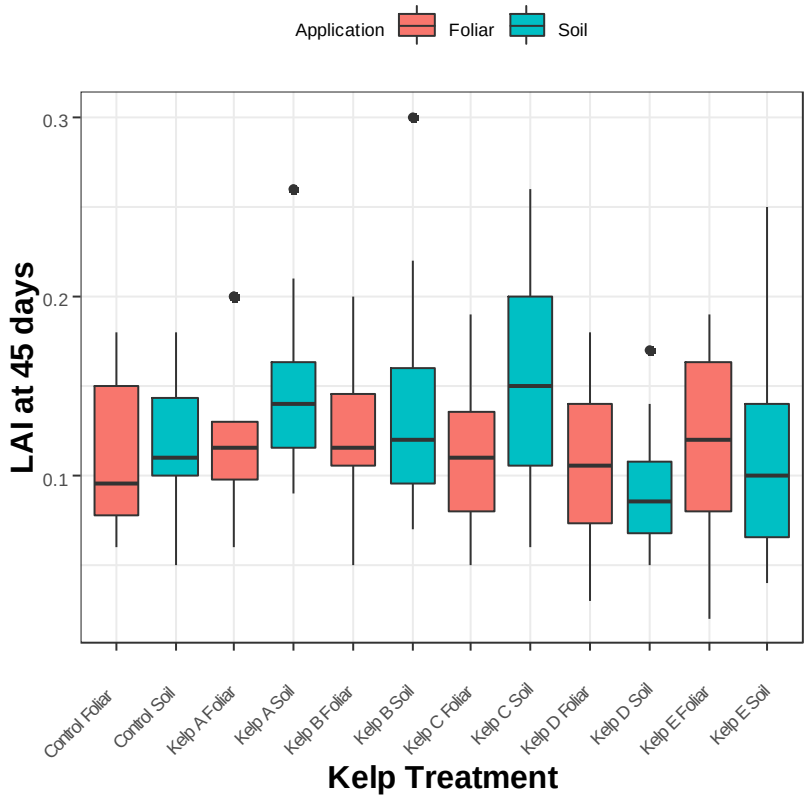

Fig 2.1.1 *Leaf Area Index at 45 days from planting, showing no significant response for any of the kelp treatments above no kelp application, when applied either as a soil drench or as a foliar application. Each box represents the 50% of the data for the treatment (first quartile to third quarter). The line inside the box represents the median. Whiskers represent the 95 percentile.*

Curd diameter at 98 days from planting of broccoli

There were no significant differences in the average curd diameter (P>0.05) among the various treatments of the broccoli plants at 98 days from planting (Fig 2.1.2). Plants were in the early stages of curd development or early reproductive growth and nutrients and plant energy were being directed to seed production.

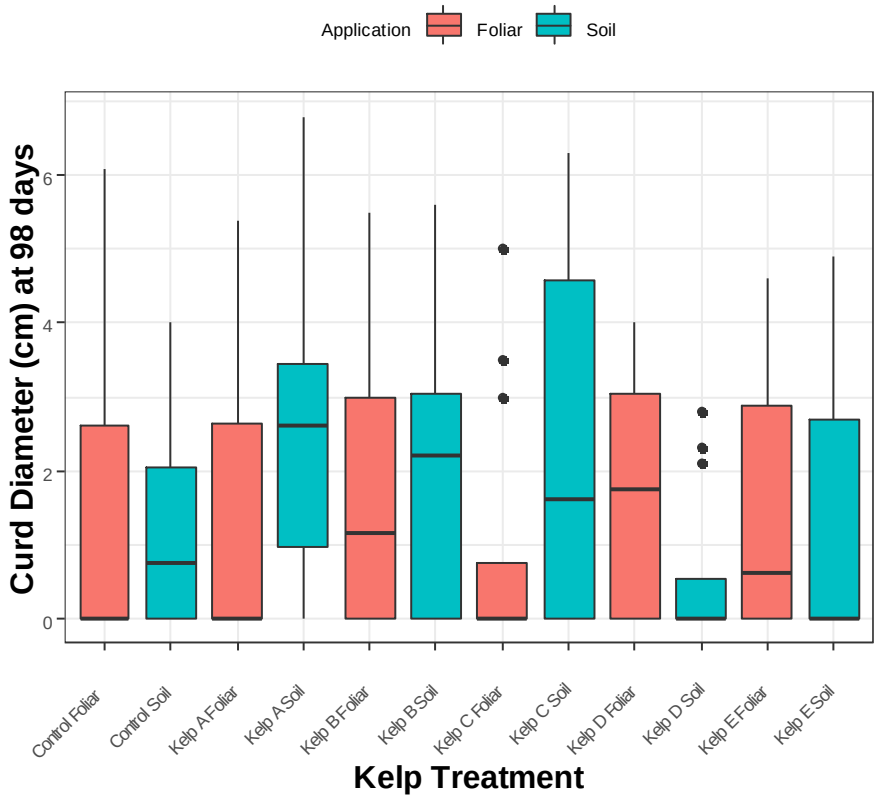

Fig 2.1.2 *Curd diameter at 98 days from planting, showing no significant response for any of the kelp treatments above no kelp application, when applied either as a soil drench or as a foliar application.*

Harvest data:

Days from planting of broccoli seedling until harvest

There was no significant difference (P>0.05) in the number of days from planting to harvest (Fig 2.1.3) of broccoli for any of the kelps when applied either to the soil or by a foliar application.

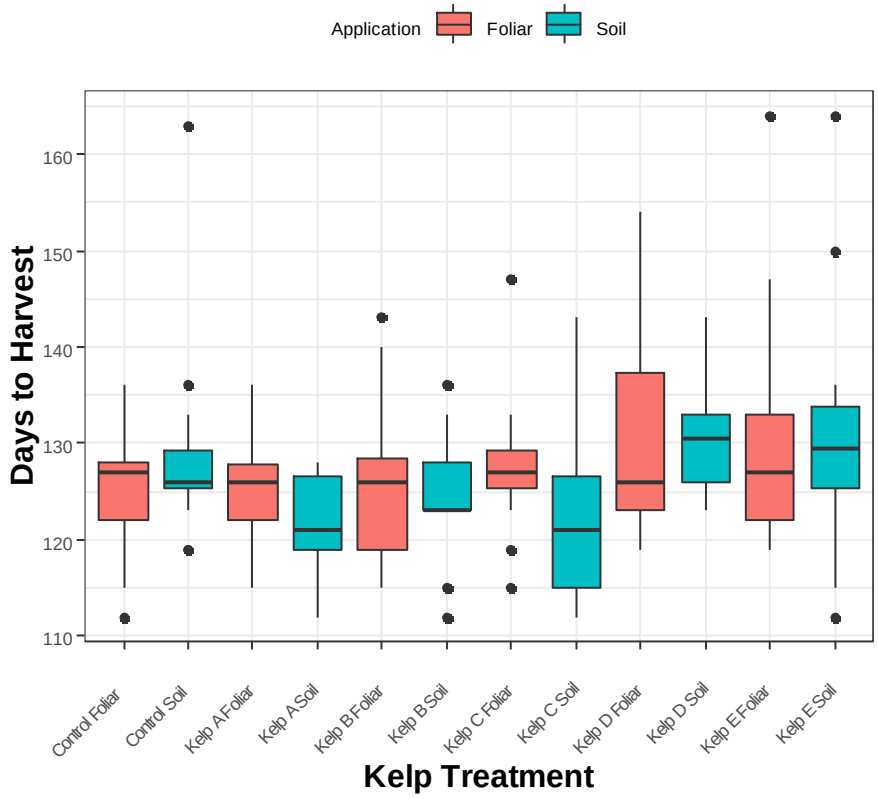

Fig 2.1.3 *Days between broccoli planting and harvest, showing no significant response for any of the kelp treatments above no kelp application, when applied either as a soil drench or as a foliar application.*

Fresh weight of broccoli curd at harvest

Broccoli curd fresh weight at harvest had no significant response (P>0.05) to any of the kelps whether they were applied via the soil or the leaves (Fig 2.1.4). There were no yield benefits for broccoli production for any of the kelp treatments.

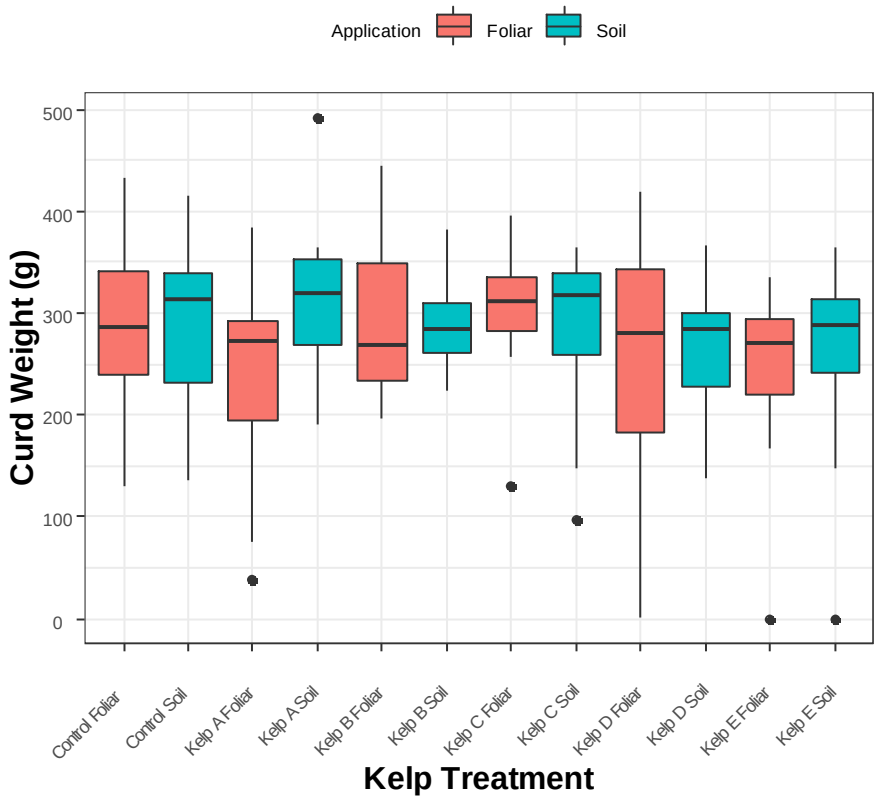

Fig 2.1.4 *Fresh Curd Weight at harvest, showing no significant response for any of the kelp treatments above no kelp application, when applied either as a soil drench or as a foliar application.*

Fresh weight of broccoli plant at harvest

Broccoli plant fresh weight at harvest showed no significant response (P>0.05) to any of the kelps whether they were applied via the soil or the leaves (Fig 2.1.5).

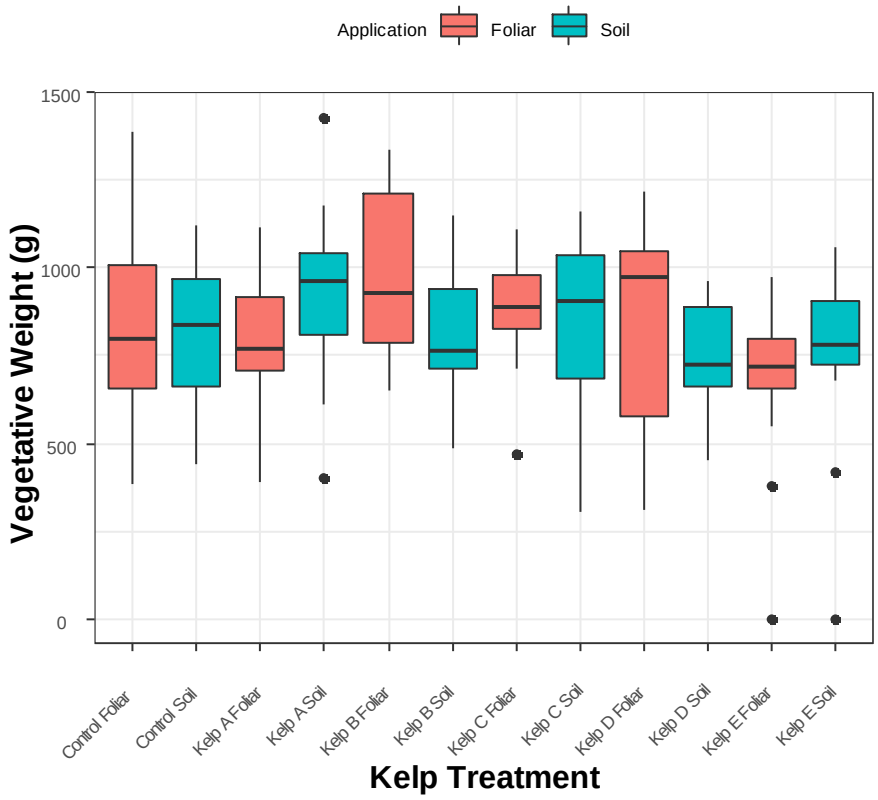

Fig 2.1.5 *Fresh Plant Weight at harvest, showing no significant response for any of the kelp treatments above no kelp application, when applied either as a soil drench or as a foliar application.*

Harvest Index

Harvest Index is the curd weight divided by the fresh plant weight and gives an indication of the resources that the plant allocates to reproductive growth as opposed to vegetative growth. The curd weight to plant weight ratio for broccoli at harvest was not significantly greater ($P<0.05$) for any of the kelps regardless of mode of application (Fig 2.1.6).

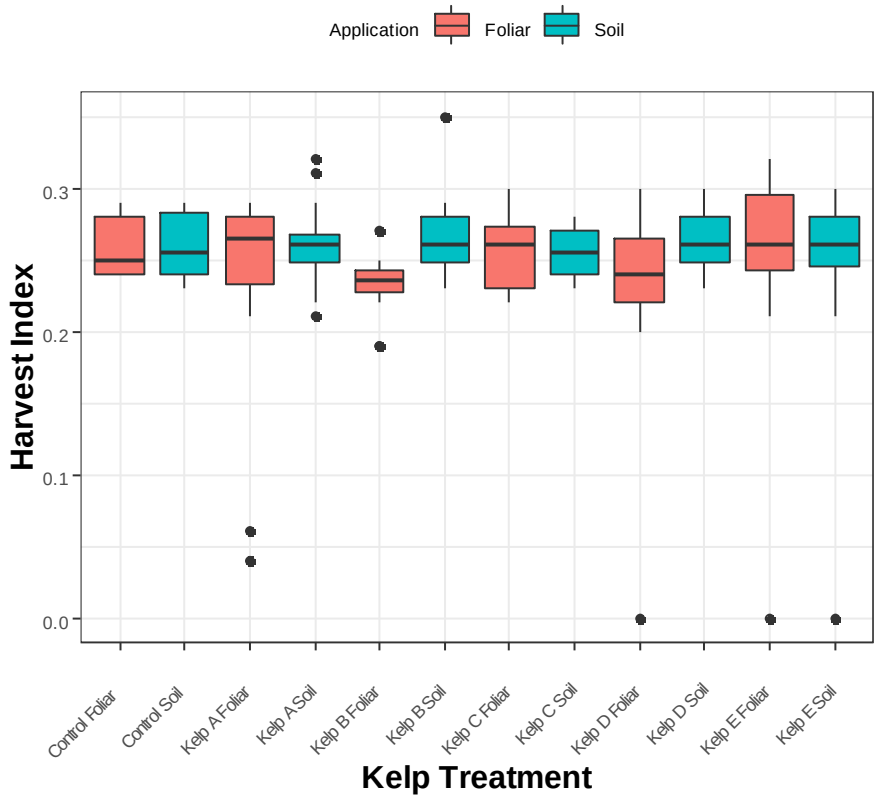

Fig 2.1.6 *Harvest Index (Curd weight/Fresh plant weight), showing no significant response for any of the kelp treatments above no kelp application, when applied either as a soil drench or as a foliar application.*

Curd Quality

According to the non-parametric Wilcoxin signed rank test, no kelp treatment resulted in significant differences (P>0.05) for Curd Quality (Fig 2.1.9). This is not surprising, given that there were no other responses to the kelp treatments. The quality of the curds for each treatment was measured as the percentage of each of the quality grades A to D (described in Fig 2.0.5) for each of the treatments. It is a subjective assessment of the plant's appearance and marketability.

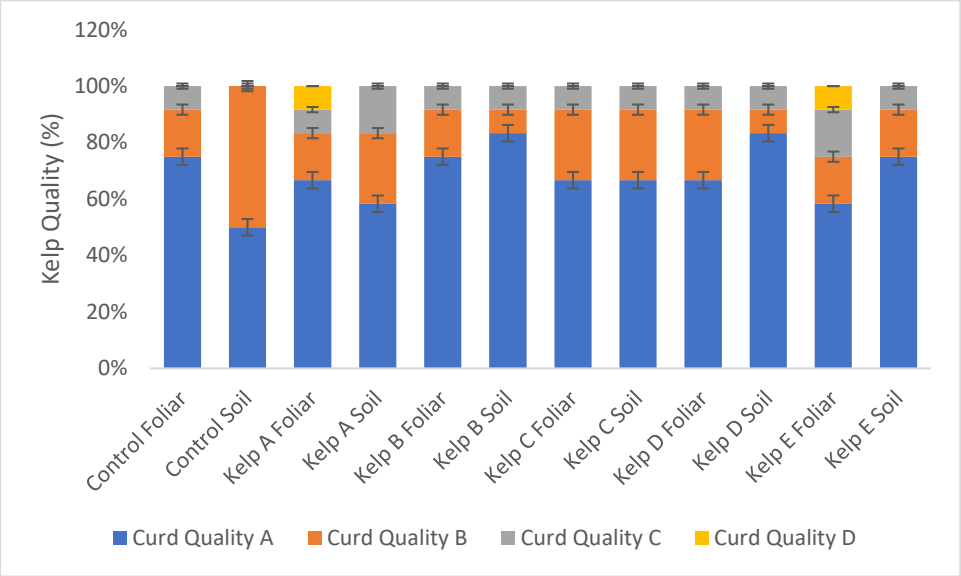

Fig 2.1.7 *Curd Quality at harvest the percentage of plants for each treatment recorded for each of the quality grades A to D described in Fig 2.0.5. Error bar indicates standard error.*

2.1.4 Discussion

Overall, there were no statistically significant responses to the kelp applications in any of the measured parameters (1. Leaf Area Index at 45 days from planting, 2. Curd Diameter at 98 days, 109 days, 114 days and 123 days from planting, 3. Curd Diameter at harvest date, 4. Days between planting and harvest, 5. Fresh Plant Weight at harvest 6. Fresh Curd Weight at harvest and 7. Curd Quality). Furthermore, two-way ANOVAs showed no statistically significant differences between soil and foliar applications.

Responses of broccoli to kelp biostimulants have been reported in the literature (Battacharyya et al, 2015), and it is important to recognise that the results presented here should not be taken as evidence of a lack of an effect of kelp extracts on broccoli growth. Rather they should be seen to demonstrate a lack of evidence of an effect of kelp extracts on broccoli growth under the conditions of the experiment. There are multiple potential reasons why no significant effect was found. The first is that the kelp extracts did not influence the broccoli at all and this possibility cannot be ruled out on the evidence of this experiment. However, it should be noted that across all treatments, broccoli growth was good, as evidenced by an average yield of broccoli curd at harvest of 19.2 tonne ha^{-1}, a very good commercial yield (Hilton, 2018). This suggests there was not a strong environmental limit on broccoli growth that kelp extract addition could rectify. The following sections (2.2 and 2.3) describe experiments designed to impose macronutrient limitations (P and N, respectively) to broccoli growth under otherwise near identical conditions. It should also be noted that variation among replicates within each treatment was relatively high (Figs 2.1.1 – 2.1.7) and this would limit the magnitude of an effect that could be detected. A likely

contributor to this variation, small patches of waterlogging across the experimental site, is discussed below, and an attempt to circumvent this limitation in a pot experiment is described in Chapter 3.

Organic matter reduces slaking by binding mineral particles and by slowing the rate of wetting. Because of the binding properties of alginate in the extract, it was expected that the kelp extracts would have a similar effect. However, even with the generous rates of kelp application, there was no discernible response to the application of any of the commercial kelps at this site. Given the nature of the soil at this site, the expectation was that the kelp extract treatments would result in improved structure of the slaking soil, thus eliciting a response in broccoli production. This soil is known to have low soil aggregate stability because of past agricultural practices and the addition of organic matter can improve this structure. Likewise, the addition of brown seaweed extracts to this soil was expected to bind the soil with cross-linking calcium bonds, hence improving soil structure and aeration.

While soil structure was not analysed at harvest, observations throughout the growing season indicated variability across the site in terms of waterlogging and subsequent hard setting of the soil as it dried. As mentioned above, yield across the experiment was good by industry standards, and it may be that the poor soil structure was not limiting to the broccoli. Perhaps the plant roots had established sufficiently in the warm soil prior to waterlogging and hence the lack of treatment effects would indicate that the control plants were not limited in any way by conditions that could be improved by the application of kelp.

This experiment provides no evidence of a benefit for kelp when nutrients are supplied at a relative high rate; the following two experiments deal with situations where one macronutrient is found to be limiting.

2.1.5 References

Battacharyya D, Babgohari MZ, Rathor P, Prithiviraj B (2015) Seaweed extracts as biostimulants in horticulture. Scientia Horticulturae 196, 39–48.

Hilton S (2018) Improving Processing Vegetable Yields Through Improved Production Practices. https://www.horticulture.com.au › project-reports.

Hurd C, Harrison P, Bischof K, Lobban C (2014) “Seaweed Ecology and Physiology.” (Cambridge University Press, Cambridge).

2.2 Broccoli Field Experiment 2. looking for kelp interaction with phosphate

2.2.1 Preamble

Essential nutrients not only have to be available in sufficient quantities in the soil, but they must also be in a form chemically available to the plant. The soil pH, physical composition, and the presence of other edaphic factors can affect the availability of nutrients as well. Kelp application has been implicated as having a positive effect on plants under different nutrient conditions (Craigie, 2011). There is research that suggests that interactions may occur between kelp extracts and P to improve uptake and availability of P, and a number of potential mechanisms for kelp facilitating this uptake have been proposed (Chatzissavvidis and Therios, 2014). A field experiment was conducted at the Waite Campus of the University of Adelaide, Urrbrae, South Australia, on a soil prone to slaking, to study the effect of kelp extract on the uptake of P by broccoli. Plant-available (Colwell) P analysis of the soil at this site showed 120 mg kg^{-1} available phosphorus (P) before P addition. As discussed below, this would usually be interpreted to be in the “high” range and unlikely to be limiting to the growth of most crops. However, the results presented here clearly show there was a response of broccoli to addition of further P in the form of mono-ammonium phosphate fertiliser.

Results from this study are presented in the following manuscript, prepared for submission to The Journal of Horticultural Science and Biotechnology for publication. The manuscript is reproduced as submitted to the journal. There is some variation from the format adopted for the body of the thesis. The manuscript is a stand-alone document, so there is necessarily some repetition from the body of the thesis.

2.2.2 Statement of Authorship

Title of Paper	No Help from Kelp, While Phosphorus Promotes Early Maturity of Broccoli on a Slaking Soil
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Prepared for submission to the Journal of Horticultural Science and Biotechnology

Principal Author

Name of Principal Author (Candidate)	Graeme Anderson		
Contribution to the Paper	Conception, acquiring data, knowledge, analysis, drafting		
Overall percentage (%)	80		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	1/3/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ronald Smernik		
Contribution to the Paper	knowledge, analysis, drafting		
Signature		Date	1st March, 2023

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Contribution to the Paper	knowledge, analysis, drafting		
Signature		Date	1st March, 2023

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2.2.3 Manuscript

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Funding, Data Availability and Conflict of Interest statement

This work is a component of the studies for Doctor of Philosophy by Graeme Anderson at the University of Adelaide, School of Agriculture, Food and Wine. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. The authors declare no conflicts of interest.

Abstract

The mechanisms for claimed benefits of brown kelp (class *Phaeophycaea*) in horticulture are not well defined. A field experiment was conducted at the Waite Campus of the University of Adelaide, Urrbrae, South Australia, on a soil prone to slaking, to study the effect of kelp extract on the uptake of phosphorus (P) by broccoli (*Brassica oleracea*). Broccoli seedlings were planted in a randomised block design, with P applied at 0, 5.5, 16.5, 33 and 66 kg ha^{-1}, with and without kelp extract, and with 12 replications of each treatment. Data were collected for Leaf Area Index (LAI) at 45 days, days to Curd Emergence (CE), Days to Harvest (HD), Fresh plant Weight at harvest (FW), Fresh Curd Weight at harvest (FCW) and curd quality. There were significant ($P < 0.05$) responses to applied P in plant maturation (LAI, CE and HD) but no significant responses to kelp throughout the development of the broccoli. Applied P improved quality of the broccoli curds and reduced time for emergence and growth to curd harvest. Phosphorus applications of 5.5 kg ha^{-1} and above significantly increased LAI and reduced CE and HD. The increased rate of broccoli curd maturation from increased P commenced early in broccoli development. The dramatic reduction in time to broccoli harvest has important economic benefits. The lack of observed response to kelp application highlights the need for further research.

Key words: Kelp, *Ascophyllum nodosum*, curd, broccoli, phosphorus, slaking soil
Non standard abbreviations: Leaf Area Index for broccoli at 45 days (LAI), Days to curd emergence (CE), Days to harvest (HD), Fresh plant weight at harvest (FW), Fresh curd weight at harvest (FCW)

Introduction

There is widespread interest in the use of extracts of brown kelp (class *Phaeophycaea*) as plant biostimulants in horticulture and viticulture, even though the mechanisms for the actions of these extracts are not fully understood (El Boukhari et al 2020). Kelp extracts are applied to crops either as foliar sprays or as soil applications, and multiple studies have claimed wide ranging beneficial effects, including improved plant health, yield, fruit quality and resistance to pathogens (Blunden et al 1992). The particular beneficial effects of kelp when applied to soils are addressed in the reviews of Calvo et al (2014), Craigie (2011) and Shukla et al (2019), with increased availability of nutrient elements to plants, and increased soil aeration and water holding capacity of the soil identified as possible mechanisms.

Kelp extracts have been applied to a range of crops with variable results. Some researchers have concluded they provide no observable benefit (e.g. Miers and Perry 1986; Edmeades 2002). Others, while acknowledging a lack of benefit in some situations, take heart in the studies that do report benefits and argue that more research is needed to understand when and why kelp extracts may be beneficial (e.g. Arioli et al, 2015; Abbott et al 2018). Broccoli has been identified as a crop for which benefits have been reported in Australia. Mattner et al (2013) showed that broccoli seedlings respond to kelp extracts but the benefit varies with soil type. Application of kelp extract as a drench to a clay-loam soil significantly increased the leaf number, stem diameter and leaf area of broccoli seedlings irrespective of application rate between 2.5 and 25 L ha^{-1}; while for a sandy soil, only the kelp applied at 25 Lha^{-1} significantly increased the leaf area of the broccoli seedlings. The difference in response was attributed to differences in the cation exchange capacity, organic matter and/or leaching properties of the different soils. The clay-loam soil may have a greater capacity to bind the kelp extract, while the sandy soil is unable to retain the kelp.

A number of potential mechanisms for kelp benefits have been proposed. Chatzissavvidis and Therios (2014) suggested that observed plant responses to kelp extracts may be due in part to improved nutrient uptake through the chelating capacity of extract components. A chelating agent facilitates the easy entry of ionic compounds into the plant by conversion to a more chemically available form. Soil pH, composition, and the presence of other elements can also affect the availability of nutrients. There is some evidence that plant responses to kelp are consistent with improved plant uptake of phosphorus (P). In a study with potatoes in which seaweed mulch, rather than extract, was added to the soil, the availability of P increased (Eyras et al, 2008; López-Mosquera and Pazos 1997). This effect was attributed to alginic acid chelating cations, especially Al^{3+} and Fe^{3+}, effectively reducing the concentration of these free ions in solution. Since these cations precipitate phosphate, decreasing their concentration in soil solution can increase the solubility and availability of P (Lopez- Mosquera and Pazos 1997). Papenfus et al (2013) experimented with okra seedlings and found that an extract from the brown kelp *Ecklonia*

maxima was effective in relieving P and potassium (K) deficiency under greenhouse conditions in quartz sand. Crouch et al (1990) noted that under nutrient element deficient conditions, kelp extracts had no effect on ion uptake by lettuce plants, but at optimum nutrition levels of K, Mg and Ca, ion uptake increased notably with kelp application. Application of kelp extract on grapevine also increased N, P, K, Ca and Mg uptake into leaves under optimum nutrient element conditions (Turan and Köse 2004).

Phosphorus is deficient in many of the world's soils, and P is the most limiting nutrient in agricultural settings after N (Kooyman et al 2017, Menzies 2009). Cutliffe et al (1968) demonstrated the importance of P in broccoli yield. They found that for maximum yields, rates of 175 to 250 kg ha^{-1} of N and 100 to 150 kg ha^{-1} of P were necessary. Phosphorus applications were also shown to affect the rate of maturation of field broccoli. Phosphorus application increased the number of mature curds at mid-season by 11% at one site and by 67% at another. This difference was evident soon after transplanting. Islam et al (2010) reported an application of 87 kg ha^{-1} P in a silty loam soil in Bangladesh resulted in broccoli curd initiation 2.3 days earlier than for unfertilized plants.

Here we present the results of a study in which we evaluated the impact of kelp application on the maturation of broccoli grown under commercial conditions on a poorly structured soil, prone to crusting and waterlogging, over a range of P application rates. Specifically, we aimed to assess whether the rate of P application would influence the response of broccoli to the application of a commercial kelp extract. Our hypothesis was that there will be an interaction between the level of applied P and the kelp application on total production and on rate of curd maturation.

Methods

Site location

The experiment was conducted at the Waite Campus of the University of Adelaide, Urrbrae, South Australia (34.97° S, 138.63° E), from 27 April 2020 to 1 October 2020. The climate at this site is typically Mediterranean with a mean annual rainfall of 626 mm, which falls mainly between the Austral Autumn to Spring.

Soil

The soil at the Waite Campus is a Red Chromosol (Isbell 1996) with a fine sandy loam surface texture. This soil slakes readily, thus resulting in water logging when wet and hard setting when dry. Soil $pH_{1:5}$ is 5.9 and there is a negligible amount of calcium carbonate present. Red Chromosols typically have inadequate P, and phosphatic fertilisers are essential to maximise yield (Grace et al 1995). The site had been sown to faba beans in 2019. Plant-available (Colwell) P analysis of the soil in 2020 prior to treatment showed 120 mg kg^{-1} available P, which is assumed to be a legacy of P application to previous crops.

Weather data

Daily rainfall and daily temperature data were obtained from the nearest Bureau of Meteorology observation site, station number 23105 (35.0S, 138.6E), located 3 km away. Rainfall was supplemented with irrigation by sprinkler (Fig 1) to ensure a minimum average water addition of 25 mm per week (as recommended by Hilton, 2018.

Daily maximum temperatures were cool throughout June, July and August, but conditions were unseasonably warm during September (Fig 2). These conditions accelerated maturation of the broccoli unharvested to this point in time. Broccoli development is influenced by temperature rather than photoperiod (Tan et al 2000), and Salter et al (1984) found that plants grown during the cooler months have more uniform growth than those grown during warmer weather.

Site design

Broccoli seedlings were planted in a randomised block design, with five levels of P fertilization, two treatments of kelp extract (no addition, addition), and 12 replications of each treatment. The site was disc cultivated on 27 April 2020. Broccoli (*Brassica oleracea cv Prophet*) seedlings obtained from Virginia Nursery, Virginia, SA, were transplanted on 7 May 2020, when 21 days old. Plants were spaced 0.3 m apart in rows 0.5 m apart, in accordance with commercial practice (Hilton, 2018).

A cylindrical core of soil 10 cm deep by 2 cm diameter was removed from the planting site for each seedling. Solutions of the P source and the kelp extract were added to the hole at the required rate for each treatment. The hole was then backfilled with soil as the seedling was planted.

Phosphorus was applied in the form of mono-ammonium phosphate (MAP, 10:22:0) prior to planting the broccoli seedlings. Application rates were equivalent to 0, 5.5, 16.5, 33 and 66 kg ha^{-1} P. A soluble powder extract of the kelp species *Ascophyllum nodosum* was dissolved in de-ionised water and applied to the soil at a rate of 12.5 kg ha^{-1} prior to planting. Nitrogen (N) was applied uniformly as a side application of a 50:50 blend of urea and calcium nitrate post planting across the site to deliver 140 kg N ha^{-1} to every plant over 8 weeks as per industry recommendation (Pers. com. Platinum Ag Services, Virginia). A hoe was used for manual weed control. The site was monitored for insect pests, but no control was required throughout the growing period.

Monitoring plant growth

Leaf Area Index (LAI)

The leaf area index (LAI) is a dimensionless quantity that characterises plant canopies. It expresses the leaf area per unit ground or trunk surface area of a plant and is commonly used as an indicator of the growth rate of a plant. Treated plants were photographed on 20 June 2020, 45 days after planting, with a Canon 400D digital camera mounted on a steel frame to position the camera 1 m above the soil surface. The area of the leaf canopy was determined using ImageJ, a public domain Java image processing program that measures leaf area in digital images. Leaf Area Index was calculated as area of the plant canopy divided by area per individual plant (0.15 m^2).

Curd emergence

Plants were monitored throughout the growing period to register timing of curd emergence, defined as when the curd first appeared from the heart of the plant. The crop was inspected twice weekly (Tuesday and Friday), and the date when the curd was first visible was recorded. Curd emergence was adopted as a non-destructive approximation of curd initiation.

Harvest

A visual grading system was introduced (Fig 3). Based upon commercial criteria, plants were harvested when the curd diameter was 12 cm or greater, or before curd quality deteriorated to C grade. For uniformity, the curd was cut from all plants at 6 cm below the lowest floret. Harvest date was recorded and Fresh Plant Weight and Curd Weight were determined. Curds were dissected in the lab, and a portion of each curd dried at 60^{o}C for 72 hours to determine Curd Dry Weight. The quantifiable parameters recorded were 1. Leaf Area Index (LAI), 2. Days to Curd Emergence (CE), 3. Days to Harvest (HD), 4. Fresh Plant Weight at harvest (FW), 5. Fresh Curd Weight (FCW) at harvest and 6. Curd Quality. All statistical analyses were performed using R statistical software, version 4.0.2 (R Core Team, 2019). Data for parameters 1 to 5 were analysed using two-way analysis of variance (ANOVA). Pairwise comparisons were made using the Tukey HSD test to make comparisons between treatments. Curd quality was analysed using the non- parametric Wilcoxin signed rank test.

Results

Response of the broccoli crop at various stages of development to applications of kelp and the specified rates of applied P are summarised graphically in Figure 5 and quantitatively in Table 1. Table 1 shows mean, standard deviation (sd) and coefficients of variation (CoV) and distribution groupings for each treatment. There were significant responses in plant maturation ($P < 0.05$) to applied P but no significant responses ($P > 0.05$) to kelp and no significant interaction between kelp and P throughout the development of the broccoli. Applied P improved quality of the broccoli curds and reduced time for emergence and time to curd harvest. Phosphorus deficiency was evident for broccoli plants receiving no or low-level P applications (5.5 kg ha^{-1}), as indicated by the purpling of leaves (Fig 4) due to a build-up of excess carbohydrate (Marschner 1995). The responses to added P for the maturity parameters of broccoli development are strongly interconnected.

Leaf area index at 45 days increased with P application rate up to 33 kg ha^{-1} P (Fig 5a). Two-way ANOVA showed the increases in LAI were significant for P addition ($P < 0.001$), but not statistically significant for kelp addition ($P = 0.16$). There was no interaction between kelp and P ($P = 0.28$) for the rates applied. Table 1 shows the mean LAI was lowest for no added P, for which leaf coverage was <10% of area. For 5.5 and 16.5 kg ha^{-1} P, leaf

coverage was between 10% and 20% of area and for 33 and 66 kg P ha^{-1} leaf coverage was approximately 20% or greater.

Time until curd emergence (CE) decreased with increasing P application rate (Fig 5b). Two-way ANOVA showed the decreases in CE were significant for P addition (P<0.001), but not for kelp addition (P = 0.83) and there was no interaction between kelp and P (P = 0.13) for the rates applied. Table 1 shows P fertilization at 5.5 and 16.5 kg ha^{-1} P resulted a significant reduction in the average number of days to CE relative to no P addition, with a further significant reduction recorded for P fertilization rates of 33 and 66 kg ha^{-1} P. Across the range of P rates, CE decreased by approximately 33 days from 107 days for the unfertilized treatment to 74 days for plants treated with 33 and 66 kg ha^{-1} P.

Time to broccoli harvest (HD), which was carried out when curd diameter was 12 cm or greater, also decreased with increasing P application rate (Fig 5c). Two-way ANOVA showed the decreases in HD were significant for P addition (P = <0.001), but not for kelp addition (P = 0.64). The interaction between kelp and P (P = 0.05) was not statistically significant at the 0.05 level. Tukey HSD analysis showed decreases in HD were significant between 0 and 5.5 kg ha^{-1} P and between 16.5 and 33 kg ha^{-1} P. The HD was reduced by 20 days, from 126 days at 0 kg ha^{-1} P, to 106 days at 33 kg ha^{-1} P.

There was no statistically significant difference in the above ground plant biomass between P treatments (P = 0.80) or kelp treatments (P = 0.08) at the time of harvest (Figure 5d). There was no significant interaction between kelp and P addition (P = 0.42). Similarly, there was no statistically significant difference in the curd weight between P treatments (P = 0.18) or kelp treatments (P = 0.24) at the time of harvest (Figure 5e). There was no significant interaction between kelp and P addition (P = 0.53).

Curd quality

The distribution in curd quality is shown in Figure 6. Most plants were considered 'A' grade, but quality tended to decline with late harvest. The Wilcoxin signed rank test gave a probability of 0.08 that there was no difference in curd quality due to the application of kelp, so that the Null hypothesis could not be rejected at the 0.05 level. The effect of P was highly significant in the improvement of curd quality (P <0.001).

Discussion`

At this site there was a significant effect of P application on the rate of broccoli maturity, the timing of the broccoli harvest, and curd quality, with no significant response to kelp application (P < 0.05), or significant interaction between kelp and P application. Three maturity parameters were assessed, and they are strongly interconnected.

It is important to note that the recorded responses occurred with a pre-treatment soil plant-available (Colwell) P of 120 mg kg^{-1}. This value would not typically be expected to be limiting for many crops (APAL Soil Interpretation Guide). Colwell P is a test that measures the potentially available P in the soil solution and critical Colwell P values are dependent upon soil type. The data on critical P values for broccoli production in the literature are limited. The APAL Soil Interpretation Guide does not provide a value for broccoli growing in a Red Chromosol soil, but suggests that for a cereal crop the critical Colwell P is 18 to 35 mg kg^{-1} P and 65 mg kg^{-1} P for a potato crop in a similarly buffered soil. Blaesing (2018), while not referring specifically to broccoli, states that soils with 70 – 100 mg kg^{-1} P are generally considered adequate for vegetables. From their work in Canada, Cutliffe et al (1968) found that for a soil with initial Total P of 38 mg kg^{-1}, application rates of 100 to 150 kg ha^{-1} of P were necessary for maximum broccoli yields. This implies that the demand of broccoli for P may be greater than that of many other plant species, but could also imply that the plant more readily responds to the P added to the soil than to the P suggested by the Colwell test to be available in the soil.

The overall yield achieved in this experiment was within the range typical of Australian commercial broccoli production. Total broccoli yield, including untreated buffer plants, was 108.2 kg of broccoli curds, equivalent to a yield of 19.2 t ha^{-1}. In Tasmania the average yield for transplanted broccoli crops is 22 t ha^{-1} (Hilton, 2018). Reported Australian mainland broccoli yields are variable, ranging from 7.1 t ha^{-1} in 2009, to 13 t ha^{-1} in 2011-12 (Mulcahy 2016).

The impact of the different rates of P application was evident throughout broccoli development. The first assessment of broccoli growth was taken with the measurement of LAI at 45 days from planting. The average LAI of plants treated with 33 kg P ha^{-1} was 0.23, more than treble that of the untreated plants (0.07), indicating early vegetative growth response to P. The time to curd emergence was significantly reduced by all levels of added P. Curd emergence commenced at an average of 74 days after planting for the 33 kg ha^{-1} P treatment compared to an average of 107 days for the control treatment, i.e. the addition of 33 kg ha^{-1} P reduced the growing period by 33 days. This response in the early maturity of broccoli to added P is much greater than previously recorded for brassicas. Islam et al (2010) reported 2.3 days difference in broccoli curd initiation between a P deficient and a high P regime, on a well tilled alluvial floodplain in Bangladesh.

Recording the time of curd emergence was chosen as a non-destructive means of measuring curd initiation. Two stages prior to CE have been identified in brassicas: a period of vegetative growth leading to leaf initiation, and the curd initiation phase, at which point leaf initiation ceases (Hand and Atherton 1987). This period includes meiosis and floret development. Phosphorus deficiency would be expected to be a limiting factor in CE and development of the florets to harvest. Phosphorus plays a vital role in all plant metabolism that involves energy transfer, including meiosis, as well as all other aspects of plant growth and development (Griffith, 2011). Phosphorus is a key molecular component of genetic reproduction and inadequate levels result in impaired genetic processes such as cell division and plant growth. Hence, P deficient plants mature at a slower rate than plants with adequate amounts of P (Marschner 1995). The results found at this site suggest that the delay in harvest under low P conditions results from retarded development up to the stage of curd emergence.

The reduction in time to CE was partly reflected in the time to broccoli harvest (HD). The addition of 33 kg ha^{-1} P at this site significantly reduced the HD by an average of 23 days. Cutliffe et al (1968) reported harvest delay of only 1 to 2 days when P was deficient, as compared to a high P regime, for broccoli grown in a fertile fine sandy loam on Prince Edward Island, Canada. The high P regime in the current study also showed less variance within the treatments than the low P treatment, indicating a more uniform HD than for no or low addition rates of P. The reduction in HD with added P is less than the reduction in time to CE, suggesting that the later maturing plants grew faster during the warmer September weather (Fig 2). There was also greater uniformity in time to harvest at higher rates of P.

There was no significant difference in the fresh weight (FW) of the broccoli plants at the time of curd harvest. The plants with little or no P treatment developed more slowly prior to CE but FW for treated and untreated plants were similar at harvest. This suggests that the level of P in untreated soil was adequate for broccoli development, but less available than the added soluble P fertiliser. Furthermore, contrary to the findings of Eyras et al (2008) under the conditions at this site, the kelp treatment has not enabled the plant to access this P more quickly.

There are economic benefits of adding P fertiliser at this site. Tan et al (1998) state that the time to harvest is important for farmers in their forward marketing projections. Seasonal conditions and interstate supply impact upon the market price for broccoli in Australia. Uncertainty in time to harvest causes problems in marketing and labour organization (Booij 1987). A shorter growing season reduces input costs, and opportunity costs for the producer.

Influence of kelp

This study was specifically targeting plant production on a poorly structured soil prone to slaking. Broccoli seedlings have been shown to respond to kelp extract (Mattner et al 2013) and there are reports in the literature of kelp extracts being beneficial to soil health. Khan et al (2009) claim that alginates from the kelp cell wall are responsible for enhancing soil health by improving moisture holding capacity and by promoting the growth of beneficial soil microbes. The gelling and chelating properties of alginates and their hydrophilic nature give them important soil conditioning qualities (Blunden, 1991). When calcium is added to alginate, it forms strong insoluble gels. In the soil, the alginate chains are broken into smaller chains, still forming gels with calcium. Thus the addition of brown seaweed extracts to this slaking soil would be expected to improve aeration and soil structure where soil aggregates are unstable. The P deficiency at this site was transient for mid-range (5.5 – 16.5 kg ha^{-1}) P applications, suggesting that more mature plants were eventually able to source less available forms of P from the soil. While the sequestering nature of alginic acid would be expected to release P more quickly (Eyras et al, 2008), there is no evidence of this in the current study. The kelp addition did not significantly benefit any of the parameters that were evaluated.

Kelp applied to this soil did not significantly interact with P uptake to reduce time to curd maturity in broccoli. Even the differences observed for kelp application with added levels of P above 33 kg ha^{-1} P were not statistically significant. The conditions of the soil at this site were very different from those locations where positive responses have been recorded. The results of López-Mosquera and Pazos (1997) were achieved in Spain on a humic Cambisol soil (FAO 1990) with a pH of 5.3. That site had an eight-year history of mulched seaweed additions (average dose 40 t ha^{-1}). In two other reports, positive responses were achieved in artificial soil media. Papenfus et al (2013) saw an interaction between kelp and P in the glasshouse in quartz sand at an unspecified pH, while Turan and Köse (2004) demonstrated higher uptake of P with added P in optimum nutrient conditions in a neutral perlite medium in the glasshouse.

The problem of slaking in soils is reduced by organic matter, which binds mineral particles together and slows the rate of wetting (Tisdall and Oades 1982). Polysaccharides in organic matter act as major binding agents to form stable micro-aggregates in soils and thus prevent slaking. In this experiment, the data does not support polysaccharides in kelp noticeably mimicking the plant polysaccharides from organic matter in stabilising the slaking soil.

The results here reflect the need for a greater understanding of kelp interaction within the soil to understand the conditions, including soil type, required for kelp to be beneficial. With no response in crop production, addition of kelp extract is not a viable alternative to the addition of organic matter for improved soil structure at this site. Nor is there evidence of chelating properties of the kelp improving P uptake at this site. Evidence based data are essential for horticulture to develop effective strategies for the use of seaweed extracts.

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Figures and Tables

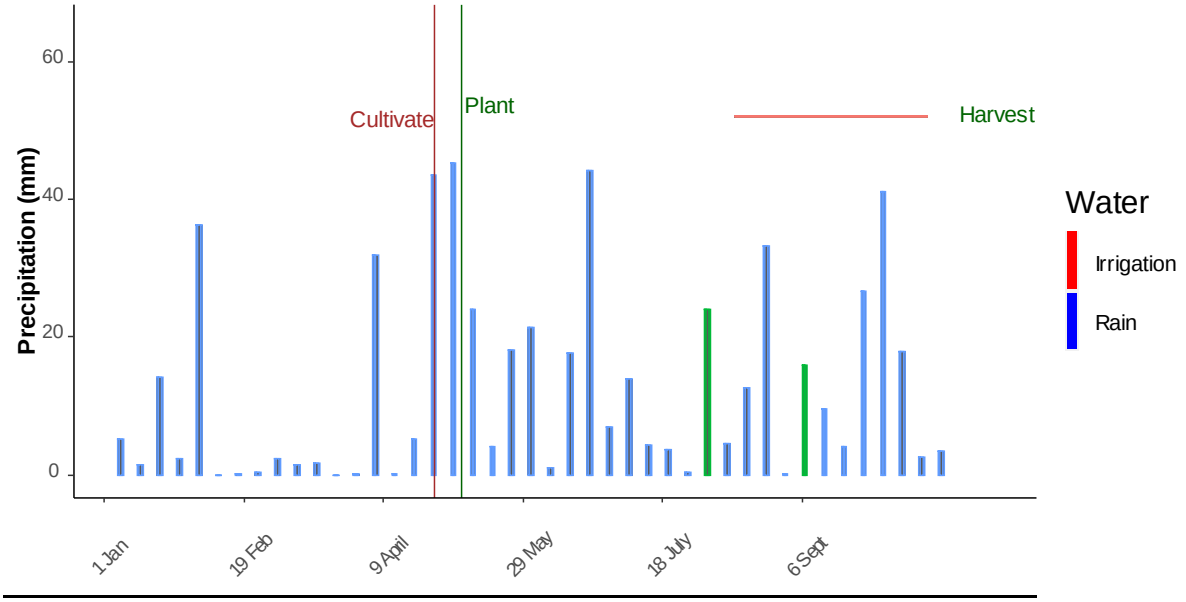

Fig 1 *Rainfall and supplementary irrigation at the field site. Bureau of Meteorology observation site, station number 23105 (35.0S, 138.6E)*

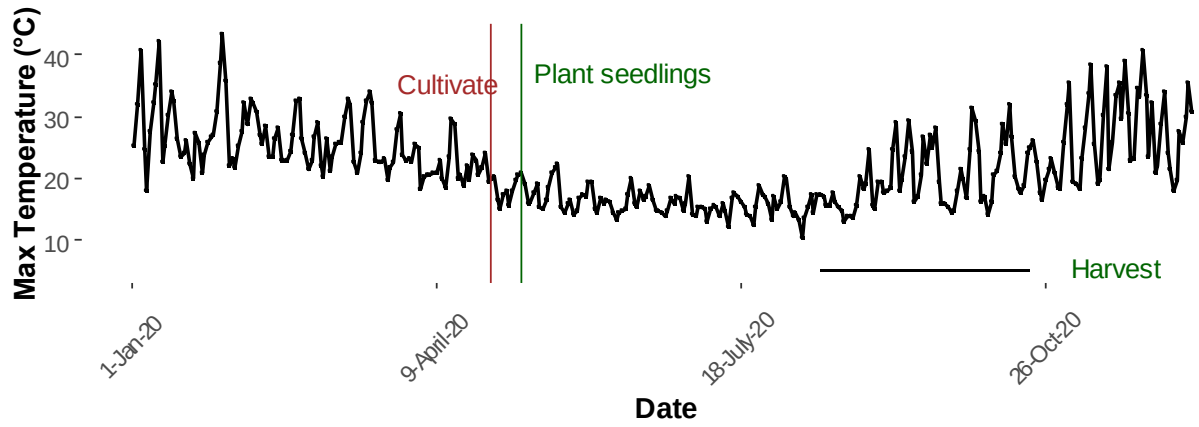

Fig 2 *Daily maximum temperature at the field site, Bureau of Meteorology observation site, station number 23105 (35.0S, 138.6E)*

A grade: Fresh, compact head, tight florets

B grade: Compact, but with florets emerging from the head

C grade: More open appearance

D grade: Very open and uneven

Fig 3 *Grading protocol for broccoli at harvest*

Fig 4 *Purple leaves of broccoli plants with little or no added P fertiliser. Purple leaves in broccoli plants are indicative of plant stress, including phosphorus deficiency. Waite Campus, University of Adelaide, 2020.*

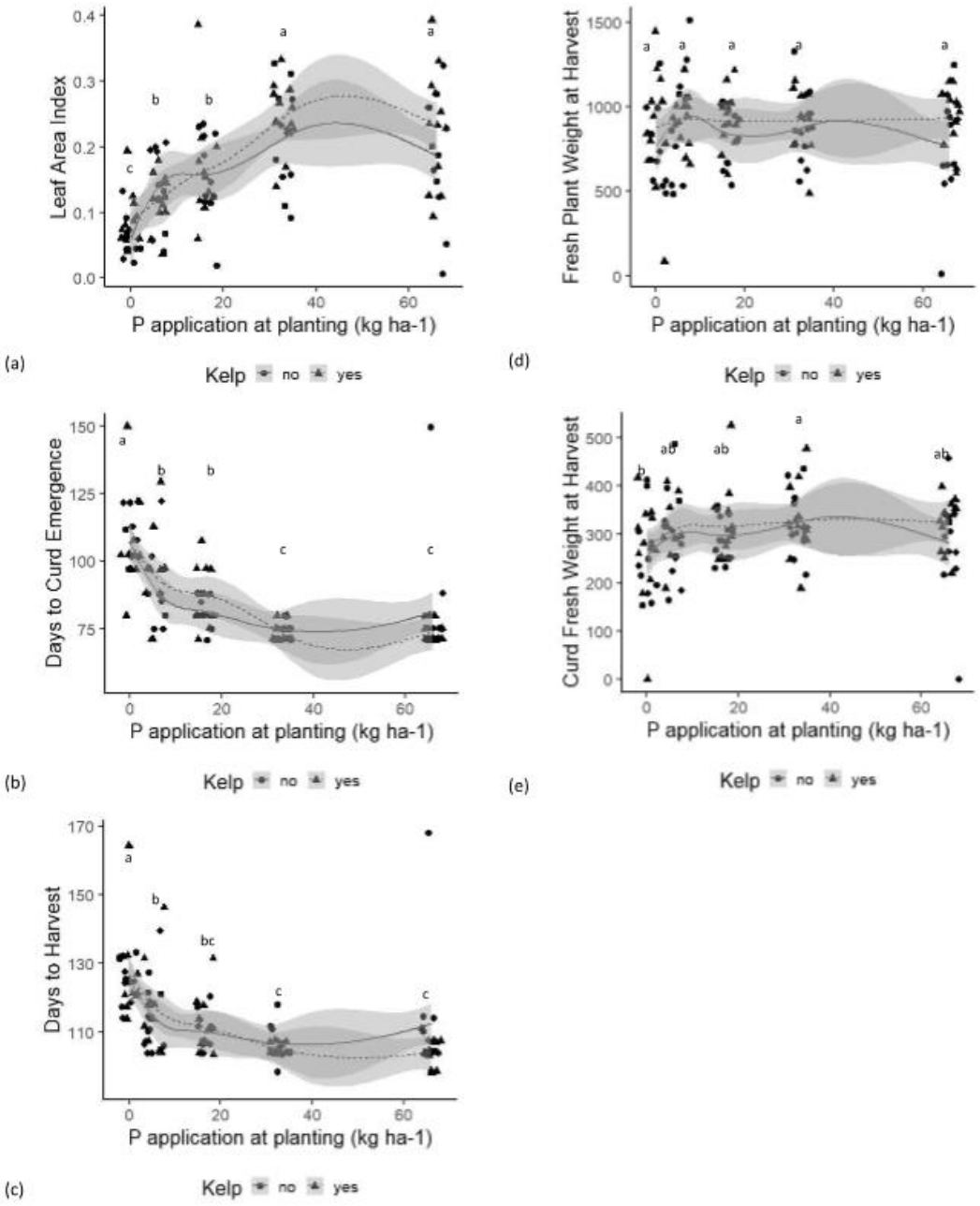

Figure 5. Effect of kelp and P applied at 0, 5.5, 16.5, 33 and 66 kg ha^{-1} to field broccoli growing on a slaking soil at the Waite Research Institute. (a) Leaf Area Index at 45 days from planting; (b) Days to curd emergence; (c) Days to harvest at prescribed stage of development; (d) Fresh weight of curd at harvest; (e) Total plant fresh weight at harvest . Smoothing lines with 95% confidence bands are overlapped upon the scatterplots in order to visualise trends. N=12, means followed by the same letter are not significantly diff at the *P*<0.05 level.

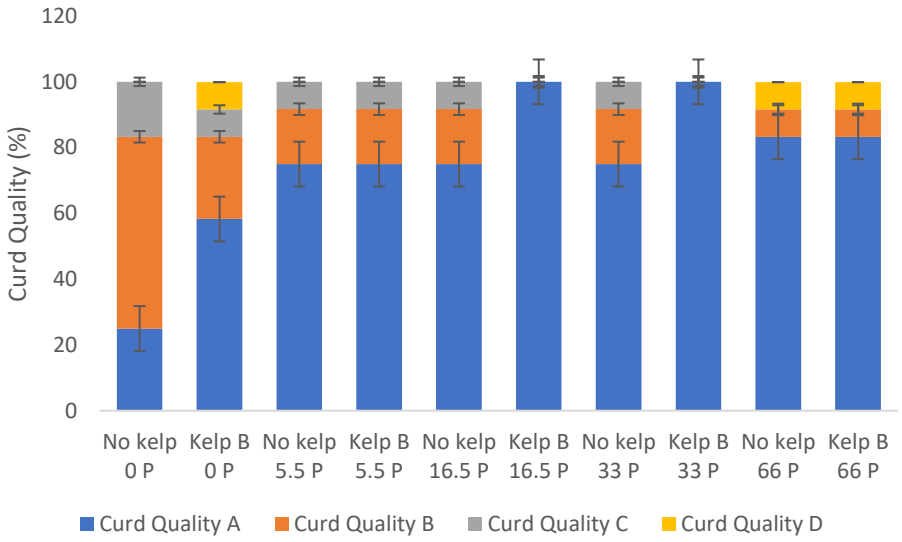

Fig 6 *Curd quality of harvested broccoli. Waite Campus, University of Adelaide, 2020. P levels are equivalent to kg ha^{-1} P.*

Table 1 *Response of the broccoli crop at various stages of development to applications of kelp and P. Mean, standard deviation (sd), coefficients of variation (CoV) and significance groupings for each treatment. Waite Campus, University of Adelaide, 2020.*

P rate (kg ha^{-1})	**0**		**5.5**		**16.5**		**33**		**66**	
Kelp (+/-)	-	+	-	+	-	+	-	+	-	+
					LAI (45 days)					
mean	0.06	0.09	0.14	0.12	0.16	0.17	0.21	0.25	0.19	0.23
sd	0.03	0.04	0.06	0.04	0.06	0.08	0.08	0.05	0.09	0.09
CoV	51%	45%	43%	33%	38%	47%	38%	20%	47%	39%
Significance	c		b		b		a		a	
					Curd Emergence (days)					
mean	107	107	89.7	94.4	81.3	87.4	74.6	73.5	75.2	73.9
sd	8.7	17.5	12.8	15.9	5.3	10.8	3.7	3.5	5.1	4
CoV	8%	16%	14%	17%	7%	12%	5%	5%	7%	5%
Significance	a		b		b		c		c	
					Harvest (days)					
mean	125.6	126.8	114.4	117.3	110.2	111.4	106.7	105	107.6	104.3
sd	6.4	12.9	10.7	11.9	5.3	8.2	4.9	1.5	3.8	3.8
CoV	5%	10%	9%	10%	5%	7%	5%	1%	4%	4%
Significance	a		b		bc		c		c	
					Aboveground biomass at Harvest: Plant Fresh Weight (g)					
mean	764.5	878.4	930.8	917.7	836.2	912.7	877.9	913.2	831	930.8
sd	230.3	354.57	315.4	158.1	176	175.3	214.8	187.9	227.1	183.5
CoV	30%	40%	34%	17%	21%	19%	24%	21%	27%	20%
Significance	a		a		a		a		a	
					Harvest: Fresh Curd Weight (g)					
mean	255.5	268	297.2	308.5	297.2	315.5	325.3	327.1	307.7	323.9
sd	85.7	106.2	91.6	58.2	44.9	80.9	65.2	76.6	66.5	53.7
CoV	34%	40%	31%	19%	15%	26%	20%	23%	22%	17%
Significance	b		ab		ab		a		ab	

2.2.3 Broccoli Field Experiment 2b Rock Phosphate

2.2.3.1 Introduction

There is no available information in the scientific literature regarding any possible interactions between kelp extracts and reactive phosphate rock (RPR). Given that the limiting factor to the use of RPR in Australia is the availability of P from this source under Australian conditions (Bolun et al 1990), it may be that kelp addition could be useful here. Reactive phosphate rock (RPR) also known as soft rock phosphate, is considered by the organic farming community to be preferable and more environmentally acceptable to "chemically processed soluble fertilisers such as superphosphate" (Pacific Fertilisers; https://pacificfertiliser.com/338/338, 2020). Reactive phosphate rock does not contain soluble phosphate, and acid soil conditions combined with an active soil biology are required to release P to a plant available form. Australian research typically has shown that both reactive and unreactive phosphate rock have been less effective sources of P relative to superphosphate (Bolun et al 1990). This is in contrast to New Zealand experiences and has been attributed to a range of differences, including soil pH, pH buffering capacity and rainfall frequency. Kumari and Phogat (2008) have reviewed amendments to RPRs which have been used to improve P availability. Success has been achieved through composting with farm manure, green manuring, partial acidulation of RPR, and the use of P solubilising organisms. It was found by de Amaral Leite et al (2020) that adding selected bacterial strains could enhance P availability from biochar-based RPR fertiliser. Khan et al (2009) claim that alginates and fucoidans are responsible for enhancing soil health by improving moisture holding capacity and by promoting the growth of beneficial soil microbes. This experiment

was conducted to explore the possibility of kelp enhancing the conversion of applied RPR to available phosphate in the soil.

2.2.3.2 Materials and methods

(Refer to 2.0.2. Methods common to all broccoli field experiments)

Site design as it relates to rock P

Broccoli seedlings were planted in a split plot randomised block design, with 12 replications per treatment. A commercially available RPR with analysis of Total P 13%, Citrate P 4.7%, Ca 37%, S 1% was compared with P in the form of mono-ammonium phosphate (MAP, 10:22:0). The RPR was applied at 130 kg ha^{-1}, equivalent to 16.9 kg ha^{-1} P. MAP was applied at 75 kg ha^{-1} P, equivalent to 16.5 kg ha^{-1} P. The control had no added P and each treatment was +/- kelp extract. A soluble powder extract of the kelp species *Ascophyllum nodosum* was dissolved in de-ionised water and applied to the soil at a rate of 12.5 kg ha^{-1} prior to planting.

Broccoli were harvested at optimum development, with 12 cm curd radius. Reduced days from planting to harvest was chosen as the measure of product performance based on the results of the kelp × P experiment described in section 2.2.2.

2.2.5.3 Results

In this acidic soil (pH = 5.9), there was no significant difference between the addition of 17 kg ha^{-1} P as MAP or RPR. (Fig 2.2.3.1) The mean of Days between planting seedlings and broccoli harvest was 126 days for the control (with or without kelp), 111 when MAP was applied and 108 when RPR was applied. There was no significant effect of kelp application for control or either of the P applications. Across all treatments, the average number of days to harvest was 114 days, with or without kelp.

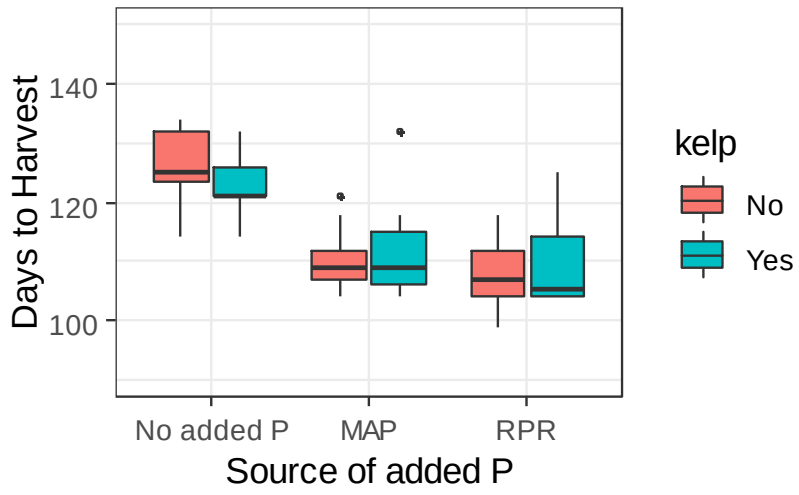

Fig 2.2.3.1 *Mean of Days between planting seedlings and broccoli harvest following treatment with P as MAP or RPR. MAP was added at 75 kg ha^{-1} adding approximately 17 kg ha^{-1} P and RPR was added at 130 kg ha^{-1}, also adding approximately 17 kg ha^{-1} P.*

2.2.3.4 Discussion

These results show that the response of the broccoli to the P from the RPR was equivalent to the response to the P from MAP. With a soil $pH_{1:5\ H2O}$ of 5.9, this is not surprising, since P from the RPR is released by acidification. There was no significant response to the addition of kelp for either of the P applications in RPR or MAP. Since there was no difference between the response of broccoli to either source of P, it is not surprising that there is no difference in the response of the two sources to kelp application.

2.2.3.5 References

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2.3. Broccoli Field Experiment 3. Looking for kelp interaction with nitrogen

2.3.1 Introduction

Nitrogen (N) plays many important roles in plant physiology. Structural and storage proteins, enzymes, amino acids and amides, nucleic acids, and plant hormones all contain N. A shortage of N not only affects yield, but also the quality of the produce. Nitrogen is absorbed by the plant mainly as nitrate or ammonium ions and, in general, N from organic N sources must be transformed to these inorganic forms before it becomes available to plants. Nitrate is reduced to ammonia in the roots or in the leaves and ammonia is metabolised into glutamine and subsequently into other amino acids.

The behaviour of N in the soil system is complex, changing from one form to another under the influence of the chemical, physical and biological environment. Nitrogen fertiliser application in agriculture is generally inefficient due to losses to the atmosphere or through leaching down the soil profile (Anas et al 2020). Nitrogen can be lost through volatilization as ammonia, or denitrification to nitrous oxide or dinitrogen (Fig 2.3.1). Nitrate-N is water-soluble and nitrate fertilisers can be leached below the crop rooting zone by rainfall or irrigation.

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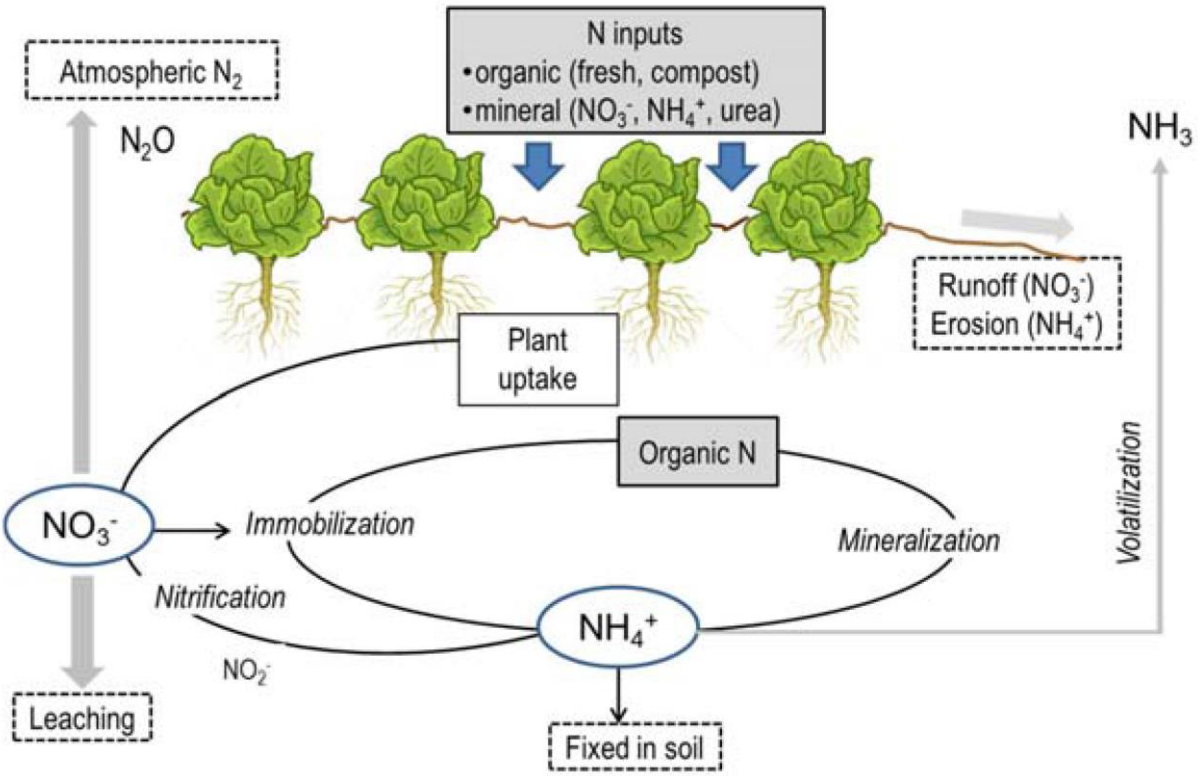

Fig 2.3.1 *The nitrogen cycle showing the main pathways for nitrogen loss (Cameira and Mota; 2017)*

Broccoli has a high demand for N. Cutliffe et al (1968) found that under conditions in Canada, rates of 175 to 250 kg ha^{-1} of N were required for maximum yields of broccoli, but only when N was accompanied by adequate P. In Turkey, Yoldas et al (2008) reported yield increases of broccoli with increasing application rates of N to 300 kg ha^{-1} N.

If kelp extracts interact with N, then they may allow growers to manage N for more profitable and environmentally friendly crop production. There are many references in the literature regarding the interaction between kelp extracts and N to improve crop yield and quality. For example, Turan and Köse (2004) claim that the application of seaweed extract on grapevine increased N, P, K, Ca and Mg uptake under optimum nutrient element

conditions. Various mechanisms have been proposed for the activity of kelp. There is a school of thought that the response from the kelp extract of increased protein levels in plants is due to organic molecules such as organic acids, methionine and polyamines in the extract increasing nutrient absorption in plants (Beckett et al, 1994). The extraction process disrupts the kelp cell wall molecules to produce organic acids, and it has been suggested that these molecules chelate the available nutrients and increase their absorbance (Papenfus et al, 2013; Chatzissavvidis and Therios, 2014). It may be that the alginates derived from brown seaweed are the mechanism for kelp extracts enhancing assimilation and basal metabolism of N in plants (Khan W et al, 2011; Sarfaraz et al, 2011). There is evidence of seaweed extracts impacting upon the level of the expression of mRNAs and causing changes to the metabolome of the treated plant (Jannin et al, 2013; Nair et al, 2012). An extract of *A. nodosum* has been reported to upregulate the expression of a nitrate transporter gene NRT1.1. This gene improved N sensing and auxin transport (Krouk et al, 2010; Castaings et al, 2011). There are other examples of kelp applications upregulating gene expression in plants (Battacharyya et al, 2015).

Protein content in wheat has been found to increase when the plants were sprayed with extracts of red algae (Papenfus et al, 2013) and Singh and Chandel (2005) reported that the application of an extract of *A. nodosum* increased protein content in wheat grain. They suggested the response may be due to promotive effects on root proliferation and thus higher uptake of nutrients required in protein synthesis (N, P, and S).

The experiment reported here aimed to determine whether an interaction between kelp and N can be found in a commercial broccoli crop. Two forms of N fertiliser were chosen,

urea and calcium nitrate, because they are taken up from the soil at different rates and kelp may interact with the source to alter uptake. Two forms of *A. nodosum* extract were compared, Kelp C, extracted via a hot caustic process, and kelp D, produced through fermentation.

2.3.2 Materials and methods

Experimental design

Broccoli seedlings were planted in a randomised block design, with two different forms of N fertilization (urea and calcium nitrate) and treatments of two different kelp extracts. There were nine replications of each treatment. Broccoli (*Brassica oleracea* cv *Prophet*) seedlings obtained from Virginia Nursery, Virginia, SA, were transplanted on 11th May 2020, when 26 days old. Plants were spaced 0.3 m apart in rows 0.5 m apart, in accordance with commercial practice (Hilton, 2018).

Phosphorus was applied in the form of mono-ammonium phosphate (MAP, 10:22:0) prior to planting the broccoli seedlings, at an application rate equivalent to 16.5 kg ha^{-1} P. Hence 7.5 kg ha^{-1} N from MAP was applied as a basal rate. The kelp treatments were dissolved in de-ionised water and applied to the soil at a rate of 20 L ha^{-1} prior to planting. For the N treatments a solution of the appropriate treatment of either urea or calcium nitrate or control was applied uniformly as side applications post planting over eight weeks. Treated plants received 140 kg N ha^{-1} above the basal rate of 7.5 kg N ha^{-1} added with MAP. Treatments were applied weekly.

Commercial Extracts for comparison

Two commercially available kelp extracts were tested.

Kelp C. Extracted from the species *A. nodosum*, using a hot caustic extraction process.

Kelp D. Extracted from the species *A. nodosum*, using a fermentation process.

2.3.3 Results

Curd diameter at 94 days from planting of broccoli

Average diameter of the broccoli curd at 94 days from planting was approximately 80% greater for each of the N treatments when compared to the controls (Fig 2.3.2). This response to either form of N was significant ($P<0.05$), but there was no significant difference between the two forms of N. There was no significant response of broccoli to either form of kelp and there was no interaction between kelp and N ($P>0.05$).

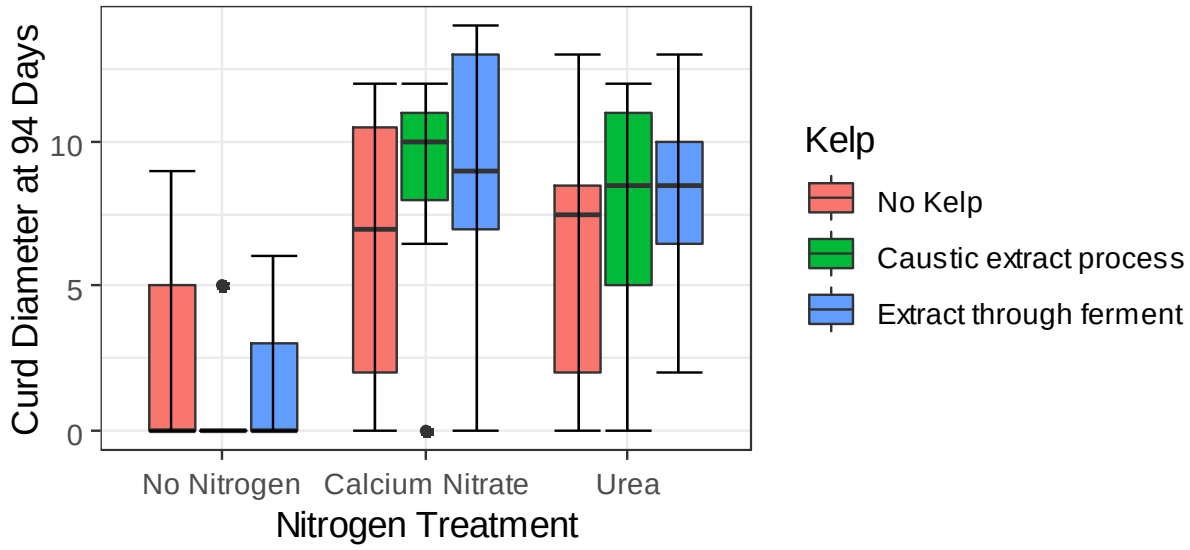

Fig 2.3.2 *Curd Diameter at 94 days. Standard Error across the experiment was pronounced. Waite Campus, University of Adelaide, 2020.*

Days from planting of broccoli seedling until curd emergence

The reduction in time to curd emergence of approximately 30% in response to either form of N was significant ($P<0.05$), but there was no significant difference between the two forms of

N (Fig 2.3.3). There was no significant response of broccoli to either form of kelp and there was no interaction between kelp and N (*P*>0.05).

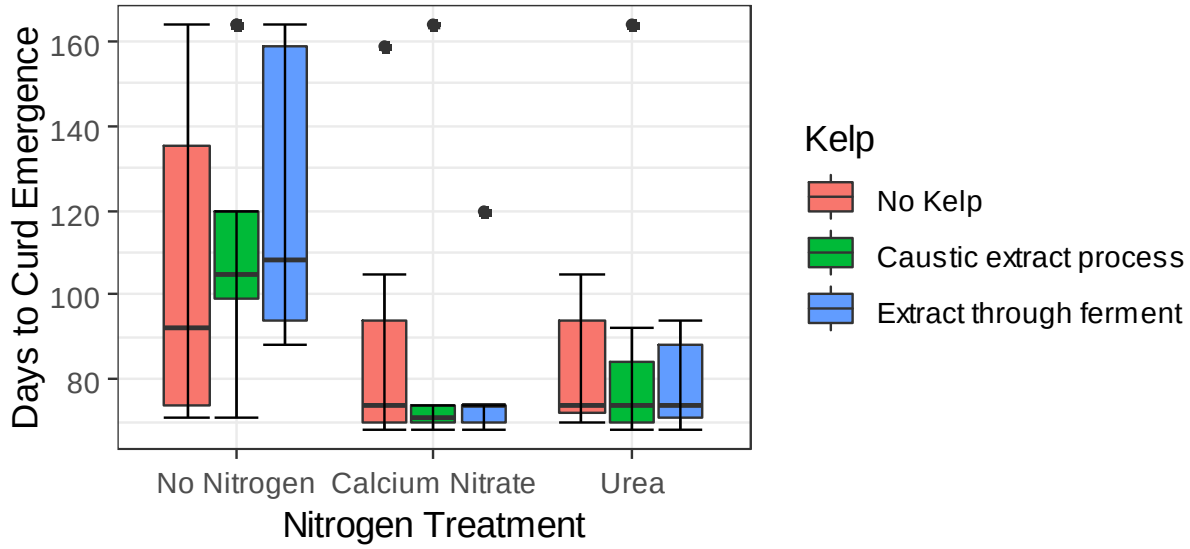

Fig 2.3.3 *Days from planting of broccoli seedling until curd emergence. Waite Campus, University of Adelaide, 2020.*

Days from planting of broccoli seedling until harvest

The 18% decrease in time to harvest of broccoli in response to either form of N was significant (*P*<0.05), but there was no significant difference between the two forms of N (Fig 2.3.4). There was no significant response of broccoli to either form of kelp and there was no interaction between kelp and N (*P*>0.05).

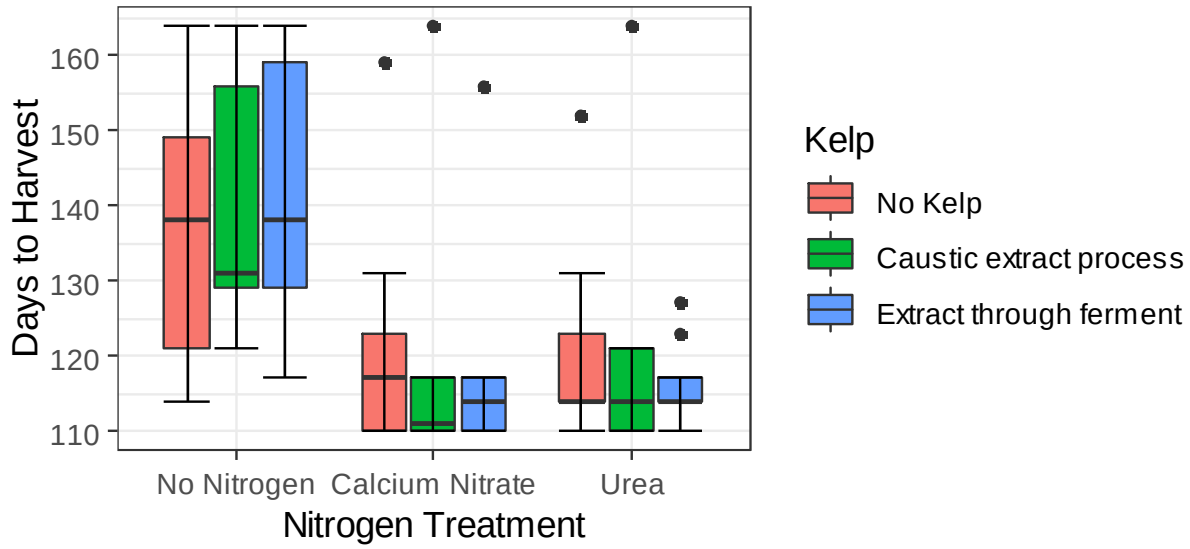

Fig 2.3.4 *Days from planting of broccoli seedling until harvest. Waite Campus, University of Adelaide, 2020.*

Curd diameter at harvest

The average diameter of the broccoli curd at harvest was 22% greater for either form of N compared with the controls and this difference was statistically significant ($P<0.05$). There was no significant difference between the two forms of N. There was no significant response of broccoli to either form of kelp and there was no interaction between kelp and N ($P>0.05$) (Fig 2.3.5). While the protocol was to harvest when curds reached a diameter of 12 cm, many of the low N treatments were deteriorating in quality at a lesser diameter and would not have grown to reach 12 cm. They were harvested before the curd quality became commercially unacceptable (i.e., before the curd reached the D grade status shown in Fig 2.0.7).

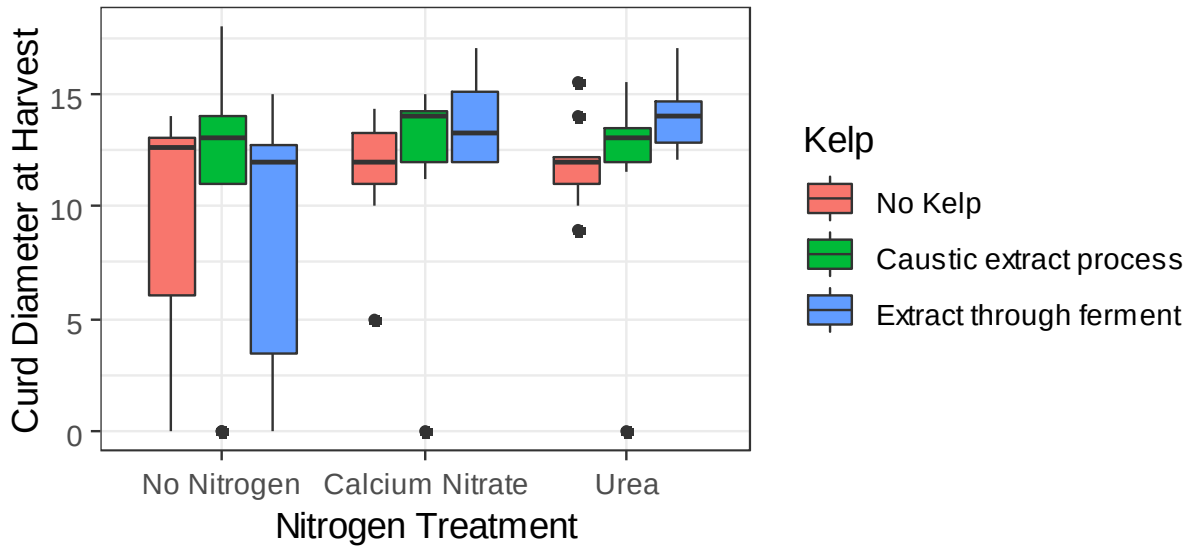

Fig 2.3.5 *Curd diameter at harvest. Waite Campus, University of Adelaide, 2020.*

Fresh weight of broccoli plant at harvest

Neither form of N application resulted in a significant response (*P*>0.05) in broccoli fresh weight at harvest. Harvest of broccoli occurred when the curds had reached a specified size, no significance in fresh weight was expected. There was no significant response of broccoli to either form of kelp and there was no interaction between kelp and N (*P*>0.05) (Fig 2.3.6).

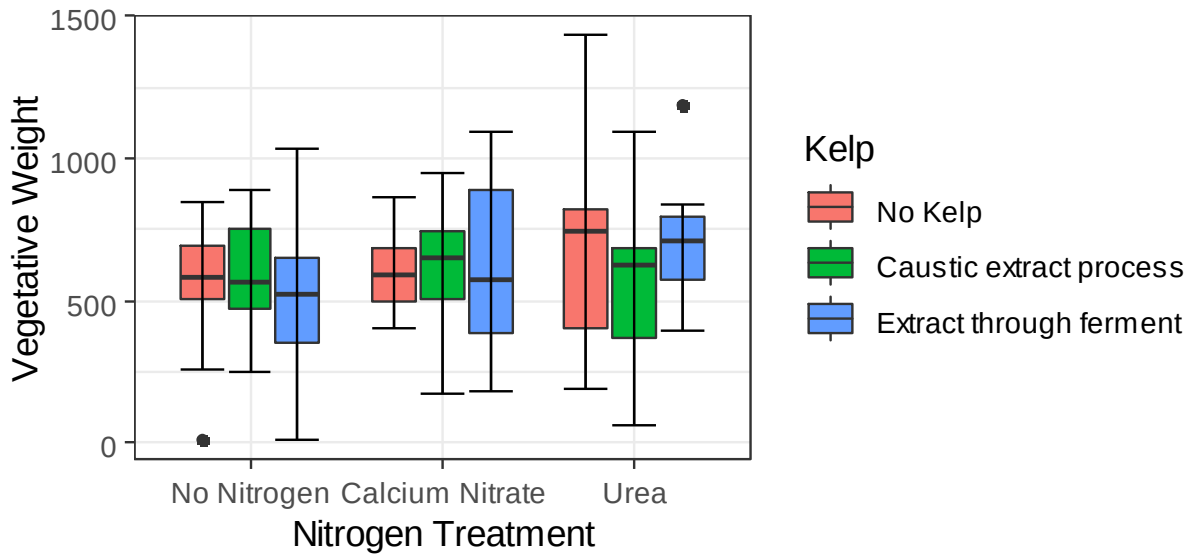

Fig 2.3.6 *Fresh weight of broccoli plant at harvest. Standard Error across the experiment was pronounced. Waite Campus, University of Adelaide, 2020.*

Fresh curd weight at harvest

The weight of the broccoli curd at harvest was significantly greater than control for the Urea treatment ($P<0.05$) by 32% but not for the calcium nitrate treatment ($P>0.05$). There was no significant difference between the two forms of N ($P>0.05$). There was no significant response of broccoli to either form of kelp and there was no interaction between kelp and N ($P>0.05$) (Fig 2.3.7).

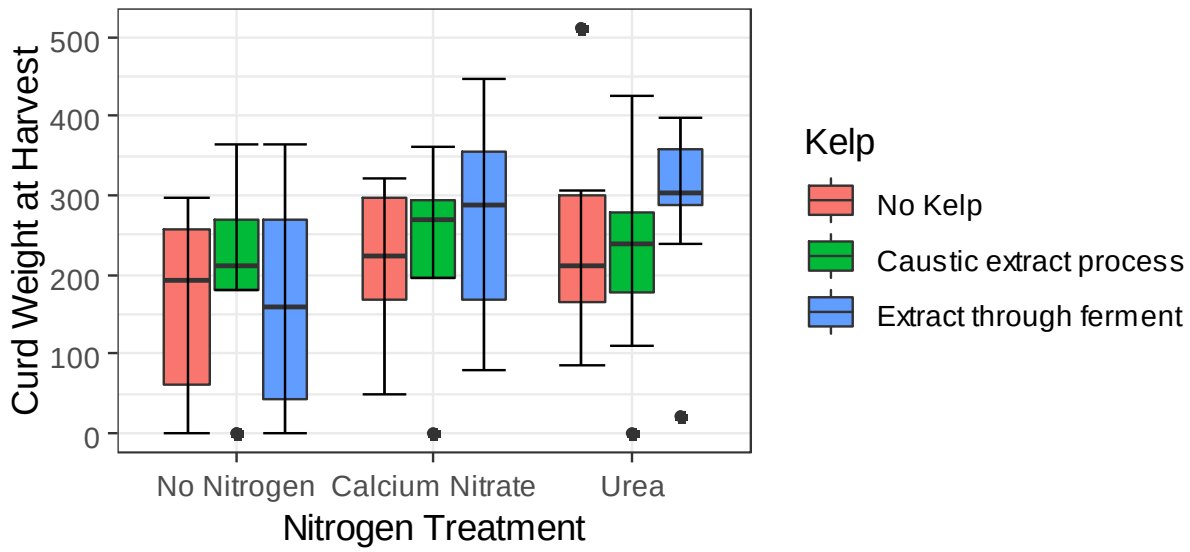

Fig 2.3.7 *Fresh curd weight at harvest. Standard Error across the experiment was pronounced. Waite Campus, University of Adelaide, 2020.*

Harvest Index

The curd weight to plant weight ratio for broccoli at harvest was significantly greater for both forms of N ($P<0.05$), but there was no significant difference between the two forms of N (Fig 2.3.8). There was no significant response of broccoli to either form of kelp and there was no interaction between kelp and N ($P>0.05$).

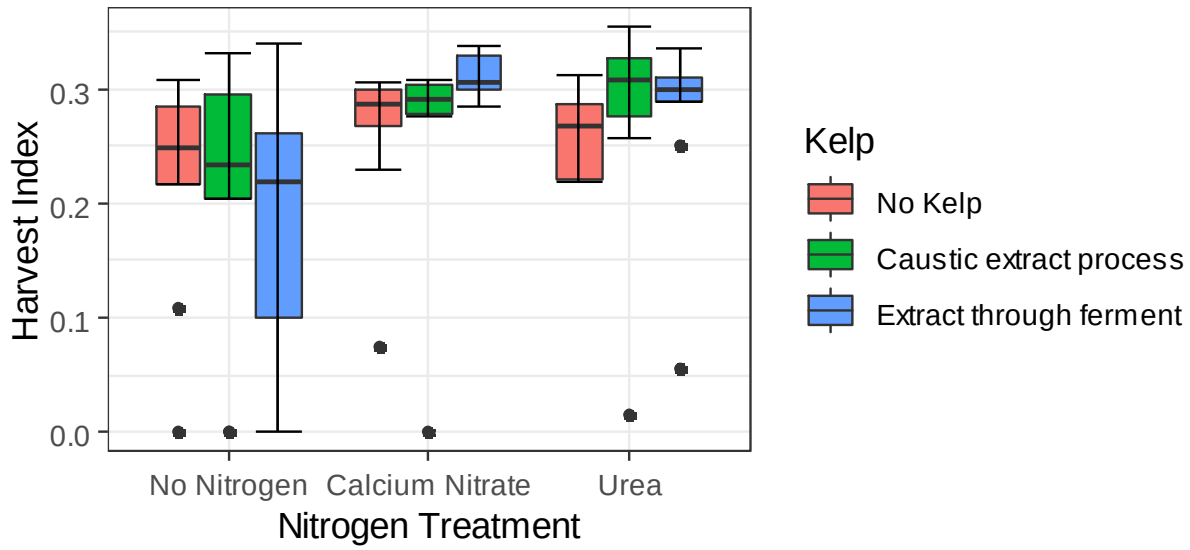

Fig 2.3.8. *Harvest Index. Waite Campus, University of Adelaide, 2020.*

Curd Quality

The non-parametric Wilcoxin signed rank test showed that the treatments did not result in significant differences (P>0.05) for Curd Quality (Fig 2.3.9). However, the percentage of "A" quality curds was below 50% for this experiment, which was lower than for the other experiments. The curds tended to be more open in this experiment.

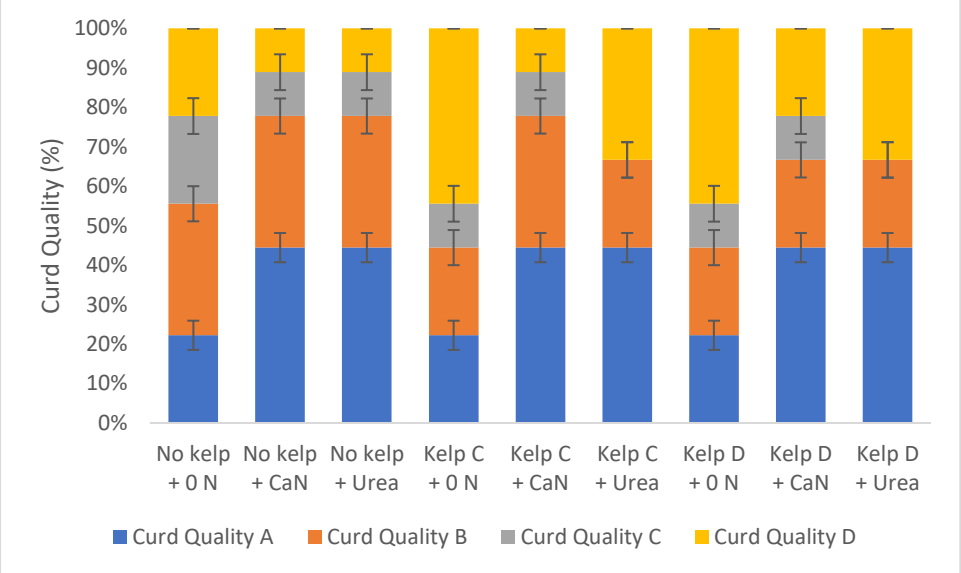

Fig 2.3.9. *Curd Quality. Waite Campus, University of Adelaide, 2020.*

2.3.4 Discussion

The outcome of the N experiment was very similar to that of the P experiment in that there was a significant increase ($P<0.05$) in the rate of maturity of the broccoli following increased nutrition but there was no significant response to the kelp applications. The addition of 140 kg ha^{-1} of N, as either urea or calcium nitrate, throughout the growing period of the broccoli decreased the date to curd emergence by approximately 32 days, a 28% reduction, and the date to harvest by 21 days, a 15% reduction. In Canada, on a fine sandy loam soil, Bakker et al (2009) reported a five-day reduction in maturity of broccoli for rates of 50 to 400 kg ha^{-1} N over a no applied N control. Westerveld et al (2003) reported that low N rates added in a grey-brown Luvisol soil delayed cabbage harvest by 12 to 18 days. However, in another Canadian study, Cutcliffe et al (1968) found that in a fine sandy loam the number of broccoli curds mature by mid-season was significantly decreased by increasing the rates of N from 0 to 200 or 300 kg ha^{-1}, but that this increase was accompanied by yield increases. Bakker et al (2009) suggested that the conflicting data may be due to the N status of the crop at the time of application of N. The form of N made no perceivable difference in this experiment. Reducing the days to crop maturity provides benefits to growers, as crop management costs such as pesticides and irrigation would be expected to be reduced and reduced time to market would benefit cash flow and business planning.

As with the P results (Section 2.2), the data in this experiment show that the impact of added N on broccoli maturity had commenced by curd emergence. In fact, after this stage, the later plants matured faster, so that the gap was reduced from 32 days to 21 days. Response to N would be expected to be detected early because it forms the building blocks

of plant proteins and enzymes and is thus essential from the commencement of plant development. Nitrogen plays a critical role in all facets of plant development. The significant results for the response of Harvest Index to added N show that the addition of N makes the broccoli plant more efficient in turning energy and inputs into curd production rather than vegetative growth.

Many reports (e.g., Khan et al, 2011; Sarfaraz et al, 2011) cite examples of kelp extracts interacting positively with plant uptake of N. There is also evidence in the literature of potential mechanisms within the plant to explain the effect of kelp interacting with N at the cellular level within the treated plant (Jannin et al, 2013; Nair et al, 2012). In this experiment two products, both extracted from *A. nodosum* via very different processes, were evaluated for their interaction with N applied to broccoli plants, but no interaction was detected. These results are consistent with the view that responses to kelp treatments are dependent on multiple plant, soil, and environmental factors and the conditions here did not meet all of the requirements. The results of Cutcliffe et al (1968), Westerveld et al (2003) and Bakker et al (2009) showed responses to higher levels of N than applied here. Perhaps macronutrients must be optimal before the benefits of kelp are realised. In order to achieve discernible effects from kelp, it is important to understand the conditions necessary for response and to identify which factors are limiting.

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2.4 Broccoli Field Experiment 4. Comparing kelp extract with alginate

2.4.1 Preamble

A field experiment was conducted to study the importance of the alginate component of kelp on broccoli production on a poorly structured slaking soil. The results of this study are presented in the following manuscript, submitted to The Journal of Horticultural Science and Biotechnology for publication. The manuscript is reproduced as presented to the journal. There is some variation from the format adopted for the body of the thesis. The manuscript is a stand-alone document, so there is necessarily some repetition from the body of the thesis.

2.4.2 Statement of Authorship

Title of Paper	Alginate alone explains kelp stimulus to broccoli growing in a slaking soil
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Prepared for submission to the Journal of Horticultural Science and Biotechnology

Principal Author

Name of Principal Author (Candidate)	Graeme Anderson		
Contribution to the Paper	Conception, acquiring data, knowledge, analysis, drafting		
Overall percentage (%)	80		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	1/3/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ronald Smernik		
Contribution to the Paper	knowledge, analysis, drafting		
Signature		Date	1/3/2023

Name of Co-Author	Timothy Cavagnaro		
Contribution to the Paper	knowledge, analysis, drafting		
Signature		Date	1st March, 2023

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2.4.3 Manuscript: Alginate alone explains kelp stimulus to broccoli growing in a slaking soil.

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Funding, Data Availability and Conflict of Interest statement
This work is a component of the studies for Doctor of Philosophy by Graeme Anderson at the University of Adelaide, School of Agriculture, Food and Wine. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. The authors declare no conflicts of interest.

Abstract
Despite the long history of seaweed use in agriculture, the mechanisms by which commercial extracts prepared from brown algae benefit plants remain under debate. Originally it was believed that polysaccharides from the algal cell wall interacted with the soil to improve structure, but alternative modes of action have been proposed for the kelp extracts to stimulate plant growth. This field study was undertaken to determine the importance of alginates from the cell wall of brown algae on the development of broccoli growing in soil susceptible to slaking. It was found that the addition of kelp extract (20 L ha^{-1}) or kelp cell wall alginate (10 L ha^{-1}) or a combination (20 L ha^{-1} plus 10 L ha^{-1}) applied to the soil resulted in similar statistically significant increases in broccoli production than in the control group. The curds of treated broccoli were wider throughout development, and heavier at harvest. The fresh plant weight of broccoli at harvest was 34-40% heavier for treated plants than the control and the fresh curd weight was 52-60% greater. Differences in curd quality were not significant and mineral concentration within the curd could not be explained by the treatments. The data are consistent with kelp cell wall alginates improving the structural stability of the slaking soil eliciting the response in broccoli production.

Key Words: brown kelp, alginate, slaking soil, broccoli

Introduction

Seaweed has been used as a soil ameliorant in agriculture for a very long time. In cold coastal regions of the northern hemisphere, its use dates back to pre-Roman times (Guiry, 1989). Records from Iceland, Norway, Great Britain and France show kelp (brown algae) was harvested for use as a soil conditioner. Commercial seaweed products were introduced in the early twentieth century, initially as a dried pulverised meal (Milton, 1952). Milton released the first commercial seaweed concentrate in 1949, prepared as an extract from the brown algae, *Ascophyllum nodosum*. Milton believed that polysaccharides from the algal cell walls acted in the soil to improve aeration and soil structure, thus stimulating soil micro-organisms and plant root systems, ultimately improving plant growth. Despite this long history of use, there remains considerable gaps in our understanding of when and how these products improve plant growth.

Since Milton, other modes of action of kelp extracts have been suggested to explain reported benefits in plant production. Mineral fertilisation by the addition of kelp extracts has been considered, but discounted, because the quantity of mineral nutrients supplied from the kelp is insignificant (Blunden, 1977; McHugh, 2003). While addition of kelp extracts delivers insufficient quantities of mineral nutrients to directly explain the response, kelp extracts contain chelating agents which could influence the availability of nutrients present but largely unavailable in soil. A chelating agent increases solubility of cationic minerals and can facilitate the entry of such cations into the plant and hence minerals can be many times more plant-available in the chelated form (Howard and Wilson, 1993). The proven capability of alginates to bind nutrient ions in a reversible process suggests that alginates could act as chelates (Tuhy et al, 2015).

It has also been argued that plant responses to kelp application are consistent with stimulation by plant hormones, and that the kelp extracts include adequate concentrations of these compounds to elicit such a response (Blunden, 1991). Plant hormones, also known as phytohormones, are chemicals produced by plants that regulate their growth development, reproductive processes, longevity, and even death (Dilworth et al, 2017). Phytohormones, including abscisic acid, auxins, cytokinins, gibberellic acid and betaines, are also found in brown algae, where they have similar functions to those in higher plants (Bradley, 1991; Tarakhovskava et al, 2007). However, many researchers continue to support the school of thought that the plant responses are most likely due to influences of the algal polysaccharides, particularly the alginates (Michalak et al, 2017; Craigie, 2011; Calvo et al, 2014).

All commercially available seaweed extracts used in agriculture are extracted from brown algae (phylum Phaeophyta), commonly known as kelp. Brown algae are the only eukaryotes known to synthesise alginates. This ability has evolved through a complex co-evolutionary history with two bacterial genera, Pseudomonas and Azotobacter (Hay et al, 2010). The benefits to soil health proposed by Milton may relate to the gelling and chelating abilities of these polysaccharides coupled with their hydrophilic properties (Blunden, 1991). When calcium is added to alginate, cross-linked polymers are formed which retain soil moisture and improve crumb structure (Khan et al, 2009). Monovalent ions such as sodium cannot crosslink the alginate polymers (Stiger-Pouvreau et al, 2016). In the soil, the alginate chains are broken into smaller chains, still forming gels with calcium. Hence the addition of brown seaweed extracts to the soil would be expected to improve aeration and soil structure.

Here we present results of a field study in which we evaluate the impact of applications of a kelp extract, and alginate (which contains no phytohormones) isolated from kelp, on production of broccoli growing under commercial conditions in a poorly structured soil, prone to slaking, crusting and waterlogging. Our hypothesis was that if improvement in soil structure was the principal mode of action, alginate addition would have a similar impact to the addition of kelp on broccoli production.

Materials and methods

The experiment was conducted at the Waite Campus of the University of Adelaide, Urrbrae, South Australia (34.97° S, 138.63° E), on a Red Chromosol soil prone to slaking (Tisdall and Oades, 1982). The climate at this site is typically Mediterranean with a mean annual rainfall of 626 mm, most of which falls between April and October. The site had been sown to faba beans in 2019. Colwell P analysis of the soil in 2020 prior to treatment showed 120 mg kg^{-1} available P. Daily rainfall (Fig 1) and daily temperature (Fig 2) data were obtained from the nearest Bureau of Meteorology station (number 23105, 35.0S, 138.6E), located 3 km away. Rainfall was supplemented with irrigation (Fig 1) to ensure a minimum average water addition of 25 mm per week in accordance with local commercial practice (Hilton, 2018). Brassica plants are shallow rooted and hence susceptible to water stress during plant establishment and head development, so require regular watering. The rainfall chart shows adequate rainfall prior to planting; one supplementary irrigation during the growing season was required on 3 August 2020 to ensure this crop received the minimum water addition.

Experimental design

Broccoli (*Brassica oleracea*; var Prophet) seedlings were planted in a randomised block design, with four treatments and nine replications of each treatment. The site was disc cultivated on 27 April 2020, and broccoli seedlings obtained from Virginia Nursery, Virginia, SA, were transplanted on 10 June 2020, 40 days after the seedlings were received. Plants were spaced 0.3 m apart in rows 0.5 m apart, in accordance with commercial practice (Hilton, 2018). Seedlings were supplied in trays with individual cells of approximately 2 cm^3 volume. For each seedling, a core of soil 10 cm deep by 2 cm diameter was removed from the planting site. Nutrient solutions and the kelp/alginate treatments (see below) were added to the hole at the required rate (see below) for each treatment. The hole was backfilled with soil, to approximately 2 cm from the surface, and the seedling planted. Treatments (added in a 100 mL solution) included control (water alone), 10 kg ha^{-1} sodium alginate (isolated from brown algae; Sigma-Aldrich product PHR1471), 20 L ha^{-1} commercial kelp extract and a combination of alginate (10 kg ha^{-1}) and kelp extract (20 L ha^{-1}). The commercially available kelp extract was derived from a blend of kelp species (from genera *Ascophyllum* and *Durvillae*) extracted via a caustic process. The rate of 10 kg ha^{-1} sodium alginate was selected to approximate the rate of alginate equivalent to the rate applied in a kelp application (Draget, 2009).

Crop management

Fertiliser applications were based on commercial recommendations. Phosphorus (P) was applied at a rate of 33 kg ha^{-1} (150 kg ha^{-1} monoammonium phosphate), a rate that appeared promising in an adjacent experiment in which broccoli seedlings were transplanted to the field earlier (unpublished data). To avoid possible deficiency in inorganic nutrients, Yara Mila Complex®, a complete fertiliser blend, was applied at a rate of 50 kg ha^{-1} at planting; 140 kg of nitrogen (N) was applied as 50% urea-N to 50% nitrate-N as side applications over a 10-week period. Snail bait (metaldehyde 50 g kg^{-1}) was applied at 10 kg ha^{-1} to protect the young seedlings from snails.

Leaf Area Index (LAI)

Leaf area index (LAI) is a dimensionless quantity that characterises plant canopies. It expresses skyward projecting leaf area per surface area of a plant and is commonly used as an indicator of the growth rate of a plant. Broccoli plants were photographed on 26 June 2020, 16 days after planting, with a Canon 400D digital camera, mounted on a steel frame to position the camera 1 m above the soil surface. The area of the leaf canopy was measured using ImageJ software, a public domain Java image processing program. LAI was calculated as area of the plant canopy divided by area per individual plant (0.15 m^2).

Harvest

Harvest occurred on 6 September 2020 (87 days after planting seedlings), when the majority of plants had a curd diameter of 12 cm or greater and before curd quality began deteriorating. Plants were harvested at ground level and plant fresh weight was recorded. The curd was removed 6 cm below the lowest floret and curd fresh weight and diameter were recorded. The curd of each plant was graded as A, B, C or D grade (Fig 3). Grading was subjective. Value in the marketplace was the driver, so head compactness and aesthetic appearance were the main criteria. The curd was then quartered, with approximately 25% retained for freezing, and 25% retained for drying. Segments were dried at 60°C for 72 h and dry curd weight determined. The segment of each curd dried at 60°C for 72 h was ground to a fine powder using a Retsch Oscillating Mill 156 MM400. Samples, ranging from 3 to 10

g dry weight, were forwarded to Australian Precision Ag Laboratory (Hindmarsh SA) for nutrient (Total N, Nitrate-N, P, K, Ca, Mg, Na, S, B, Cu Zn, Mn, Fe, Al, Cl) analysis.

Statistical Analysis

All statistical analyses were performed using R statistical software, version 4.0.2 (R Core Team, 2019). Applying the Shapiro-Wilk normality test, data for LAI at 16 days, curd diameter at harvest (CD), plant fresh weight at harvest (FW), curd fresh weight at harvest (FCW) and harvest ratio (HR) were found to be normally distributed. These data were analysed using two-way analysis of variance (ANOVA Agricolae package). The non-parametric Kruskal-Wallis rank sum test was applied to data for curd diameter at 75 days (D75) and Curd quality (CQ). Nutrient data are presented using principal component analysis (PCA), generated using the R package FactoMineR (Version 2.4).

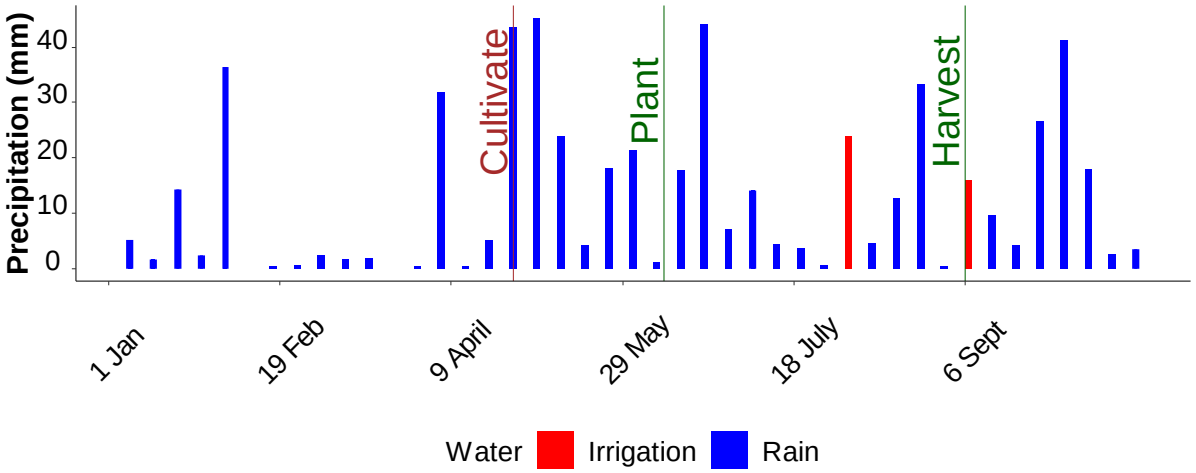


Fig 1. *Weekly rainfall (Bureau of Meteorology station 23105, located 3 km away) and supplementary irrigation for the experimental site.*

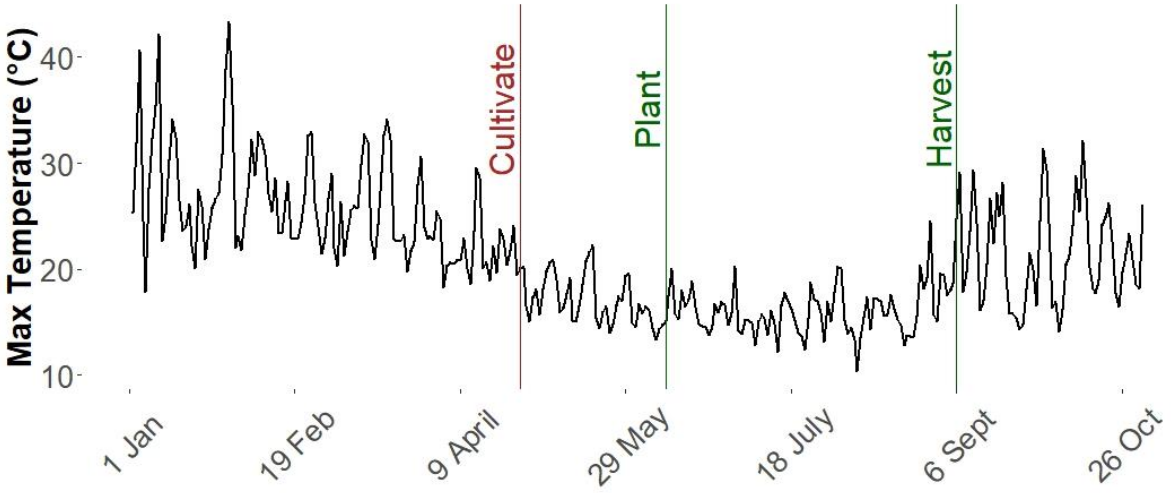
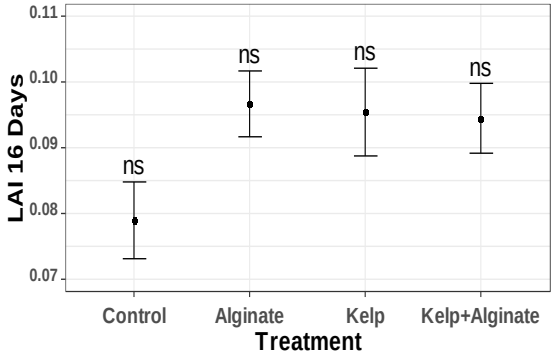


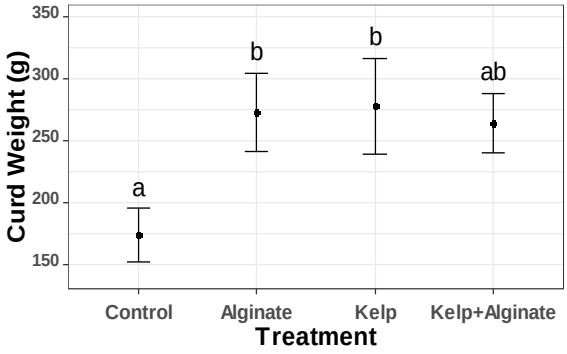
Fig 2. *Daily maximum temperature (Bureau of Meteorology station 23105, located 3 km away) for the experimental site.*

Results

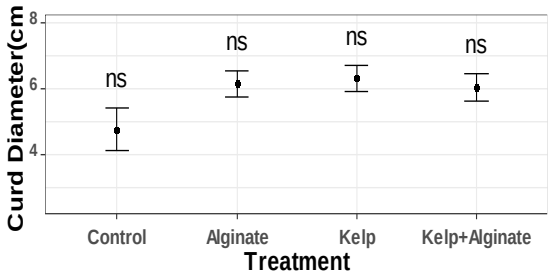
The response of the broccoli crop at various stages of development to applications of either kelp or alginate is presented in Fig 3 (tabulated values are presented in Supplementary Information; Table S1). There was a trend of greater LAI at 16 days after planting for the treatments of alginate, kelp and kelp plus alginate than the control (Fig 3a), but differences were not statistically significant ($P = 0.17$). The data for curd diameter at 75 days after planting (Fig 3b) was found not to be normally distributed (Shapiro-Wilk normality test). Treatments were 27-31% greater than control, but the application of a non-parametric test found this not to be significant ($P > 0.05$). At harvest, curd diameter of the plants treated with alginate, kelp or kelp plus alginate was 18% greater than the control plants and this was significant ($P=0.02$) (Fig 3c). Curd fresh weight at harvest (Fig 3d) and plant fresh weight at harvest (Fig 3e) were similarly significantly higher for the treated plants ($P= 0.02$). Curd fresh weight was 52-60% greater for treated plants, while plant fresh weight was 34-40% greater. The harvest index (Fig 3f), the ratio of curd weight to fresh plant weight at harvest, was significantly higher for the broccoli treated with either kelp or alginate than for control plants ($P<0.01$). The harvest index for the kelp plus alginate treatment was also higher than for the control, but in this case the difference was not significant at the 0.05 probability level. There were no significant differences between the kelp, the alginate and the combined kelp and alginate treatments in any of the plant response parameters (Fig 3a-f).



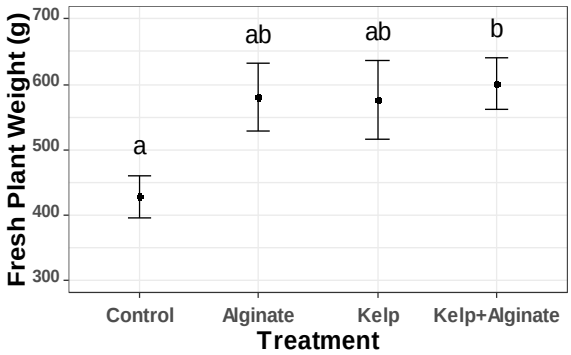
a. *Leaf Area Index at 16 days*



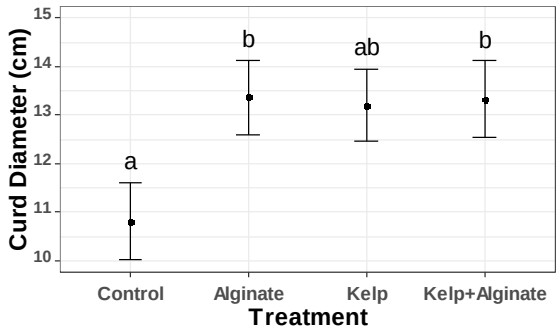
d. *Curd Weight at harvest (g per curd)*



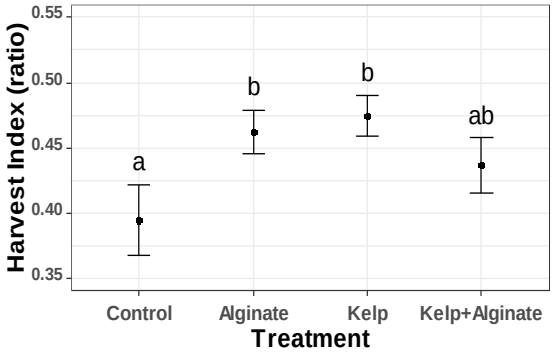
b. Diameter of curd 75 days from planting (cm)



e. Plant Fresh Weight at harvest (g per plant)



c. Curd diameter at harvest



f. Harvest Index

Fig 3. *Response of the broccoli crop at various stages of development to applications of either kelp, alginate or kelp plus alginate. Each treatment has 12 replications. (a) Leaf Area Index at 16 days from planting. (b) Diameter of curd 75 days from planting. (c) Curd diameter at harvest. (d) Curd weight at harvest. (e) Plant fresh weight at harvest. (f) Harvest index, the ratio of curd weight to fresh plant weight at harvest.*
Using the Shapiro-Wilk normality test, data for b (Diameter of curd 75 days from planting (cm)) was found to be not normally distributed, and significance was analysed using the non-parametric Kruskal-Wallis rank sum test. All remaining data was found to be distributed normally and tested for significance using ANOVA. Means were compared using Tukey HSD and means with the same letter are not significantly different at the 0.05 probability level. Bars represent standard error of the mean.

For all treatments, the majority of curds were of A quality (Fig 5); the proportion of A quality curds was lowest for the control treatment, but the non-parametric Kruskal-Wallis rank sum test indicated differences were not significant (P = 0.37).

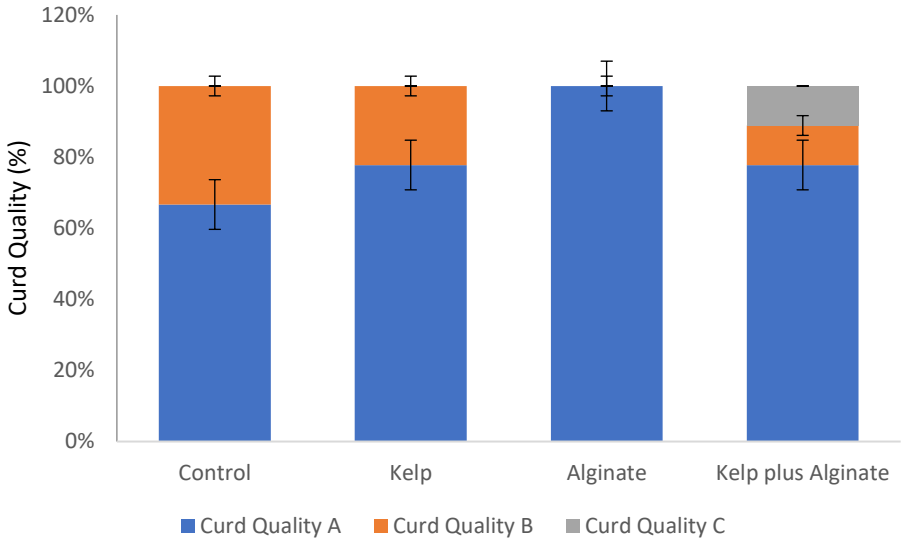


Fig 4. *Curd Quality: Frequency of the quality grades (%) for each treatment. The non-parametric Kruskal-Wallis rank sum test indicated no significant differences between the treatments (P = 0.37).*

Principal component analysis (PCA) of the curd nutrient concentrations and the plant responses (LAI at 16 days, curd diameter at 75 days, curd diameter at harvest, total curd weight, total fresh weight) showed that all plant response variables are closely aligned, consistent with the similarity seen for each individual property (Fig 5). Tabulated elemental concentrations of the curd are presented in the Supplementary Information. Vectors for most of the nutrient concentrations are approximately orthogonal to those for the plant response variables, indicating that variation in most nutrient concentrations was not correlated with plant response, meaning that most nutrient concentrations were neither higher nor lower for larger plants. Vectors for chloride and nitrate N concentrations in the curd were the most positively aligned to the broccoli growth vectors, but linear regression between these parameters indicated generally weak correlations (r^2=0.26 and 0.13, respectively; data not shown). On the other hand, vectors for Fe and Mo concentrations were the most negatively aligned to the broccoli growth vectors, but again linear regression between these parameters indicated generally weak correlations ($r^2 < 0.10$; data not shown).

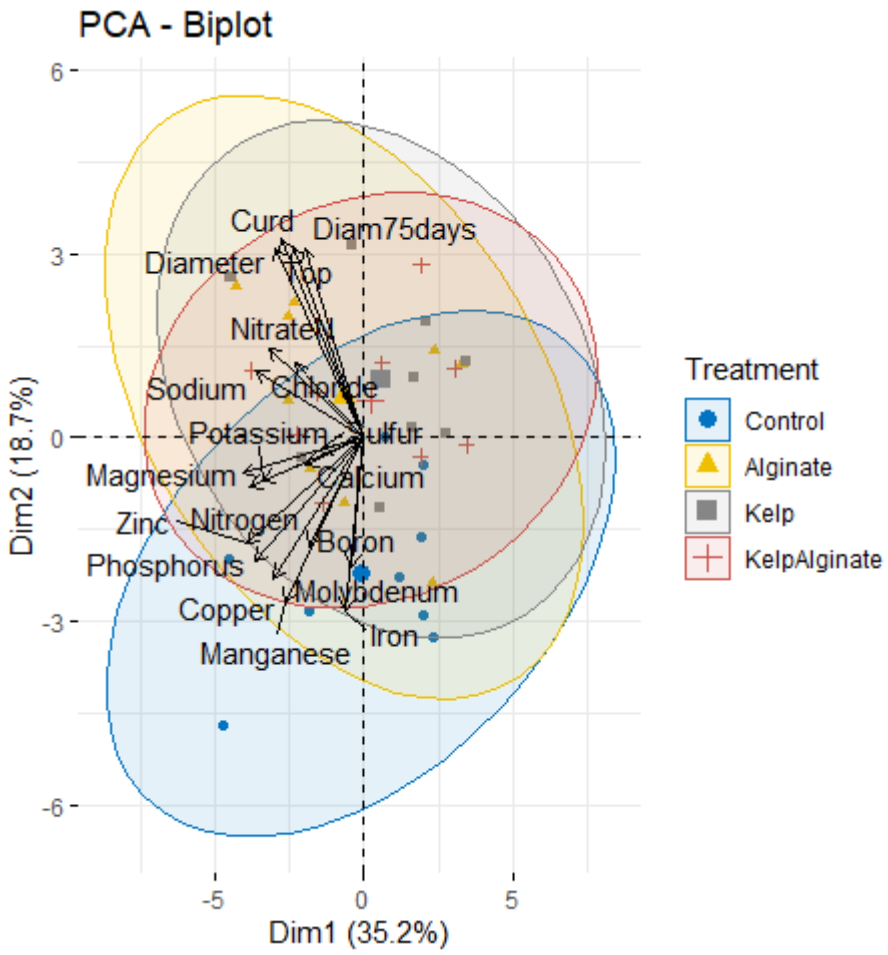


***Fig 5**. Principal component analysis biplot of measured variables, including parameters of broccoli growth and concentration of elements within the broccoli curds. The ellipses represent 95% confidence limits about the treatment mean.*

Discussion

In this experiment, the addition of kelp extract to the soil resulted in a significant increase in broccoli production. Furthermore, the data presented supports the hypothesis that much of the kelp bio-stimulus is due to the alginate component of kelp. This reinforces the views presented by Craigie (2011) and Calvo et al (2014) that the focus of kelp research should be on the carbohydrate content of kelp, specifically alginates of the cell wall. The benefits of added kelp or alginate were seen throughout the growth of the broccoli plants, with a trend emerging in LAI after 16 days. At 75 days from planting, kelp, alginate and kelp plus alginate treatments had significantly greater mean curd diameter than for curds of the control plants. At harvest, broccoli plants treated with either kelp extract or alginate were bigger, and had larger, heavier, denser curds. The ratio of curd to plant weight (harvest ratio) was greater, so the plant production was more efficient. For all parameters, the combination of alginate and kelp gave no further increase in broccoli biomass or curd development. While 33% of the heads from the control plants were rated as "B" grade, and hence would suffer a price penalty in the marketplace, 78-100% of the treated plants had heads rated as "A" grade, although non-parametric analysis of the data indicated these differences in quality were not significant.

The results at this site are not consistent with the observed bio-stimulus to broccoli growth resulting from hormones present in the kelp extract. There were no significant differences between the treatments of kelp extract, alginate and kelp plus alginate. If there were effects due to kelp-derived hormones, then these effects would be reflected in the kelp treatment but not the alginate treatment. If bio-stimulus resulted from both the alginate and enhanced plant hormone activity, then the kelp plus alginate should have benefitted from the additional alginate and outperformed both the kelp and the alginate treatments.

The results at this site are also inconsistent with the hypothesis initially proposed by Stephenson (1968) that the alginates act predominantly as a chelating agent. If alginates had facilitated chelation, we would expect higher concentrations of metal nutrients in the treated plants. Concentrations were similar for all treatments. Because concentrations are similar, this does mean that the larger treated plants do have greater overall uptake, but this is consistent with improved physical soil conditions rather than improved chemical availability. Principal component analysis of the plant response and nutrient concentrations did not indicate a correlation between uptake of any of the elements and broccoli development at this site. This reinforces the hypothesis that the observed plant responses to kelp extracts or alginates were not due in part to improved nutrient uptake through the chelating capacity of the extracts. There is no indication that nutrients were limiting growth or development, and nutrient concentrations in the broccoli curd are not correlated with any of the growth parameters that were measured.

Overall, the data presented here are consistent with the response to the kelp extract treatments at this site being due to soil 'conditioning'. This data supports the original hypothesis of Milton (1952) that polysaccharides from the algal cell walls act in the soil to improve soil structure and hence aeration, and ultimately improving plant growth. Salts of alginic acid combine with the metal ions in the soil to form high molecular weight cross-linked polymers to improve soil crumb structure (Khan et al, 2009). The soil at this site has poor structural stability and is very prone to slaking (Tisdall and Oades, 1982). When the soil is rapidly wet, from rain or irrigation, the larger soil aggregates (2-10 mm) break down into smaller sized micro-aggregates (< 250 μm). This occurs because the larger aggregates are not strong enough to withstand internal stresses caused by the water uptake. Clay particles swell and trapped air from the pores is squeezed out from the soil. Detached soil particles settle into pores and cause surface sealing and reduce water infiltration into the soil. As a consequence, the surface soil becomes waterlogged and aeration is limited. Lack of oxygen is the major cause of limited plant growth in waterlogged soils (Setter and Belford, 1990). Low concentrations of oxygen in waterlogged soil decrease nutrient uptake by slowing root growth, lowering the availability of some nutrients and by reducing the energy available within the root for active uptake. When this soil dries, it sets hard and the growth of the roots is again strongly inhibited, due to the physical resistance to root development (Passioura, 1991). Poor root extension results in poor foraging ability, thereby limiting plant's capacity to access its requirements from the soil. (Morris and Daynard, 1978; Barraclough and Weir, 1988). Overall growth is reduced.

The problem of slaking in soils is reduced by organic matter. Polysaccharides in organic matter bind mineral particles into larger, stable aggregates in soils and thus prevent slaking, as well as slowing the rate of wetting (Tisdall and Oades, 1982). In this experiment, the alginates in kelp appear to be functioning in the same manner as the plant polysaccharides found in soil organic matter in stabilising the slaking soil. Taken together, we suggest

that the alginate, and the alginates within the kelp, are most likely enhancing broccoli production by improving soil structure.

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Appendix 1. Supplementary Data

Table S1*. Broccoli parameters measured, showing mean and standard deviation for each treatment.*

Parameter		Treatment			
		Control	**Kelp**	**Alginate**	**Kelp + Alginate**
LAI	**Mean**	0.08	0.1	0.1	0.09
	SD	0.02	0.02	0.02	0.02
	% Variation from control	-	21%	23%	19%
Curd Diameter 75 Days	**Mean**	4.8	6.3	6.2	6.1
	SD	1.9	1.2	1.1	1.2
	% Variation from control	-	31%	29%	27%
Curd Diameter at Harvest	**Mean**	11	13	13	13
	SD	2.4	2.2	2.3	2.4
	% Variation from control	-	18%	18%	18%
Curd Fresh Weight	**Mean**	174	278	273	264
	SD	64	116	95	72
	% Variation from control	-	60%	57%	52%
Total Top Weight (g)	**Mean**	428	575	581	601
	SD	97	180	154	117
	% Variation from control	-	34%	36%	40%
Harvest Index	**Mean**	5.8	7.6	7.6	7.6
	SD	0.39	0.47	0.46	0.44
	% Variation from control	-	21%	18%	13%

Table S2. *Nutrient analysis. Mean concentration and standard deviation of elements in the broccoli curds.*

Treatment	Elements							
	Nitrate N		Total N		P		K	
	Mean mg/kg	SD	Mean %	SD	Mean %	SD	Mean %	SD
control	225	289	4	0.46	0.73	0.07	4.8	0.45
Kelp	335	377	3.7	0.42	0.69	0.04	4.6	0.31
Alginate	551	523	4	0.57	0.72	0.06	4.6	0.28
Kelp + Alginate	336	317	3.9	0.54	0.7	0.04	4.6	0.31

Treatment	Ca		Mg		Na		Cl	
	Mean %	SD	Mean %	SD	Mean %	SD	Mean %	SD
control	0.56	0.04	0.21	0.02	0.07	0.01	0.48	0.14
Kelp	0.57	0.07	0.2	0.02	0.07	0.02	0.51	0.14
Alginate	0.59	0.06	0.21	0.03	0.08	0.03	0.59	0.09
Kelp + Alginate	0.56	0.05	0.2	0.02	0.07	0.02	0.47	0.09

Treatment	S		B		Cu		Zn	
	Mean %	SD	Mean mg/kg	SD	Mean kg/kg	SD	Mean mg/kg	SD
control	0.89	0.13	29	1.48	4.38	0.46	28.89	4.34
Kelp	0.86	0.09	29	1.94	3.81	0.25	26.22	2.68
Alginate	0.89	0.1	29	1.33	4	0.19	27.56	3.32
Kelp + Alginate	0.87	0.12	29	1.45	3.99	0.48	27.56	4.19

Treatment	Mn		Fe		Al		Mo	
	Mean mg/kg	SD	Mean mg/kg	SD	Mean mg/kg	SD	Mean mg/kg	SD
control	20	2.11	92	17	12.5	5.3	1.64	0.38
Kelp	19	1.73	73	7	10	2.7	1.48	0.29
Alginate	19	1.45	72	7	9	2.5	1.63	0.38
Kelp + Alginate	19	1.12	72	8	9.5	3.4	1.5	0.32

2.5 Summary of field broccoli experiments

The set of experiments described in this chapter was conducted primarily to study the impact of kelp extracts on the production of commercial broccoli. Although statistically significant effects of kelp were absent in all but one of the experiments, the combined results of these experiments are consistent with the view that kelp treatments can have a positive effect on broccoli, but this is dependent on multiple plant, soil, and environmental factors. In this context, the conditions for the first three experiments did not meet the requirements for a statistically significant effect to be observed, whereas the fourth experiment did. The possible reasons for this outcome are explored below.

In Experiment 1 there was no significant benefit to broccoli production or maturation rate for any of the five commercial kelps under the conditions and time frame at this site. When initiated, this experiment was envisaged as the "main experiment" in which relative differences among the kelp treatments would be gauged. The result of no kelp treatment providing a benefit relative to the control shifted the focus from comparing among the kelps towards searching conditions under which kelp may provide a benefit. It should be noted that for all treatments, including the control, the broccoli grown in Experiment 1 resulted in good yields by industry standards. This can be interpreted as indicating that there was an absence of major soil and nutrition limitations that the kelp extracts could have mitigated against. The implication is that the absence of an effect of kelp reflects the strong performance of the control treatment rather than a poor performance of the kelp treatments.

Experiments 2 and 3 were designed to explore interactive effects of kelp and the macronutrients P and N. By limiting macronutrients it was hoped to elicit interactions between nutrients and kelp. In Experiment 2 it was found that the broccoli responded to levels of applied P up to 33 kg ha^{-1}, which was a rate double that applied in Experiment 1 (16.5 kg ha^{-1}). The design of the experiment, and in particular the protocol used for harvest, meant that response was mainly seen in terms of faster maturity for the higher P addition rates. Higher rates of P resulted in greater LAI at 45 days, earlier curd emergence and earlier harvest. With lower levels of P added and for the control, broccoli plants eventually achieved similar yield, although later harvested plants tended to be poorer quality due to the changing weather conditions. In the context of the discussion of the lack of a limitation to broccoli growth in Experiment 1, the clear response to P in Experiment 2 is important. The fact that there was no significant interaction between added P and kelp provides strong evidence that, under the conditions of in this experiment, the kelp extract has not aided uptake of P bound in the soil. In Experiment 3, there was no significant interaction between added N and kelp.

Experiment 4 was designed to test whether a specific component of kelp extract – alginate – may be responsible for effects observed in the other experiments. Ironically, this was the only experiment of the four in which a clear effect of kelp was observed. In Experiment 4, the addition of either kelp extract or alginate extracted from kelp to the soil resulted in a significant increase in broccoli production, but there was not an additive effect when both were applied. These results support the hypothesis that much of the kelp bio-stimulus is due to the alginate component of kelp. The benefits of added kelp or alginate were seen throughout the growth of the broccoli plants, with a trend emerging in LAI after 16 days. At

harvest, treated plants were bigger, and had greater mass, and had heavier, denser curds. There was a 30% yield increase. Unlike Experiment 2, maturation of the broccoli was apparently not retarded by deficiency of available macronutrients and kelp and alginate treated and untreated broccoli matured evenly. The vegetative and reproductive development of the nourished broccoli appears to have responded to the ability of the treated plants to benefit from enhanced soil conditions. This experiment was harvested 87 days after planting. While the growing period from transplanting was much shorter for this experiment than for the other experiments, the seedlings were of the same age for each experiment and the later plantings spent more time in the punnet with little growth. All broccoli in Experiment 4, treated and untreated, reached harvest criteria at this time. The assessment of productivity in Experiment 4 was thus different to Experiments 1 to 3, where time to maturity was the indicator of plant growth. The implication is that improved yield in Experiment 4 is not related to nutrition.

Experiment 4 was adjacent to the two nutrient experiments but planted out 29 days later than Experiment 1 and 22 days later than Experiment 3. The differences in timing between Experiment 4 and the other experiments resulted in physical and environmental differences between the experiments. These differences included the time that the broccoli seedlings spent in the punnet, the water application before planting out and the temperature of the soil at the time of planting out. At the time of transplanting out seedlings for Experiment 4 the soil had already been waterlogged and compacted. The soil application of kelp extract acted upon this compacted soil rather than freshly tilled soil.

Differences in soil temperature can only be speculated upon, as these were not recorded. Moderate autumn temperatures would have been expected to have supported early broccoli development but not necessarily supported a response to kelp extract. Previous field experimentation with kelp extracts has noted interactions between kelp and seasonal conditions, especially temperature. Warman and Munro-Warman (1993) found in field experiments in Canada with a range of vegetables including beans, potatoes, cabbage, sweet corn and cucumbers that response to kelp extract was dependent upon soil temperature. They found that at soil temperatures of 18°C or lower, kelp extracts had positive effects on seed germination of pea and sweet corn seeds, but not at temperatures above 18°C. In Experiments 1 to 3 described here, ambient air temperatures at the site were of the order of 20°C. Soil temperatures were not recorded, but given the season and the ambient temperatures, they would have been reasonably expected to be in excess of 18°C. At the time of planting Experiment 4, ambient temperatures were cooler and soil temperatures would also have been cooler. Could soil temperatures have accounted for the differences in the impact of kelp in Experiment 4 compared to the other three experiments? This could be a topic for future work.

From these four experiments, it appears that under the growing conditions of this site, response to kelp only occurred under conditions of adequate nutrition. For this site, kelp addition did not appear to facilitate the extraction of tightly held P from the soil. Good yields by industry standards were achieved in Experiment 1, with no response to any of the kelp treatments. It was concluded that the addition of kelp to the poorly structured soil subject to waterlogging in Experiment 4 improved the soil conditions and enhanced the vegetative and reproductive development of the well-nourished broccoli.

Variability within treatments was relatively high for these experiments, even experiment 4. Because of the observation that plants growing in the localised waterlogged areas were stunted (Fig 2.0.3), the waterlogging could reasonably be expected to be contributing to the variation within treatments. Hence, waterlogging may have contributed to the lack of statistical differences in the field experiments. A glasshouse experiment (Chapter 3) was conceived to try to get around the effect of this and other small-scale field variation.

2.5.1 References

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Chapter 3

Pot experiment with broccoli

3.0 Pot experiment with broccoli

3.1 Introduction

In the field experiments with broccoli described in Chapter 2 there was considerable variation between replicates in a number of the measured parameters. It was hypothesised that small scale variation in microtopography and/or soil properties might be responsible for masking small effects of kelp extract applications. In particular, it was noted that small areas (one to several square metres) were susceptible to becoming waterlogged after larger rainfall events and poor performing plants tended to be located in the waterlogged areas where their roots were likely to be episodically deprived of oxygen. This chapter describes an experiment in which broccoli was grown in pots under controlled conditions in the glasshouse, using the same soil as used in the field experiment. It was expected that this would result in reduced variation between replicates and provide an opportunity to achieve statistical significance with smaller treatment effects. The experiment involved kelp x P treatments and was chosen based on the strong P response detected in the broccoli field experiment (Experiment 2). In Experiment 2, no significant kelp or P x kelp interaction was detected, but there was, for example, a non-significant trend of 1- 6% higher curd weight for the kelp treatments for each of the five P fertilization levels (Table 1, Section 2.2). The aim of the experiment described here was to test for a response of broccoli to kelp extract under controlled glasshouse/pot conditions using the same soil as used in the field experiments.

3.2 Materials and methods

Broccoli seedlings (var. Spinks, sourced from Lefroy Valley Seed Company) were purchased from Virginia Nursery. This variety is described by the producer as being suitable for transplanting in autumn – winter for target harvest in spring. Because of seasonality, the Prophet variety that was used in the field experiments described in Chapter 2 was not available when seedlings were purchased.

Tapered plastic pots, 12 cm in diameter and 12 cm high, holding 1.2 kg of soil collected from the site adjacent to the broccoli field experiment site (Chapter 2) were prepared for broccoli propagation. Soil was collected from the top 10 cm of soil in the field, dried at 60°C for 3 days, mixed thoroughly and sieved to less than 2 mm. The soil was found to have a field capacity (FC) of 0.25 g/g and wilting point (WP) of 0.07 g/g.

Experimental design

The experiment included 13 rates of P addition (MAP added at rates equivalent to 0, 5.5, 11, 16.5, 22, 27.5, 33, 38.5, 44, 49.5, 55, 60.5, 66 kg P ha^{-1}). These rates cover the range of P treatments in Experiment 2 in the field and were chosen in order to identify possible correlation between added P and broccoli development and possible interaction between kelp and P. Two replicate pots were treated with Kelp E at a rate equivalent to 50 L ha^{-1} and two control replicates received the equivalent volume of water. Nitrogen was added to each pot in the form of urea to achieve an overall rate of added N equivalent to 140 kg ha^{-1}. At the commencement of the experiment, one broccoli seedling was planted in each pot. The position of the broccoli pots in the glasshouse was randomised weekly.

While the field experiment included 120 broccoli plants, with five rates of P x 2 kelp treatments (with and without added kelp extract) x 12 replications, this experiment included 52 plants (13 rates of P x 2 kelp treatments x 2 replications). It was anticipated that more rates of P would improve the chances of detecting an interaction, while the more homogenous nature of the glasshouse and hence lesser coefficient of variation, would compensate for the reduced number of replications. The experiment was also designed to be analysed differently, with a more or less "continuous" variation in P rate with minimal (i.e., 2) replications. This design effectively precludes statistical comparison of individual P rates to each other, but the large number of P rates along with the expected lower random variability should facilitate detection of the "shape" of the P response and detect interactive effects in the case that such effects only occur over a limited range of P addition rate.

Environmental conditions in the glasshouse

This experiment was conducted at the Waite Research Institute, in Glasshouse 7 (GH7) of the glasshouse facilities managed by the South Australian Research and Development Institute (SARDI), commencing 7 May 2021. Temperature in GH7 is controlled, nominally between 17 and 22°C. When outside temperatures exceed this range, the maximum temperature control is +/-10°C. Throughout the period of the experiment, the minimum temperature was below 7°C on 24 occasions. The lowest outside minimum temperature was 2°C on 27th August, so the glasshouse temperature was unlikely to fall below 12°C throughout the experiment. The highest maximum temperature was 31°C, so it is unlikely that the glasshouse exceeded 22°C during the course of the experiment.

Watering regime

At the commencement of the experiment, each pot was watered to 1.3 kg (gravimetric water content 0.204 g/g) and subsequently watered to this weight thrice weekly throughout the experiment. Water content was maintained between a minimum of 0.127 g/g and maximum of 0.204 g/g throughout the experiment, well within the "available" range between the WP (0.07 g/g) and FC (0.25 g/g). In the field experiment (Chapter 2) irrigation was supplied to ensure total water added (precipitation + irrigation) was 25 mm per week or higher. At times of high precipitation, the surface soil of the field site became waterlogged in patches. With the controlled watering regime in this experiment, waterlogging did not occur in the pots (Fig 3.1), and thus, was not a factor as it had been in the field (Chapter 2).

Fig 3.1 *Soil and roots of broccoli in pot at harvest (10.9.2021). Note that roots filled the entire pot volume and there was no indication of waterlogging or disease.*

Plant development

Broccoli seedlings were planted in pots in the glasshouse on 7 May 2021. Plant development was monitored in the same manner as the field experiment.

Leaf area (LA)

Leaf area was measured and assessed with ImageJ 21 days and 41 days after planting. This was before and after meiosis based on the method of D. Tan (Sydney University, *pers. comm.*) in which the timing of "cupping" of the broccoli leaves is used (Thistlethwaite et al, 2020).

Curd emergence, Flower emergence and harvest

Time to curd emergence was recorded. Curd emergence is used as a non-destructive approximation of curd initiation. As described in Section 2.2, curd initiation is a significant event in plant demand for P.

Broccoli plants in these pots were found not to develop as observed in the field. Differences between development in the glasshouse and in the field were noticed after about six weeks. With no significant difference between P and Kelp treatments at the six-week stage, it was decided to extend the experiment to continue observations. Time to flowering was recorded. Harvest of plants above ground occurred on 10 September 2021, 126 days after planting, when all plants had flowered. Above ground plant fresh and dry weights were recorded.

3.3 Results

LA at day 21 and day 41

Leaf Area (LA) was measured on 28 May 2021, 21 days after planting (Fig 3.2) No cupping had been observed, and the plants were assessed to be pre-meiosis (D. Tan, Sydney University, *pers. comm.*).

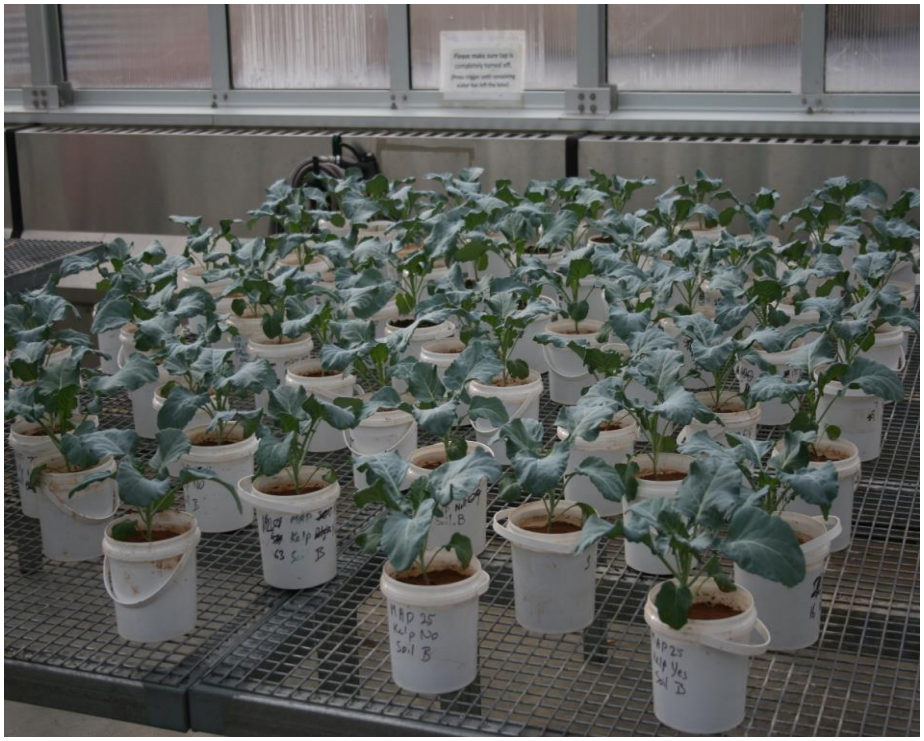

Fig 3.2 *Broccoli pots 21 days after planting. LA was assessed at this time. WRI glasshouse, 28 May, 2021*

No significant relationships were found between the rate of applied P and the leaf area (LA) 21 days after planting out the broccoli seedlings ($P > 0.05$), either without added kelp ($R^2 = 0.18$) or with added kelp ($R^2 = 0.11$) (Fig 3.3). No interaction was found between P

application rate and the addition of kelp extract (*P*= 0.5). The overall mean LA for all pots after 21 days from planting was 348 cm^2 (Table 3.1), so initial plant growth in the glasshouse was much more rapid than for broccoli in the field, which reached an LA of 225 cm^2 after 37 days. The average coefficient of variation (standard deviation divided by mean) for LA in the glasshouse was 10% after 21 days (Table 3.1), which was much lower than the average coefficient of variation for LA in the field after 37 days (42%; Table 3. 2).

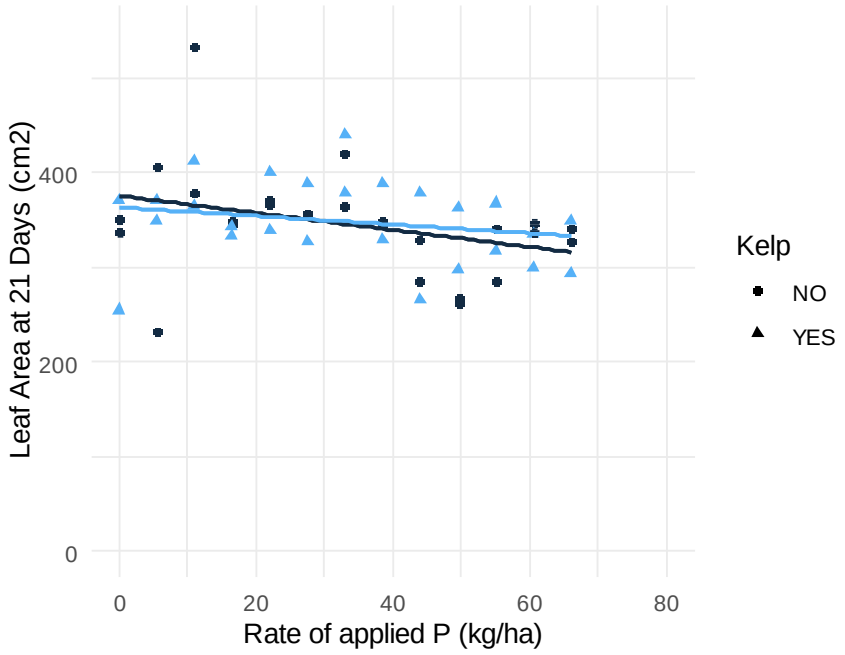

Fig 3.3 *Leaf area of broccoli plants after 21 days from seedlings being transplanted. No significant relationship (P > 0.05) without added kelp (R^2 = 0.18) or with added kelp (R^2 = 0.11).*

Broccoli grew substantially in the week prior to the 16 June 2021 (41 days) and it appeared at this time that the leaves had "cupped", indicating meiosis. No significant relationships were found between the rate of applied P and the leaf area after 41 days from planting out the broccoli seedlings and the application of kelp to the pots ($P > 0.05$), either without added kelp ($R^2 = 0.30$) or with added kelp ($R^2 = 0.03$) (Fig 3.4).

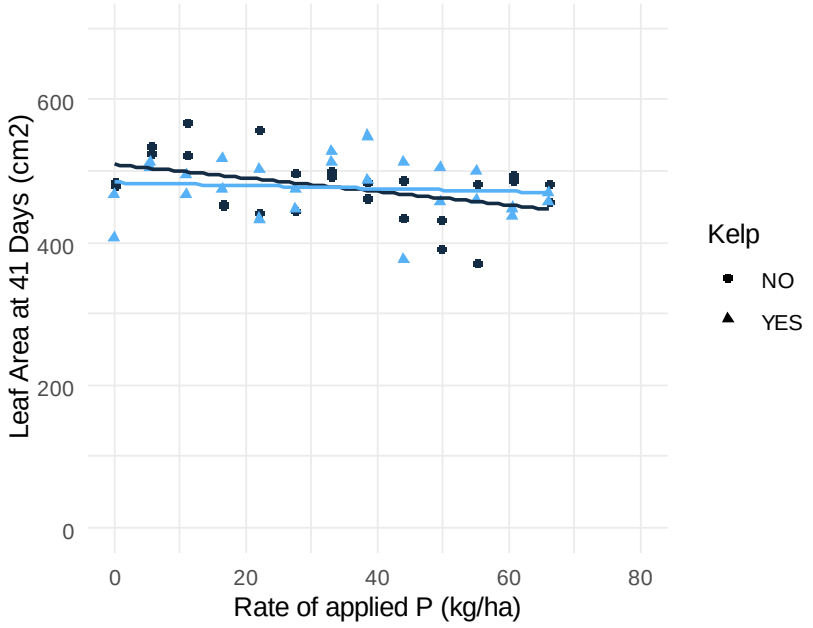

Fig 3.4 *Leaf area of broccoli plants after 41 days from seedlings being transplanted. This was deemed to be post "cupping". No significant relationships either without added kelp ($R^2 = 0.30$) or with added kelp ($R^2 = 0.03$).*

No significant interaction between P and the addition of kelp extract was detected ($P = 0.18$) Mean LA for all broccoli after 41 days in the glasshouse was 478 cm^2, while after 45 days in the field, mean LA for all broccoli was 348 cm^2. The average coefficient of variation in the glasshouse for LA was 8% after 41 days (Table 3.1.1), while the average coefficient of variation in the field for LA was 42% after 45 days (Table 3.1.2).

Table 3.1. *Leaf Area for broccoli in the glasshouse after 21 days and 41 days, showing rate of P and kelp addition. These timings were assessed to be pre and post meiosis.*

	Glasshouse broccoli LA 21 Days (cm^2)				**Glasshouse broccoli LA 41 Days (cm^2)**			
	No Kelp		**Added Kelp**		**No Kelp**		**Added Kelp**	
P	**Mean**	**CoV**	**Mean**	**CoV**	**Mean**	**CoV**	**Mean**	**CoV**
0	345	3%	313	26%	482	1%	436	10%
5.5	320	38%	360	4%	531	1%	509	1%
11	457	24%	389	9%	545	6%	481	4%
16.5	347	1%	339	2%	454	0%	496	6%
22	368	1%	370	11%	501	17%	468	11%
27.5	358	0%	358	12%	471	8%	460	4%
33	392	10%	409	11%	497	1%	520	2%
38.5	347	1%	359	11%	474	3%	518	8%
44	307	10%	322	25%	460	8%	445	22%
49.5	265	2%	331	14%	412	7%	480	7%
55	314	13%	343	11%	427	18%	480	6%
60.5	343	2%	318	8%	490	1%	443	2%
66	335	3%	322	12%	471	4%	464	2%
Mean	**346**	**8%**	**349**	**12%**	**478**	**6%**	**477**	**7%**

Table 3.2. *Leaf Area for broccoli in the field (2020) after 37 days and 45 days, showing rate of P and kelp addition.*

	Field broccoli LA 37 Days (cm^2)				**Field broccoli LA 45 Days (cm^2)**			
	No Kelp		**Added Kelp**		**No Kelp**		**Added Kelp**	
P	**Mean**	**CoV**	**Mean**	**CoV**	**Mean**	**CoV**	**Mean**	**CoV**
0	90	49%	132	47%	150	57%	200	48%
5.5	203	43%	177	34%	288	47%	273	40%
16.5	203	43%	249	49%	316	39%	321	26%
33	203	43%	368	22%	448	32%	514	22%
66	279	51%	346	39%	438	63%	537	39%
Mean	**196**	**46%**	**254**	**38%**	**328**	**48%**	**369**	**35%**

By 24 June 2021, 48 days from planting, it was clear that the broccoli plants were not developing as in the field.

Curd emergence

Curd emergence began for the broccoli plants on 6 August 2021 (82 days after planting) and curd emergence was complete for every plant by 15 August 2021 (91 days after planting). No significant relationships were found between the rate of applied P and the number of days from planting out the broccoli seedlings to curd emergence ($P > 0.05$), either without added kelp ($R^2 = 0.09$) or with added kelp ($R^2 = 0.004$) (Fig 3.5). No interaction was found between P application and the addition of kelp extract ($P = 0.44$).

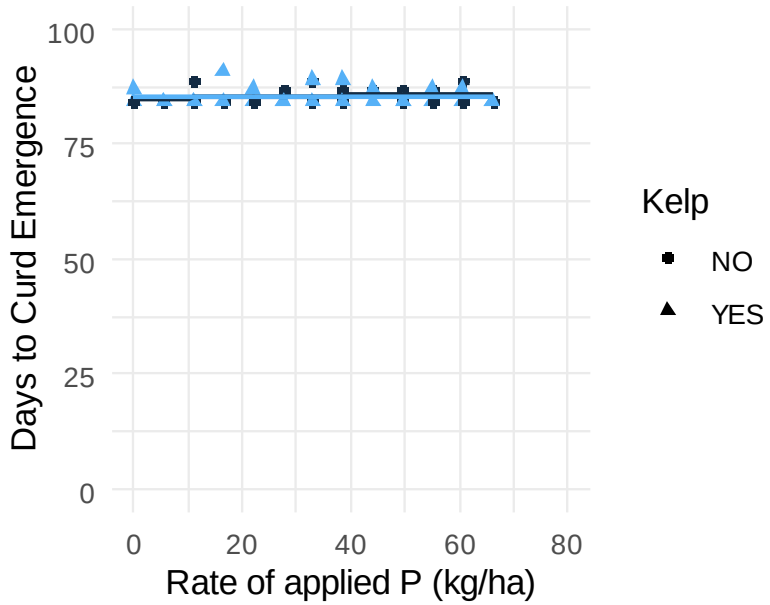

Fig 3.5 *Days to curd emergence for broccoli in the glasshouse. No significant relationships either without added kelp ($R^2 = 0.09$) or with added kelp ($R^2 = 0.004$).*

While the field broccoli showed significant differences in timing of curd emergence for P treatments and the glasshouse broccoli showed no such differences, the overall mean time to curd emergence for the glasshouse (Table 3.3) and the field (Table 3. 4) was 86 days. The average coefficient of variation for timing of curd emergence in the glasshouse was 2% (Table 3.3) and 10% for the field (Table 3.4).

Table 3.3. *Days to curd emergence for broccoli in the glasshouse, showing rate of P and kelp addition.*

	Glasshouse broccoli Curd Emergence (Days)			
	No Kelp		**Added Kelp**	
P	**Mean**	**CoV**	**Mean**	**CoV**
0	84	0%	86	2%
5.5	84	0%	84	0%
11	87	4%	84	0%
16.5	84	0%	88	6%
22	84	0%	86	2%
27.5	87	0%	84	0%
33	87	4%	87	4%
38.5	86	2%	87	4%
44	87	0%	86	2%
49.5	86	2%	84	0%
55	86	2%	86	2%
60.5	87	4%	86	2%
66	84	0%	84	0%
Mean	**86**	**1%**	**86**	**2%**

Table 3.4. *Days to curd emergence for broccoli in the field, and coefficients of variation, showing rate of P and kelp addition.*

	Field broccoli Days to Curd emergence			
	No Kelp		**Added Kelp**	
P	**Mean**	**CoV**	**Mean**	**CoV**
0	107	8%	107	17%
5.5	90	14%	94	17%
16.5	81	6%	87	13%
33	75	5%	74	5%
66	75	7%	74	5%
Mean	**86**	**8%**	**87**	**11%**

Flower emergence

With no significant difference between P and kelp treatments at curd emergence, it was decided to extend the experiment through to flowering. Plants first commenced flowering on 3 September 2021, 110 days after planting, without the curds developing into harvestable broccoli. By 17 September 2021 (124 days) all plants had commenced flowering. Linear regression (Fig 3.6) showed that for broccoli in the glasshouse, days to flowering was not related to the level of added P, either without added kelp ($R^2 = 0.06$) or with added kelp ($R^2 = 0.03$). No interaction was found between P application and the addition of kelp extract (*P*= 0.74).

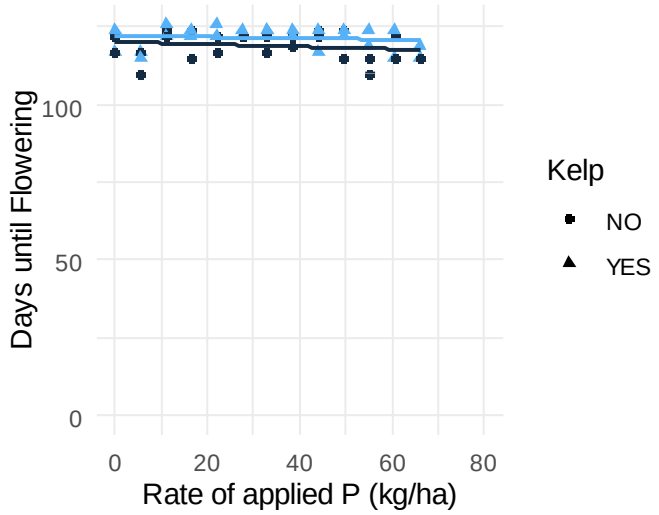

Fig 3.6 *Days to flower emergence for broccoli in the glasshouse. No significant relationships either without added kelp (R^2 = 0.06) or with added kelp (R^2 = 0.03).*

The overall mean days to flower emergence for the glasshouse (Table 3.4) was 121 days. The average coefficient of variation for days to flower emergence in the glasshouse for LA was 3%.

Table 3.4. *Days to flower emergence for broccoli in the field, and coefficients of variation, showing rate of P and kelp addition.*

	Glasshouse broccoli Flower Emergence (Days)			
	No Kelp		**Added Kelp**	
P	**Mean**	**CoV**	**Mean**	**CoV**
0	120	3%	121	4%
5.5	114	4%	116	1%
11	123	1%	124	2%
16.5	120	5%	123	1%
22	120	3%	124	2%
27.5	122	0%	123	1%
33	120	3%	124	0%
38.5	121	2%	123	1%
44	123	1%	121	4%
49.5	120	5%	123	1%
55	113	3%	122	3%
60.5	119	4%	120	5%
66	115	0%	117	2%
Mean	**119**	**3%**	**122**	**2%**

Plant harvest

On 10 September 2021, 126 days after planting, broccoli plants were cut at ground level and plant fresh weight recorded. Plants were subsequently dried and weighed again.

Linear regression (Fig 3.1.6) showed a negative relationship between the rate of applied P and the fresh plant weight (FPW) at harvest ($P < 0.05$), but no significant response to the application of kelp extract ($P = 0.1$). Specifically, a 0.07% decrease in FWP was found for every 1% increase in P. There was no significant interaction between P addition rate and the addition of kelp extract ($P = 0.15$). The linear correlation for both no added kelp ($R^2 = 0.56$) and added kelp treatments ($R^2 = 0.37$) (Fig 3.7) were moderate.

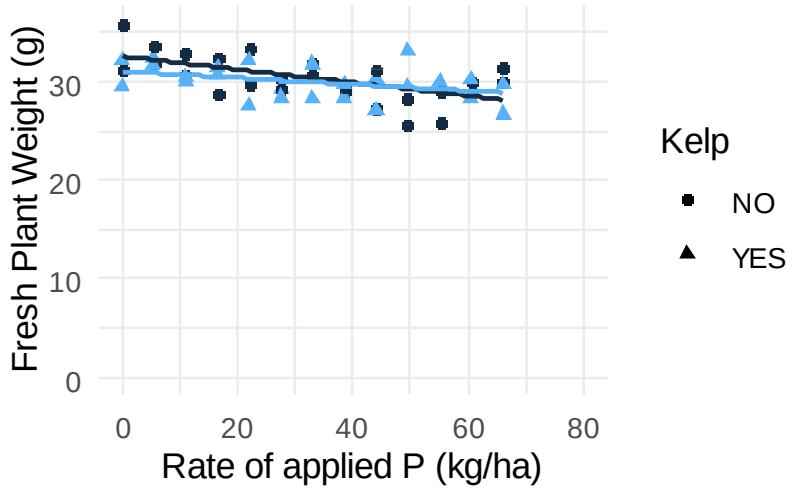

Fig 3.7 *Fresh Plant Weight for broccoli in the glasshouse. The negative response to added P is significant (P<<0.05) but the relationships either without added kelp extract ($R^2 = 0.56$) or with added kelp extract ($R^2 = 0.37$) are not strong.*

The overall mean FPW for the glasshouse after 126 days of growth (Table 3.5) was 30 g. The mean coefficient of variation for FPW for the glasshouse was 6%.

Table 3.5. *Plant fresh weight for broccoli in the glasshouse, and coefficients of variation, showing rate of P and kelp addition.*

	Glasshouse broccoli Fresh Weight at harvest (g)					
	No Kelp			**Added Kelp**		
P	**Days to harvest**	**Mean weight (g)**	**CoV**	**Days to harvest**	**Mean weight (g)**	**CoV**
0	126	34	10%	126	31	6%
5.5	126	33	3%	126	32	2%
11	126	32	5%	126	30	1%
16.5	126	31	9%	126	31	2%
22	126	32	9%	126	30	11%
27.5	126	30	2%	126	29	2%
33	126	31	3%	126	30	8%
38.5	126	29	2%	126	29	3%
44	126	29	9%	126	29	8%
49.5	126	27	7%	126	31	8%
55	126	28	8%	126	30	0%
60.5	126	29	2%	126	29	5%
66	126	31	4%	126	28	8%
Mean	**126**	**30**	**6%**	**126**	**30**	**5%**

The harvest dates for broccoli in the field were based upon maturity of the curd, and prior to the flowering stage. The mean number of days to harvest was 113 (Table 3.6), and mean FPW of harvested broccoli was 880 g, with an average coefficient of variation of 25%.

Table 3.6 *Plant fresh weight for broccoli in the field, and coefficients of variation, showing rate of P and kelp addition.*

	Field broccoli Fresh Weight at harvest (g)					
	No Kelp			**Added Kelp**		
P	**Days to harvest**	**Mean weight (g)**	**CoV**	**Days to harvest**	**Mean weight (g)**	**CoV**
0	126	765	30%	127	878	40%
5.5	114	931	34%	117	918	17%
16.5	110	836	21%	111	913	19%
33	107	878	24%	105	913	21%
66	108	831	27%	104	931	20%
Mean	**113**	**848**	**27%**	**113**	**911**	**23%**

3.4 Discussion

This experiment was an attempt to further explore aspects of the findings from the field experiment in which the response of broccoli to a range of P additions, with and without applied kelp extract, was studied (Section 2.2). While the progression of broccoli plants in this experiment was abnormal, the objective of decreasing random variation was achieved in the glasshouse as compared to the field. Average coefficient of variation for measurements of leaf area in the field were of the order of 50%, compared with less than 10% in the glasshouse. Average coefficient of variation for days to curd emergence were of the order of 10% in the field but 1% and 2% in the glasshouse. For plant fresh weight, mean coefficients of variation were 27% and 23% compared with 6% and 5% in the glasshouse. Overall, this suggests that approximately 80% of variation among replicates in the broccoli field experiment, across a range of parameters, was due to small-scale variation of soil condition in the field plot.

Unfortunately, the broccoli production in this glasshouse experiment was too different from production in the field to generate meaningful information on broccoli growth beyond 6-8 weeks, which roughly coincides with initiation of meiosis. Broccoli growth in the glasshouse was initially much faster than in the field. Mean LA in the glasshouse was 348 cm^2 after 21 days while broccoli in the field reached mean LA of 225 cm^2 after 37 days (Table 3. 2). Mean LA after 41 days in the glasshouse was 478 cm^2, while after 45 days in the field, mean LA was 348 cm^2. So, at 21 days, plants in the glasshouse had grown at a rate of 16.6 cm^2 per day, and by 41 days, this had decreased to 11.7 cm^2 per day. The plants in the field were growing at 6.1 cm^2 per day after 37 days and this had increased to 7.7 cm^2 per day after 45

days. The glasshouse is designed to maintain temperatures as much as possible between 17°C and 22°C, while Fig 2.1.2 shows that in 2020 minimum temperatures in the field ranged between 1 and 15°C, never reaching 17°C. The much warmer temperatures in the glasshouse explain the early faster growth of the broccoli in the glasshouse compared to the field. Another important difference between the glasshouse and field experiment was the response to P. In the field, increasing available P to the broccoli plant significantly increased leaf area by 45 days and plants with high available P developed curds earlier and reached harvest size earlier. In the glasshouse, there was no positive response of LA to P.

Interestingly, the mean number of days to curd emergence was 86 days for both the field and for the glasshouse, although given the contrasting growth patterns, this is coincidental. In the field, curds emerged significantly ($P < 0.05$) earlier for broccoli with high rates of added P and later for broccoli with low or no added P. In the glasshouse, there was no significant difference in timing to curd emergence, regardless of added P. Plants failed to progress “normally” beyond curd emergence and did not develop harvestable curds.

Broccoli plants in the glasshouse were very undersized at harvest. The glasshouse plants were harvested after 126 days, and mean plant fresh weight was 30 g, just 3% of the fresh weight of plants harvested in the field after an average of 113 days. Plant growth in the glasshouse showed a slight negative response to added P at harvest, the opposite response to the field, where there was a significant positive response to overall production for higher available P. This very different response to P may infer poor assumptions regarding the relevance of the rate of P addition in the pot compared to the field. There is much greater contact of plant roots to added P in these small pots.

A potential cause of, or at least contribution to, the aberrant progress of the broccoli plants in the glasshouse is the absence of cold conditions. Although broccoli crops are grown as annuals, broccoli plants are biennial plants. They require low temperature exposure (vernalisation) to induce them to flower (Heisswolf et al, 2004). The vernalisation requirement prevents the biennial plant from flowering at an inappropriate time. Boersma (2009) described broccoli as a "facultative plant with a preference for after effect vernalisation". That is, vernalization of the broccoli seedling causes early flowering, whereas an obligate plant requires vernalization for a time period to induce flowering. Fujime and Hirose (1980) concluded in their work that the cold stimulus in broccoli plants is cumulative for curd production and reduced but not nullified by a subsequent high temperature. The cardinal vernalisation temperatures required for broccoli appear to be cultivar dependent (Farnham and Bjorkman, 2011). There is no documentation for the cardinal requirements for the cultivar used here (Spinks), but Phillips et al (2020) of the Michigan State University Extension Unit have a general recommendation for the ideal temperature schedule for raising broccoli. They suggest initially maintaining broccoli seedlings above 21°C in the greenhouse for establishment, then transplanting them early enough to accumulate vernalizing temperatures at night, with warmer daytime temperatures. This will encourage vegetative growth over a few weeks to initiate flower formation at a gradual pace. Broccoli seedlings are considered receptive to vernalisation from beyond the four-leaf stage. The cardinal temperature range given for vernalisation is from a minimum of 0°C to a maximum of 20°C, with an optimum temperature of 5°C. Two to four weeks exposure is required. The glasshouse certainly did not meet these requirements, so optimum vernalisation requirements were not met.

Besides poor conditions for vernalisation, there were likely to have been other aspects of growth conditions that were sub-optimal in this glasshouse experiment. It is difficult to replicate the environmental and climatic conditions of the field in the glasshouse. Soil structure in the pot is not the same as in the field. Limpens et al (2012) found that in their studies of carbon sequestration capacity of peat bogs that glasshouse experiments were not reliable proxies for field experiments due to differences in the behaviour of N. They concluded that while glasshouse experiments were useful for studying some interactions, field experiments were needed in order to properly quantify responses of plants to N. Forero et al (2019) were even more emphatic in the shortcomings of glasshouse experiments not being correlated with field experiments. In their studies of plant-soil feedbacks, they employed five different studies in both greenhouse and field conditions. They found that for 36 plant species, values measured in the greenhouse were not positively correlated with values measured in the field. They discuss morphological and physiological responses of plants to varying container sizes and the consequences of root restricting conditions. However, they note that differences for species in varying container sizes have not been thoroughly explained. It would be interesting and beneficial to conduct further experimentation with broccoli propagated in larger pots and implement the recommendations of Phillips et al (2020) of the Michigan State University to allow the seedlings a vernalisation period.

3.5 References

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Chapter 4

Growth and production of tomatoes in the greenhouse

4.0 Growth and production of tomatoes in the greenhouse

4.1 Introduction

There are many reports of tomato plants (*Solanum lycopersicum*) benefitting from treatment with kelp extracts (e.g. Blunden, 1972; Khan et al, 2009; Calvo et al, 2014). Benefits have been observed throughout the plant and fruit development stages and under varying regimes of soil type, nutritional status and disease pressure. Responses have been observed for either soil drench or foliar spray applications. These benefits have been observed for a range of kelp products from different kelp species and extraction methods. There are no recorded comparisons of the commercially available kelp products.

Crouch and van Staden (1992) reported that a kelp extract from the brown kelp *Ecklonia maxima* significantly improved the growth of tomato seedlings when applied as a soil drench but not as a foliar spray. Then, typical of inconsistencies and variabilities observed with kelp, in a second experiment with a foliar application of 0.4% concentration of the same extract, a significant response was achieved. This time they found that in response to foliar application, tomato fruit ripened significantly earlier, the number of tomatoes harvested was improved by approximately 10% and total fruit fresh weight was increased by 17% over the control. The foliar application also increased flower numbers, root:shoot ratios, biomass accumulation in tomato seedlings and reduced transplant shock in seedlings. In a previous study, Crouch et al (1990) attributed a response in tomato production to *E. maxima* to an increase in Mn uptake. An extract from the same kelp (*E. maxima*) has also

been reported to trigger early flowering and fruit set in a number of other crop plants (Featonby-Smith and van Staden, 1987; Arthur et al, 2003).

Ali et al (2015) reported on the benefits of foliar and soil drench applications of an extract from the brown kelp *Ascophyllum nodosum* on tomato plants grown under tropical field conditions. They observed significant increases in plant height, root size and fruit yield. In a greenhouse experiment, fruit from foliar treated plants showed significant increases in size, colour, firmness, total soluble solids, ascorbic acid levels and nutrient levels. Treated tomato plants had larger root systems and increased mineral concentrations in the shoots. Whapham et al (1993) reported on the positive effect of soil or foliar applied *A. nodosum* extract on chlorophyll content of tomatoes and speculated that this was due to reduced chlorophyll degradation in the leaves. Eyras et al (2008) found a beneficial effect of a seaweed compost extract from *A. nodosum.* Total weight and number of tomatoes, and vegetative plant biomass were significantly higher for the compost treatments than those of the control. They attributed the increased production of seaweed-treated plants to a combination of higher nutrient availability (mainly P together with increases in readily available K), slight increases in pH of the soil and improved soil physical conditions through increased pore size. Kelp treated plants bore mature fruits, on average, nine days earlier and presented higher resistance to diseases than controls. Arioli et al (2015) reported on the benefit of seaweed extract comprising *A. nodosum* and *Durvillae potatorum* on the development of tomato roots.

Beneficial results have been reported for tomatoes from many other brown kelp species: *Sargasssum crassifolium* (Sutharsan et al, 2014), *Sargassum johnstonii* (Kumari et al, 2011),

Gracilaria textorii (Rao and Chatterjee, 2014), *Hypnea musciformis* (Rao and Chatterjee, 2014), *Kappaphycus alvarezii* (Zodape et al, 2011), *Ulva lactuca* (Khan et al, 2009) and *Padina gymnospora* (Khan et al, 2009)**.** In these studies, responses of kelp applications by tomatoes have typically been associated with nutrient availability and uptake.

Kelp extracts have been shown to be beneficial in assisting tomato plants under disease pressure. Featonby-Smith and van Staden (1983) studied the effect of kelp extract on the growth of tomato plants in nematode-infested soil. An extract from *E. maxima* at a dilution of 1:500, applied as foliar applications or as a soil drench, significantly improved the growth of tomato plants. Root growth was significantly improved, and root-knot nematode infestation was reduced in all cases where the seaweed concentrate was applied. In their review, Kahn et al (2009) discussed some less consistent results and suggest that kelp extract may impart nematode resistance by altering the auxin: cytokinin ratio in the tomato plant. Wu et al (1997) report on suppression of root rotting fungi and root knot nematode in tomatoes through the application of *A. nodosum* extract.

Arbuscular mycorrhizas are known to have positive benefits for tomatoes (Cavagnaro et al, 2006) and there is evidence of kelp extract having a positive relationship with arbuscular mycorrhizas (Suhail, 2013). At the time of planning the research reported here, no studies had been reported of kelp and mycorrhizal associations improving tomato growth, but Suhail (2013) showed synergy between a kelp extract and arbuscular mycorrhizas when applied to cucumber (*Cucumis sativus L*.). Growth and yield of cucumber were significantly improved when the kelp product was applied to the leaves of cucumber growing in a clay loam soil inoculated with a mixture of fungus mycorrhiza (*Glomus fasciculatum +*

Acoulospora laevis). Reports of kelp extract addition affecting mycorrhizal associations in tomato have been published since the time that this experiment was designed (Hussain et al, 2021).

The work reported here was originally designed as two experiments, with the first dedicated to studying the interaction between kelp, and the colonisation of arbuscular mycorrhizas and the second to compare the effects of five commercial kelp extracts on the growth and development of tomato plants through to maturity.

In the first experiment, kelp extract was to be applied to the non-mycorrhizal tomato *rmc mutant* and its wild type parent *76R*. It was planned to implement a three-level factorial design, incorporating with or without kelp extract x with or without AMF inoculation x 2 tomato genotypes. It was planned to grow the tomatoes in an autoclaved sand: soil mix of 9-part fine sand and 1 part Waite Arboretum soil. The soil would have needed to be autoclaved to remove mycorrhizal colonization from the arboretum soil. The combination of sand and arboretum soil in preference to potting soil or soil from the cultivated field on Waite Campus used in the experiments described in Chapter 2 was to minimize the level of soil phosphorus (P). High levels of P discourage mycorrhizal colonization of the 76R genotype (Watts-Williams and Cavagnaro, 2012). Restrictions in place at the university due to Covid-19 curtailed these plans. With the restrictions, it was no longer possible to access facilities to autoclave the soil and to inoculate with AMF and thus no longer possible to study interactions between kelp and arbuscular mycorrhizas. With these restrictions and uncertainty in access to glasshouse facilities in early 2020 due to the COVID-19 pandemic, the experiment was modified (see below). Instead, one combined experiment was carried

out in which the effect of addition of a range of kelp extracts on the growth and development of mycorrhizal (76R) and non-mycorrhizal (*rmc*) tomato plants was assessed from seedlings through to the start of tomato production.

4.2 Materials and methods

A glasshouse experiment was carried out to compare the effect of the five commercial kelps that were used in the broccoli field experiments (Kelp extracts A, B, C, D, E in Chapter 2) on the production of tomato plants. The experiment was conducted as a randomised block design with twenty blocks. Within each block, there were six tomatoes in individual pots, including a control treatment and treatments of each of the five commercial kelps.

As discussed, prior to COVID-19 pandemic restrictions, two experiments were designed, one of which was to compare the effect of commercial kelp extract on the production of two tomato genotypes, a wild-type genotype, 76R, and a nearly isogenic non-mycorrhizal mutant, *rmc*, in the glasshouse. Seedlings of the two tomato genotypes had already been germinated, so all seedlings were incorporated into a single experiment to study growth and production of tomatoes under the various kelp regimes.

Kelp extracts

Four commercially available liquid kelp extracts (Kelp A, C, D and E) and one granulated commercial kelp extract (Kelp B) were compared with the control (equivalent volume of water added in place of kelp extract solution).

Kelp A. Extracted from the species *E. maxima*, using a cold cell burst extraction process. For vegetables, a fortnightly foliar application commencing at the 3 to 4 leaf stage at a rate of 2 L ha^{-1} is recommended, but there is no indication that higher rates are detrimental.

Kelp B. Extracted from the species *A. nodosum*, then dried and granulated. There is no specific recommendation for vegetables. There is a general recommendation of 0.3 to 1 kg ha^{-1}, but no indication that higher rates are detrimental.

Kelp C. Extracted from the species *A. nodosum*, using a hot caustic extraction process. For vegetables, it is recommended to apply a total of 10 to 18 L ha^{-1} as soil or foliar applications throughout the season. There is no indication that higher rates are detrimental.

Kelp D. Extracted from the species *A. nodosum*, using a fermentation process. Applications of 6 to 10 L ha^{-1} are recommended. There is no indication that higher rates are detrimental.

Kelp E. Extracted from a blend of *Durvillaea* species and *A. nodosum*, using a caustic extraction process. Foliar applications of 6 to 10 L ha^{-1} are recommended. There is no indication that higher rates are detrimental.

Protocol

The seeds of the two tomato genotypes, 76R and *rmc*, were aerated for 15 minutes in a 3% sodium hypochlorite solution, then rinsed for 60 minutes in deionised water. On 24 January 2020 (Day 1), the seeds were transferred into Petri dishes and placed in an incubator set at 25^{o}C to germinate. The germinated seeds were planted out into fine sand in seedling trays in the glasshouse. After 10 days, when seedlings were established, sixty plants of each genotype were dipped into respective solutions of either water (control) or 1% dilution of

respective kelp solutions. Following treatment, the seedlings were planted out into 120 x MK12 punnets (11 cm x 10 cm x 10 cm depth) (Fig 4.1) containing 200 g of the potting soil Biogro®. BioGro® is rich in nutrients (15N: 9P: 11K + 2 Mg + trace elements) and has a high water-holding capacity.

Pots were arranged randomly within blocks in the glasshouse and randomized again on a weekly basis. Each kelp treatment was applied as a soil application equivalent to 7 L ha^{-1} on a weekly basis until transplanting to larger pots.

Fig 4.1. *Young tomato plants growing in MK12 punnets, WRI glasshouse 7, February 2020.*

After 30 days the young tomato plants were transferred to larger (25 cm diameter × 25 cm depth) pots each containing 1.5 kg of a blend of 90% Biogro® and 10% Red Chromosol soil from the Waite Campus. Plants were randomised as before on a weekly basis and treated on a fortnightly basis with soil applications of 7 L ha^{-1} for each of the respective kelp

treatments. Records of flowering times, flowering numbers, fruit production (numbers and weight) and vegetative weights were taken as the plants developed.

Plant development and plant health

Mite infestation (Fig 4.2) was detected 22 April 2020 (Day 89) in plants at the northern end of the glasshouse and spread apparently randomly throughout the glasshouse. On 28 April 2020 (Day 95), all plants were sprayed with insecticide for mite control.

Fig 4.2 *Mite damage to tomato plant, WRI glasshouse 7, April 2020.*

On 2 May 2020 (Day 100) the symptoms of blossom end rot (BER) were observed, where the remnant blossom was retained on the fruit (Fig 4.3, 4.4) and the end of the fruit was soft and pale (Fig 4.4). BER is a physiological disorder of tomato in which the tissue of the blossom end of the fruit breaks down and rots, due to a lack of calcium being transported to the fruit. A common remedy for BER is a foliar application of Ca^{2+} (Taylor and Locascio, 2004). Tomatoes were sprayed to run-off with a foliar application of 10 gL^{-1} Ca^{2+} (as calcium nitrate) on 7 May 2020 (Day 105).

Fig 4.3 *Remnant blossom retained on immature tomato, WRI glasshouse 7, May 2020.*

Fig 4.4 *Tomato with BER symptoms. Remnant blossom and Blossom end of fruit soft and beginning to rot, WRI glasshouse 7, May 2020.*

On 31 May 2020 (Day 138), all tomatoes received a soil application of the equivalent of 100 kg ha^{-1} Mila complex for general nutrition and to provide Ca^{2+} to ensure that BER symptoms were controlled.

Assessment of mycorrhizal colonization

On 9 July 2020 (Day 167) soil samples were taken from a single block of each treatment (6 pots) to assess mycorrhizal colonization of the roots. A 10 cm soil core was removed from each pot in the block. The tomato roots were retrieved from soil cores by rinsing with RO water. Fresh roots were fixed in ethanol and then cleared with a 10 % potassium hydroxide (*w/v*) solution at room temperature for seven days. Cleared roots were stained with 5% ink in vinegar solution at 60^{o}C for 10 minutes (Vierheilig et al, 1998) before being de-stained in acidified water for 24 hours. Mycorrhizal colonization of the roots was then determined using the gridline intersect method for at least 100 intersections (Giovannetti and Mosse, 1980).

Harvest

On 20 August 2020 (Day 209) tomato fruit was harvested (Fig 4.5). Plants were cut at ground level, weighed and dried for 3 days at 60^{o}C. The fruit was segregated into immature tomatoes and the six maturity stages of tomatoes outlined by Cantwell (2010). These stages are based upon colour and appearance (Fig 4.6). The groups were counted and weighed.

Fig 4.5 *Tomato plant at harvest, WRI glasshouse 7, August 2020.*

Fig 4.6 *Example. Immature tomatoes and the six ripening stages of tomato (Cantwell, 2010).*

4.3 Results

Comparing 76R and *rmc* genotypes

The comparison of all pots in single block of the experiments showed no arbuscular mycorrhizal fungi (AMF) in the roots of either of the 76R or *rmc* tomato genotypes at Day 167. As discussed in the introduction, high levels of available P are not conducive to mycorrhizal colonization (Watts-Williams and Cavagnaro, 2012), and the nutrient rich potting soil used in this experiment was expected to not support colonisation.

Any productivity differences observed between the two tomato genotypes were found to be not significant ($P>0.05$). No significant differences were found between the two genotypes for Tomato number (Fig 4.7.a), Tomato vegetative plant weight (Fig 4.7.b) or total fruit weight (Fig 4.7.c). This result is supported by Cavagnaro et al (2006), who found no pleiotropic effects with the *rmc* mutation. Since neither of the two tomato genotypes hosted AMF under these conditions, and yields were not statistically different, then the two genotypes are effectively identical under the imposed conditions and so effectively n=20 for kelp treatments.

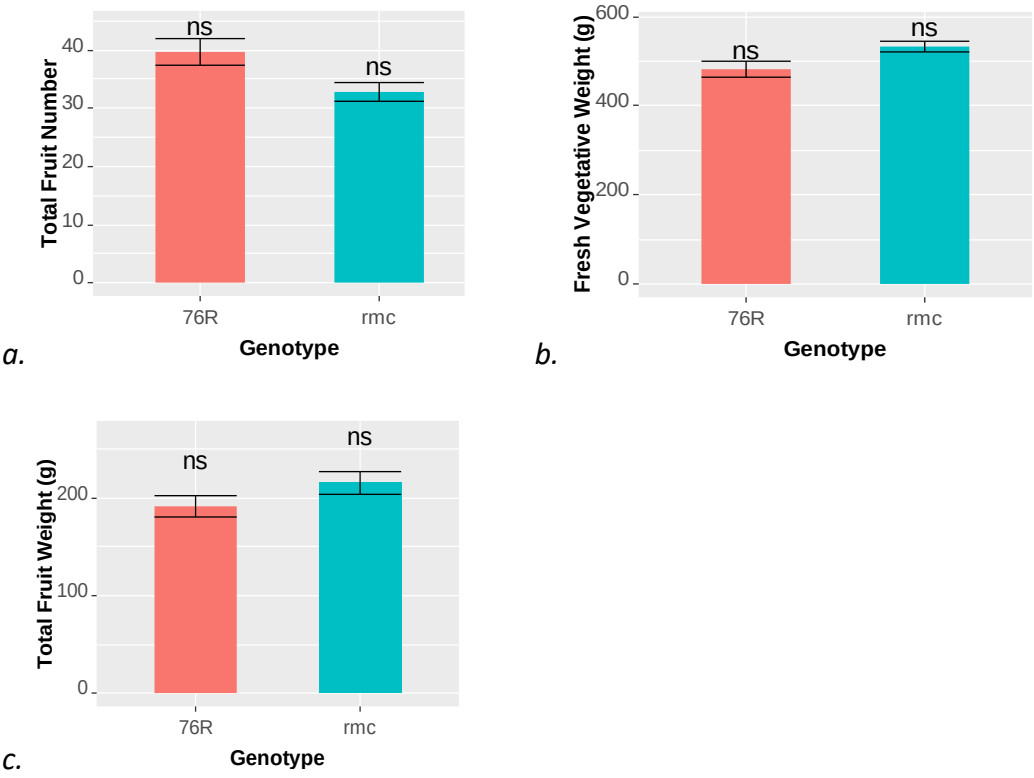

Fig 4.7 *Comparisons between the genotypes 76R and rmc at harvest. No significant differences (P>0.05) between the genotypes at harvest for a. Tomato number, b. Tomato vegetative plant weight or c. total fruit weight.*

Plant development and plant health

Flowers began to appear on Day 31 and the number of flowers increased until Day 50 (14 March 2020) after which they were no longer recorded. There were no significant differences in flower number among treatments during this time (Figure 4.8).

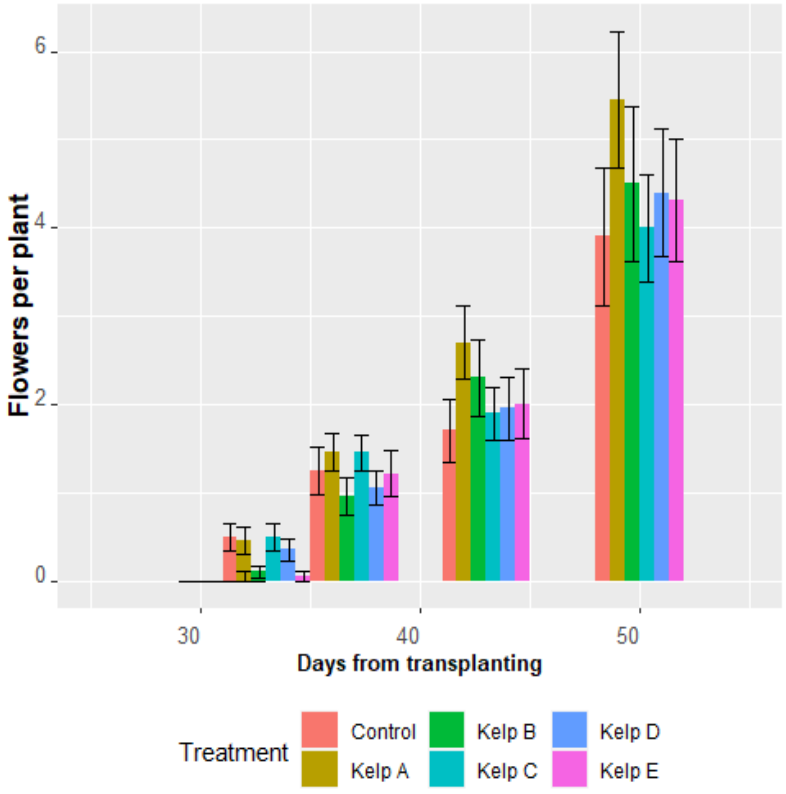

Fig 4.8 *Comparison of Flower numbers over 50 days. Flowering commenced after 30 days. At 31, 37, 43 and 50 days, no statistically significant differences (P>0.05) were found between the kelp treatments and control. Error bars indicate standard error (se).*

Fruit began to appear on Day 50. There were no significant differences (*P*<0.05) in fruit numbers between treatments at each weekly recording between Day 50 and Day 154, when fruit was harvested (Figure 4.9). No data was recorded on Day 141, due to personal Covid-19 restrictions at that time.

Note that this pattern of no significant differences continued following mite infestation detected 22 April 2020 (Day 89), BER symptoms detected on 7 May 2020 (Day 104) and calcium nitrate treatment for BER applied to plants on 15 May 2020 (Day 112). These factors affecting plant health did not cause significant differences between the kelp treatments and control.

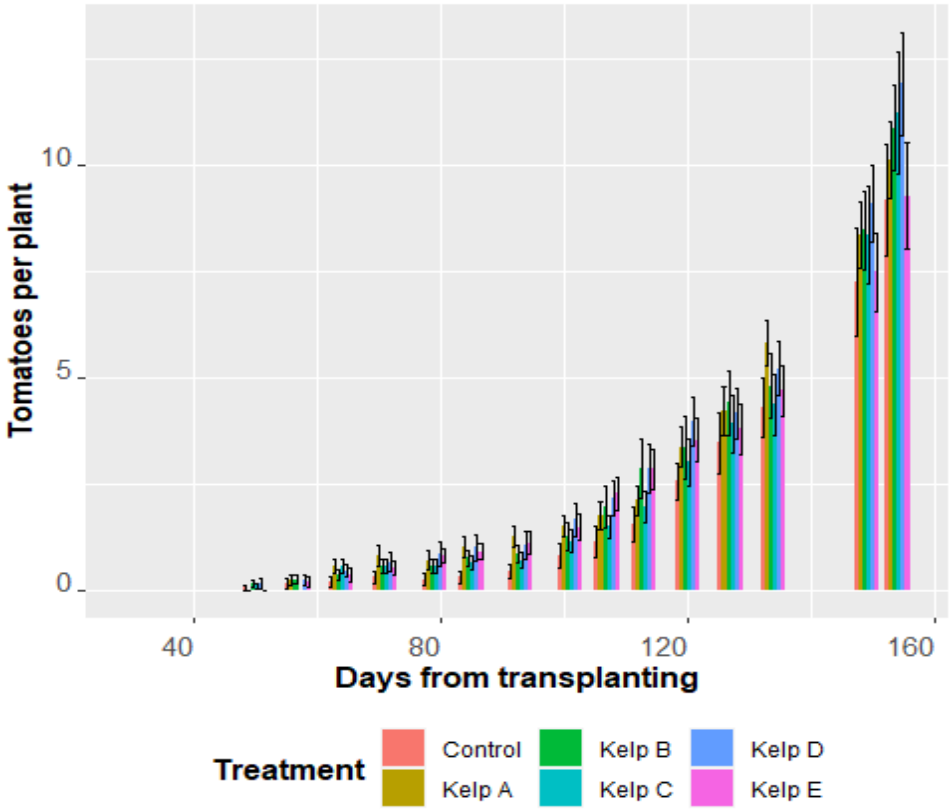

Fig 4.9 *Comparison between treatments of tomato numbers per plant at weekly intervals (Day 141 not recorded due to Covid-19 related restrictions). No significant differences (P>0.05) between the kelp treatments and control were observed. Error bars indicate standard error.*

Harvest

At harvest (Fig 4.10), there was no significant difference in tomato numbers between treatments. Neither were the numbers nor the weights statistically significant for any of the six ripening stages of tomato (Cantwell 2010) (Fig 4.11).

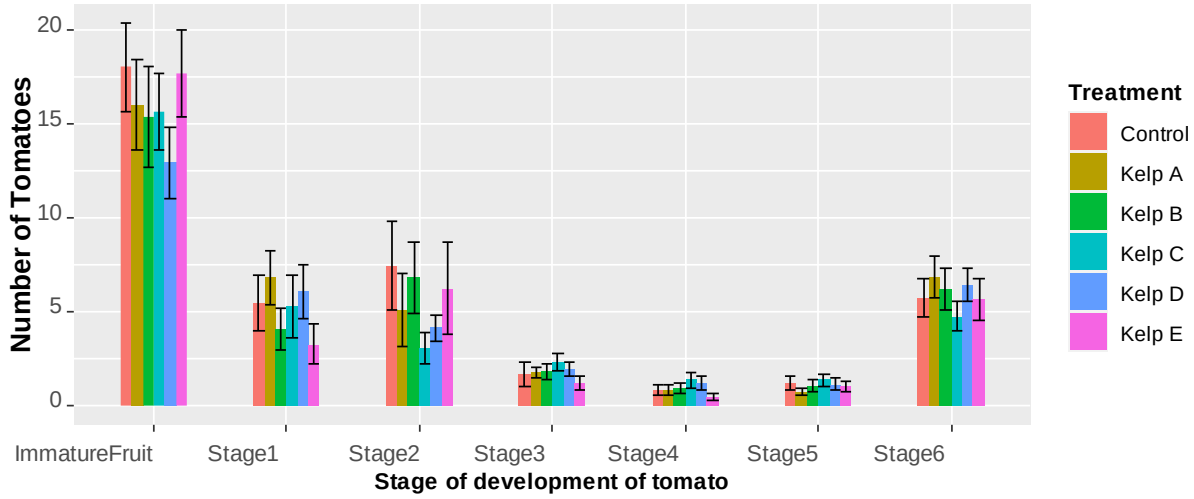

Fig 4.10 *Tomato numbers at harvest for immature fruit and each of the six stages of fruit development (Cantwell, 2010), showing no significant difference (P>0.05) between the kelp treatments and control. Error bars indicate standard error (se).*

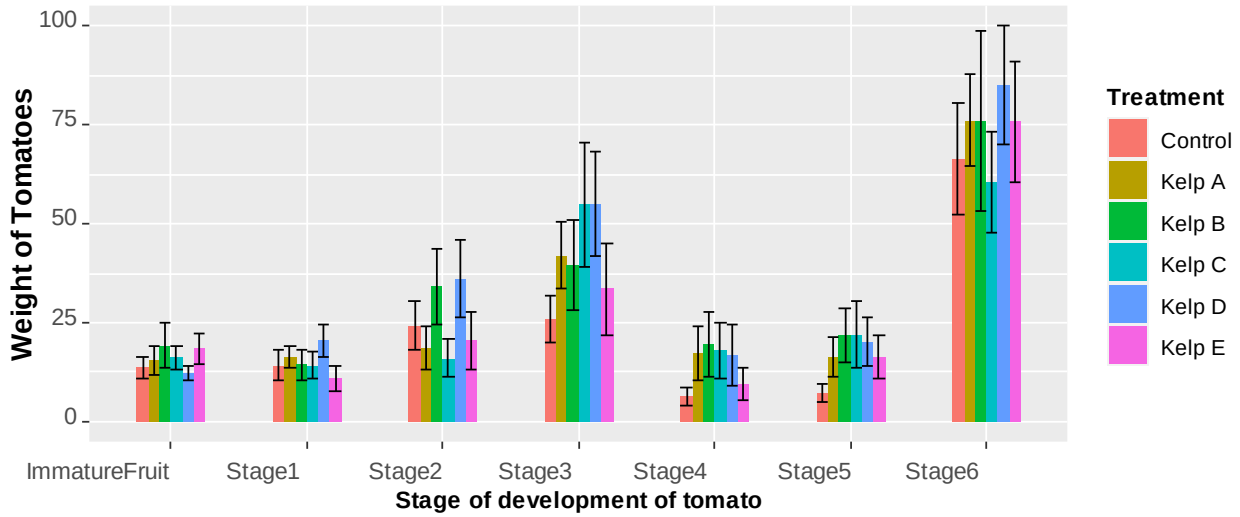

Fig 4.11 *Tomato weight at harvest for immature fruit and each of the six stages of fruit development (Cantwell, 2010), showing no significant difference (P>0.05) between the kelp treatments and control. Error bars indicate standard error (se).*

A significant increase in total fruit yield was seen at harvest. There was no significant difference (*P*>0.05) in the total number of tomatoes harvested (Fig 4.12.a), nor was there significant difference (*P*>0.05) in the vegetative weight of the tomato plants harvested (Fig 4.12.b). There was, however, a significant difference at harvest in the total weight of all tomatoes at all stages (Fig 4.12.c). The mean yield of each of the kelp treatments was greater than the control, with Kelp D showing a statistically significant increase (*P*<0.05) in the total weight of tomatoes. Mean yield for Kelp D was 56% greater than the control (Fig 4.12c). The harvest index for the tomato yield of Kelp D was 37% greater than the control, but this was not significant at the 0.05 probability level (Fig 4.12.d).

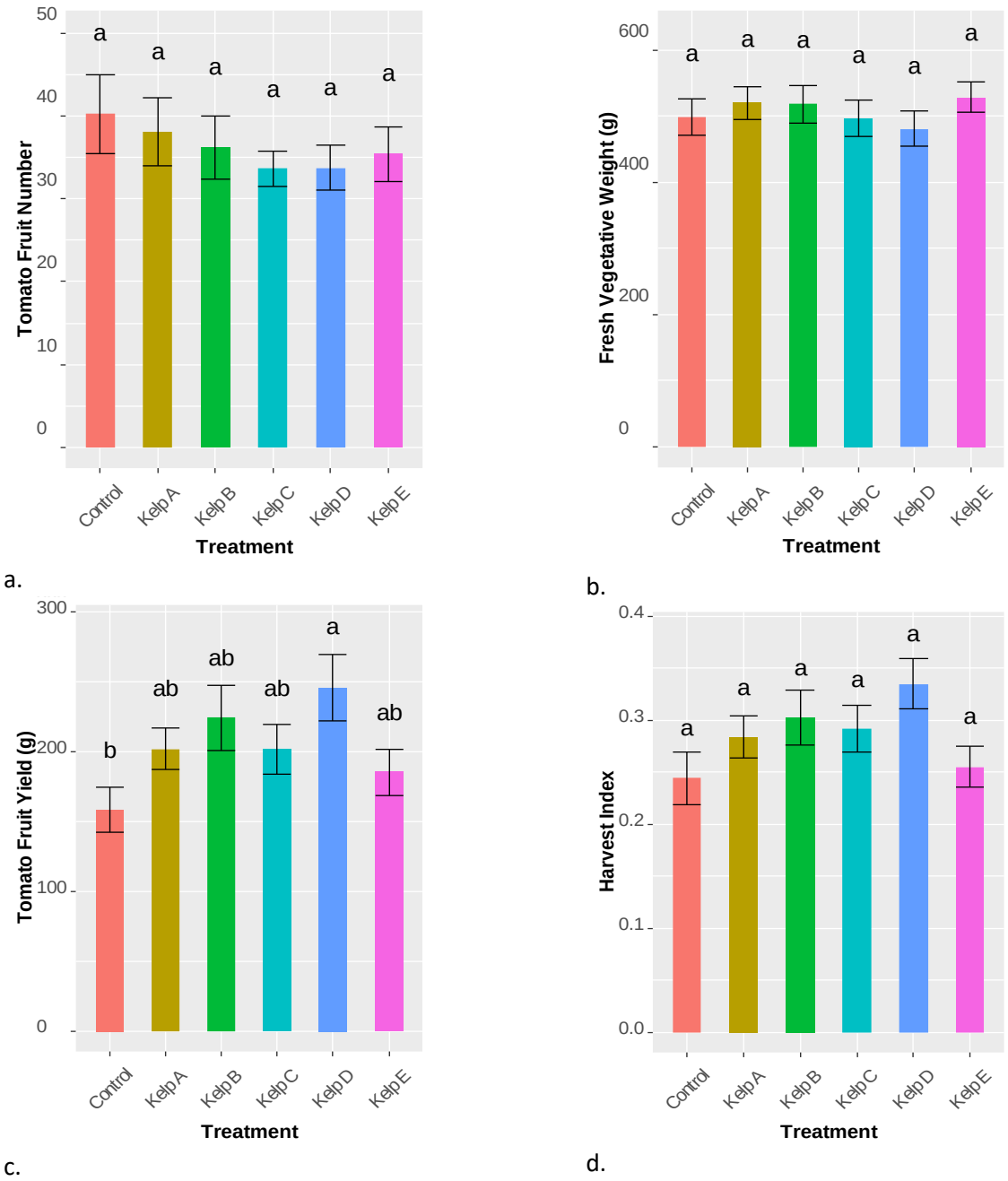

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Fig 4.12 *Tomato harvest; a. No significant difference (P>0.05) between the kelp treatments and control for the total fruit number; b. No significant difference (P>0.05) between the kelp treatments and control for vegetative weight of the tomato plants; c. Fruit production for tomatoes with Kelp D significantly greater than for control (P<0.05); d. No significant difference (P>0.05) between the kelp treatments and control for the Harvest Index. Error bars indicate standard error (se).*

Note that the coefficient of variation is very high for each of the measured parameters (Table 4.1).

Table 4.1 *Summary of tomato production at harvest, showing tomato numbers, weight and Harvest Index for control and kelp treatments*

	Total Fruit Number			**Total Fruit Weight**			**Harvest Index**		
	Mean	C of V (%)	Proportion of Control	Mean (g)	C of V (%)	Proportion of Control	Mean (ratio)	C of V (%)	Proportion of Control
Control	40	53	1	158	45	1.00	0.25	62	1
Kelp A	38	49	1.0	202	33	1.28	0.28	42	1.16
Kelp B	36	48	0.9	224	46	1.42	0.3	66	1.24
Kelp C	34	28	0.9	202	39	1.28	0.29	48	1.19
Kelp D	34	36	0.9	246	43	1.56	0.34	50	1.37
Kelp E	35	41	0.9	185	39	1.17	0.26	45	1.04

4.4 Discussion

At harvest, after 209 days of apparently no benefit from kelp treatments, a statistically significant increase in total tomato weight was found for tomatoes treated with Kelp D (*A. nodosum* extracted by fermentation) over the control. The plant weights were not significantly different, nor were there differences within the fruit ripening stages, but the total weight of tomatoes harvested for the Kelp D treatment was 56% greater than the control. Tomato yields treated with kelps A, B, C and E were also greater than control, but the differences were not statistically significant at the 0.05 probability level.

Whereas much research has found positive responses for a range of growth parameters (for example, Featonby-Smith and van Staden, 1983; Crouch and van Staden, 1992; Hussain et al, 2021), the observed significant response in this experiment was only for fruit weight at harvest. The variability in research outcomes further highlights the complexity of the activity of kelp extracts on plant growth. In the experiment reported here, there were no differences in flower numbers or fruit numbers, the plant weights were not significantly different, nor were there differences within the fruit ripening stages. The increase in fruit weight without an increase in plant size or fruit number indicates efficiency in allocation of the plant resources to fruit development, as indicated by the Harvest Index. The Harvest Index for the Kelp D treatment is 37% greater than the control.

The significant response in yield was for the treatment with Kelp D, but the other kelp treatments in the experiment also showed greater fruit weight than control. Given the high coefficients of variation for the kelp treatments, the significance for these treatments may

have been masked by the variation across the experiment. Sources of variation included, but were not limited to, the mite infestation and the occurrence of BER. It is worth noting that while kelp extracts have been found to protect plant species from disease and insect attack (e.g., Featonby-Smith and van Staden, 1983; Wu et al, 1997; Ali et al, 2015), there is no direct evidence of that occurring here. There were no significant differences in fruit production immediately following either the infestation with mites, or the occurrence of BER. Neither did the affinity of kelp alginate for Ca^{2+} interfere with the application of $Ca(NO_3)_2$ to treat for BER.

In the field experiment with broccoli, kelp and alginate (Chapter 2, Experiment 4), response to kelp extract was considered to be most likely due to the action of kelp alginates improving soil structure. In the experiment reported here, the tomatoes were grown in rich potting soil with good organic structure, so it seems unlikely that alginates would or could improve the structure of this soil. As discussed in the literature review (Chapter 1), many mechanisms for kelp extract activity have been reported. For many years phytohormones, including auxins and cytokinins, have been implicated in plant growth responses to kelp extracts (Blunden, 1972; Calvo et al, 2014). Plant hormones are contained within kelp. If indeed increased hormonal activity is responsible for stimulated tomato fruit growth, then it may be that that it is caused by incremental activity of hormones from the kelp. For this to occur, sufficient quantities of active phytohormones would need to be present in the kelp being processed and survive the extraction process employed for the kelp product. However, Tay et al (1985) concluded that the levels of cytokinins in kelp were not sufficient to produce the beneficial effects reported in their work.

There is recent work (Ali et al, 2019) that indicates that *A*. *nodosum* extract upregulates genes involved in hormone production resulting in increased auxin (*IAA*), gibberellin (*Ga2Ox*) and cytokinin (*IPT*) biosynthesis. Research shows that interactions between auxins and gibberellins determine the fruit size and weight potential of tomatoes at about the time of pollination (de Jong et al, 2009). The studies of Ali et al (2019) show that kelp extracts can regulate the expression of genes responsible for the endogenous biosynthesis of growth hormones including auxin, cytokinin, and gibberellin in tomato. This explanation is consistent with the observation in this experiment that kelp application has promoted efficiency of production of heavier fruit. It would explain the observations of other researchers such as Featonby-Smith and van Staden (1983), Crouch and van Staden (1992) and Hussain et al (2021), who saw kelp applications enhance other aspects of tomato production. Silva et al (2019) describe the role of genetic regulation and gibberellic acid hormone balance on tomato floral induction and flower development. Auxins, gibberellins and cytokinins are also involved in the regulation of tomato vegetative growth (Schwartz et al, 2016). If kelp extract application influences the development of the enzymes, it is reasonable to assume that responses will vary according to aspects of the application technique, volume of kelp extract and environmental conditions throughout the development of the tomato fruit.

This experiment was terminated prematurely due to ongoing uncertainties caused by COVID-19. With only 19% of harvested tomatoes having developed to either stage 5 or stage 6 at the time of harvest, and yet Kelp D yielding a 56% increase in weight over the control, it would have been interesting to see what running the experiment longer could have achieved. The other kelp treatments also yielded above control, but the differences were

not significant at the 0.05 probability level at the stages of fruit development at harvest. It would have been interesting to allow them to reach their potential.

As discussed in the introduction, the work with tomatoes was originally intended to include an experiment studying possible interaction between soil applied kelp extract and arbuscular mycorrhizas. In light of the work of Hussain et al (2021), showing positive benefits of a kelp extract derived from a blend of *D. potatorum* and *A. nodosum* on the biology in the soil root zone of tomatoes, it would have been interesting if this experiment had been able to proceed. Further research in this area is required to achieve a greater understanding of the effects of kelp extract on the plant-soil ecosystem.

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Chapter 5

Conclusions and suggested further work

5.0 Conclusions and suggested further work

In recent years the use of kelp extracts as biostimulants in agriculture has gained in popularity. There has been considerable research published over the past 80 to 100 years studying the mechanisms by which kelp extracts may be able to stimulate plant production. This body of research has established that there are many ways in which kelp extracts can act upon the development of terrestrial plants. What has not been clearly identified are the conditions under which the different mechanisms are effective. Nor, to the author's knowledge, have there been any peer reviewed publications which compare the effectiveness of various commercial kelp extract products available on the market today.

It is logical to expect kelp products to vary in composition for several reasons, including (i) the chemical composition of each kelp species varies when it grows under different environmental conditions; (ii) the chemical composition varies among the different kelp species used in agriculture; and (iii) different methods are used for extracting the active kelp products, resulting in varying extraction rates of different components and varying degrees of chemical transformation of some of these components.

One key aspect of this variability in composition among kelp products is the concentration of alginate, which varies within and between kelp species, depending upon factors including ocean turbulence (Stiger-Pouvreau et al, 2016), and method of extraction (McHugh, 2003). Furthermore, the composition and properties of the alginate component of kelp varies, including in the ratio of the polysaccharides D-mannuronate to L-guluronate (M/G ratio) and this affects the gelling capabilities and gel strength of the alginate, consequently affecting

the competitive advantage of kelps within their environment. Varying this ratio would also be expected to impact upon the functionality of brown kelp extracts acting upon terrestrial plants.

An initial broad aim of this project was to compare the biostimulatory performance of several commercially available kelp extracts on a range of crop species growing under different environmental conditions in order to identify conditions under which biostimulatory pathways of kelp extracts within terrestrial plants are activated. Initially I had hypothesised that the different commercially available kelp extracts would operate differently, and different pathways would operate dependent upon environmental conditions. Ultimately, I made little progress on this aspect: in Section 2.2.2, a comparison of five kelp extract products found no significant effect of any of these relative to the control on the growth or production of broccoli grown under field conditions. A similar situation was reported for tomatoes grown under glasshouse conditions in Chapter 4, in which the same five kelp extract products were compared against a control (no kelp) treatment. For all but one measured parameter, there was no significant difference between the control and any of the kelp treatments. In a promising finding, one kelp treatment did result in a significantly higher tomato fruit yield than the control at the termination of the experiment. However, it should be noted that the tomato fruit yield for this kelp treatment was not significantly higher than for any of the other four kelp treatments, so it cannot properly be said that the one kelp treatment “out-performed” the others.

An important decision in this project was which plant species to study. In the end, I narrowed my focus to two plants (broccoli and tomato). This selection was largely based upon literature reports showing response of these plants under Australian conditions (Mattner et al, 2013; Arioli et al, 2015) and multiple literature reports, particularly for tomatoes, of responses to kelp applications. Broccoli and tomatoes are both high yielding crops, and mostly grown under fairly intensive horticultural conditions, in which biostimulant addition could be economically and agronomically practical. They are also quite contrasting in many ways. They differ in the consumed component, growth habit and the fact that broccoli is a non-mycorrhizal species and tomato is a mycorrhizal species. I also carried out several "pilot" or "screening" experiments that included other plant species. These experiments are summarised in the Appendices but only reported in the main body of the thesis in the following brief summaries.

An experiment was conducted in the glasshouse to study the effect of kelp treatment on early wheat growth. While published research has shown wheat plants treated with a kelp concentrate have increased root: shoot dry mass ratio (Nelson and van Staden, 1984), these results were not replicated here. There was some evidence that tillering may have been stimulated by kelp extracts, but this was not pursued.

Appendices 2 and 3 summarise two experiments conducted to cast a wide net over a range of plant species treated with a range of commercial kelp extracts on the seed germination and plant emergence of the target species. With no significant responses to kelp treatment for the extensive range of treatments, these experiments serve to highlight the need to better define the conditions under which positive outcomes can be expected. Suggestions

have been made to adapt these experiments to remove aspects which may have confounded the results.

The experiment described briefly in Appendix 2 was designed to study the effect of kelp on the germination of a range of plant seeds, using the "Germination Index" described by Krader (2005). This experiment compared the effect of pre-soaking seeds with kelp extract on seed germination rate and early root growth in Petri dishes. Seeds of wheat (*Triticum aestivum* var. Axe), broccoli (*Brassica oleracea* var. italica), tomato (*Solanum lycopersicum L.*), canola (*Brassica napus*) and medic (*Medicago trunculata*), treated with five commercial kelp extracts at six different strengths, were studied. Overall, the results did not show any clear effect of kelp treatment, but recommendations have been made for improved techniques, and the method may be adapted for a future experiment.

Appendix 3 briefly describes an experiment designed to study the effect of kelp on the seedling establishment of the plant species studied in Appendix 2. Mattner et al (2013) reported upon the application of a kelp product enhancing seedling establishment of broccoli. Thus, the experiment described in the appendix compared the effect of the kelp products described earlier on the seedling establishment for this range of plant species. No stimulatory effects were found but several improvements have been identified for future experiments of this type.

Appendix 4 describes an ambitious experiment to study a potential impact of kelp extract upon the phytochemical composition of broccoli curds. This experiment had been devised because brassicas are valued for their nutritional benefits (Sanlier and Guler Saban, 2018).

Two experiments were undertaken to devise a method to determine a procedure to detect differences between samples in a range of phytochemicals identified as important to human nutrition. The decision not to proceed was influenced by time constraints, the impact of COVID-19 and the nature of the retained samples.

The most comprehensive experiments in this project were carried out on broccoli. I conducted four field experiments and a glasshouse experiment to study various aspects of kelp treatment upon broccoli growth. In the first broccoli experiment I compared the activity of five commercially available kelp extracted products. Experiments 2 and 3 were designed to study the potential interaction between applied kelp extract and the macronutrients phosphorus (P) and nitrogen (N). Experiment 4 was an evaluation of the importance of alginate in the activity of kelp extracts. In this experiment I took advantage of the poor structure and susceptibility to slaking of the soil on the grounds of the Waite Campus of the University of Adelaide to determine whether alginate would play an important role in kelp effectiveness under these conditions.

The results of the set of four parallel field experiments with broccoli emphasised the fickle aspects of kelp bio-stimulation of plants. In the first experiment (Section 2.1), originally intended to be the main experiment, no significant response of the broccoli was found to any of the kelp products whether applied to the soil or to the plant foliage. Experiment 2 (Section 2.2) showed a strong response of broccoli to applied P but no response to the kelp and no P $\times$ kelp interaction. Importantly, this experiment showed no evidence of the kelp extract sequestering or chelating P for uptake by the broccoli, and this contrasts with many previous studies where extracts from *A. nodosum* have reportedly aided uptake of P by

terrestrial plants. The positive response to the higher rates of P in Experiment 2 (most strongly expressed in shorter time to maturity) also revealed that the level of applied P in Experiment 1 was probably below optimum for broccoli production at this site. This response to P was unexpected based on the high level of available P in this soil (120 mg kg^{-1} Colwell P). Experiment 3 (Section 2.3) showed the expected response of the broccoli to N fertiliser, applied as either urea or calcium nitrate, but there was no interaction between either of the two kelp products with either of the two forms of added N.

In contrast to Experiments 1-3, the final experiment with broccoli in the field (Experiment 4; Section 2.4) showed a strong response of broccoli production to the applied kelp extract and also a similarly strong response to the addition of alginate at a rate calculated to be of the same order as the calculated alginate component of the kelp extract. This experiment was adjacent to Experiments 1-3 but differed from these experiments in at least two ways: P nutrition and timing. Because Experiment 2 indicated that broccoli at this site responded to high levels of added P, the level of applied P was 33 kg ha^{-1} higher than for Experiments 1 and 3. This suggests that a response to kelp might be more likely when nutrient levels are non-limiting. The result seems to contradict other work where kelp products have made nutrients more available to the plant and hence a response to kelp would be expected to be more likely under nutrient limiting conditions.

As noted in section 2.2, total P at the site was not limiting to final production, but plant-available P determined maturation rate. Under the conditions of this experiment, the applied kelp extract did not facilitate the conversion of P in the soil to available P. With the maturation rate of the broccoli varying with the rate of plant nutrition, it was decided to

adapt the time of harvest to suit the situation. In experiments 1 to 3, individual plants were harvested according to commercial requirements. Time to plant maturity is a valid commercial consideration.

The second obvious difference between broccoli field Experiment 4 and broccoli field Experiments 1-3 was the timing of the experiment. In Experiment 4, broccoli seedlings from the same batch as those used in the first three experiments (i.e., planted and germinated on the same date) were planted out in the field later, when the growing conditions were cooler than for the first three and presumably the soil temperature was also much cooler. Figure 2.0.5 shows that seedlings for this experiment were planted out immediately following the coldest week of the year. Soil temperature has been shown to be important for the impact of soil applied kelp upon seed germination (Warman and Munro-Warman, 1993), and maybe soil temperature is also important in the situation described here. Gelation properties of alginate are known to be affected by temperature (Lee and Mooney, 2012; Jeong et al, 2020), adding credence to the hypothesis that soil temperature has an important influence on the activity of kelp in the soil.

The four field experiments with broccoli are a strong demonstration of the need for ongoing research into identifying the environmental triggers for kelp stimulation of plant growth.

The variation in broccoli yield within treatments throughout the field experiments described in Chapter 2 was high (e.g., coefficients of variation were ~50% for leaf area index and ~25% for fresh weight at harvest). Furthermore, it was observed (qualitatively) that poorly performing plants seemed to coincide with small patches of localised waterlogging in low

points across the experimental plots. Hence the field experiments described in Chapter 2 were followed up with a glasshouse pot experiment (described in Chapter 3) in an attempt to remove this source of variability and provide a stronger opportunity to detect an effect of kelp addition on the growth of broccoli plants. This glasshouse experiment was set up as a kelp × P experiment due to the strong response to P found in the field experiments. In particular, the glasshouse experiment was designed with a large number of P addition rates (13), to maximise the chances of detecting an interaction between kelp and P that may occur through a small range of P concentrations. The experiment was successful in greatly reducing variability among replicates (e.g., coefficients of variation were ~10% for leaf area and ~5% for fresh weight at termination). However, broccoli growth in the pots was severely restricted from around 4-6 weeks and plants progressed abnormally from this point, with no proper curd development. When plants were terminated after flowering, average aboveground biomass per plant was <5% of that of field-grown plants at harvest.

Statistically, there was no effect of kelp on the growth of broccoli plants in the glasshouse experiment, but neither was there a positive influence of P addition. Ultimately, differences between broccoli growth in the glasshouse and broccoli growth in the field were too great to give physiologically meaningful results regarding interaction between kelp and P. The cause of the poor progress of broccoli plants grown in pots after 4-6 weeks was not determined, although a lack of vernalisation was identified as a possible contributor.

The experiment with tomatoes was conducted in the glasshouse (Chapter 4). The original intention was to conduct two experiments with tomatoes: one to compare the five commercial kelp extracts and a second to study investigating whether the kelp would

interact with mycorrhizal fungi in the soil. The latter was prevented by restrictions applied in response to COVID-19. However valuable observations were made in comparing the five kelp extracts. No significant responses of the tomato plants to any of the five kelp extract products were detected throughout the development of the tomato plants for the 200 plus days until the premature harvest. Then, at harvest, one of the five kelp treatments, Kelp D, showed a significant 55% increase in total fruit yield above the yield for no applied kelp. The other kelp extract applications also resulted in higher fruit yields (between 17% and 42% above control) but these results were not statistically significant. Kelp D is a fermented *A. nodosum* extract while kelps B and C are extracted from *A. nodosum* using caustic extraction. Many researchers (e.g., Ali et al, 2015; Eyras et al, 2008 and Whapham et al, 1993) have observed positive responses by tomatoes to caustic extracted *A. nodosum* products in many aspects of vegetative growth and fruit quality products as well as a positive fruit yield response. There is no reported research into tomato production following treatment with *A*. *nodosum* extracted by fermentation.

The observed responses to kelp extracts of broccoli in the field and tomatoes in the glasshouse appear to be due to different mechanisms. The response seen in the broccoli in Experiment 4 (Section 2.4) appeared to be a response to specifically the alginate component of the kelp extract, while the response seen in the tomato experiment in the glasshouse is suggested to be due to increased plant hormone activity. No other response to the kelp extract appears to have been triggered in the broccoli and an alginate response does not appear to have been triggered in the tomatoes. The alginate component of kelp has long been considered important as a soil ameliorant (Blunden, 1991). Alginate forms calcium bonds in the soil to improve soil particle structure and the field experiments described here

confirm that when soil structure is adversely affecting growing conditions, then the alginate has important effects. Khan et al (2009) also state that alginate elicits plant defence mechanisms, but this aspect has not been pursued here.

On the other hand, the yield increase of tomatoes in the glasshouse, growing in a well-structured potting mix, shows no response that can be attributed to alginate. The evidence suggests that the yield response is likely due to the activity of plant hormones, either increased incrementally by identical hormones in kelp, or triggered to over-produce in response to signals from the kelp extract invoking genetic pathways within the tomato plant, thus stimulating plant hormone development. The latter is suggested to be more likely. While all five kelp treatments showed greater tomato fruit yield, the response was only significant for Kelp D which is extracted via a fermentation process. The fermentation process may offer benefits over caustic extraction for triggering a plant hormone response, but this has not been pursued. The results reinforce the literature showing the many different mechanisms by which kelp extracts can act upon green plants, but as stated earlier, the observed response of tomatoes in the glasshouse differs from other reports in the literature of responses in vegetative plant growth.

Overall, my results highlighted that while kelp extracts can improve the growth and yield of green plants, there are many instances when a response is not detected. I found two different mechanisms for plant response, the effect of alginate on broccoli production, and an effect in the tomatoes likely due to increase in plant hormones either from the kelp or

stimulation of the plant by the kelp to over-produce plant hormones. Variation within treatments in the field proved to be a frustration in detecting significant differences.

It is apparent that further work is required to define the many pathways for the activity of kelp extracts and the environments and mechanisms necessary to elicit responses from kelp extracts. Unless anecdotal evidence is replaced with scientifically based data to explain the frequent non-responses to kelp observed in this work and in the agricultural environment, scepticism such as that of Edmeades (2002) will persist, uptake in the use of kelp will be compromised and money will be wasted on ineffectual applications of kelp. It is critical to understand when to use the correct kelp products and how they should be used. What can we identify which will help us to better understand environments and perhaps application techniques under which a response to kelp extracts can be more likely predicted? In the context of the work described here, the potential areas for future research include:

1. The effect of soil temperature upon the efficacy of kelp extracts, and any differences that may exist between the kelps. This is highlighted by the lack of response of broccoli to kelp extract applications in the first three field experiments, when temperatures were moderate, and the strong response in the fourth field experiment, when temperatures were at their lowest for the year. This research should lead on to a study of potential temperature and alginate interaction. If such an interaction is found, then the stability of structural changes caused by alginate in the soil could be studied to understand how temperature might affect the gelling properties of alginate and the efficacy of alginate on plant growth. Based upon the results reported in section 2.2, differences should be evident for broccoli after 45 days.

2. The potential interactions between kelp products and P need to be further investigated. As discussed in section 2.2, other researchers have found positive interactions between kelp and P, but this has not been seen in these experiments. A positive response to kelp was achieved when P was not limiting (i.e., in Experiment 4, Section 2.4), but this may have been due to specific conditions at the site, possibly timing of application, and unrelated to plant nutrition. There are economic and environmental benefits in minimising the input of P into agriculture, so there are good reasons for researching possible situations where interactions between kelp and P can occur.

3. The original intention of the study with tomatoes was to conduct two experiments: one to compare the five commercial kelp extracts and a second to study whether the kelp would interact with arbuscular mycorrhizas in the soil. Repeating the comparison experiment under modified conditions may indicate whether there is a real difference between the kelps. The problems with Blossom End Rot disease and mites during the experiment may have interfered with obvious benefits from the kelp earlier than the differences observed at harvest. It would be interesting to see whether taking fruit through to harvest at maturity may have given more clarity to the results.

4. With recent research indicating interaction between kelp extracts and arbuscular mycorrhizas, including the work of Hussain et al (2021) that showed these interactions benefitting tomato plants, there is a need for further research in this area. The work of Hussain et al (2021) was published after the tomato experiment commenced. The tomato genotypes *rmc* (AM –ve) and 76R (AM +ve) offer an opportunity to study the impact of kelp extracts upon arbuscular mycorrhizas. Waite Arboretum soil and washed fine sand (1:9)

should be used, and calcium phosphate dibasic ($CaHPO_4$) should be used for P nutrition to support good plant growth and maintain high mycorrhizal root colonization in 76R . Plants should be grown for seven weeks and then destructively harvested for measurement of biomass and nutrients for roots and shoots and assessed for mycorrhizal root colonization.

5. Metabolomics. The molecules responsible for the nutritional benefits of brassicas are well known (Appendix 2) and the application of kelp extracts has been shown to be beneficial for fruit quality (Chapter 1). Further metabolomic analyses of plant foods (e.g., broccoli) with or without kelp treatment will add to our knowledge as to the extent of the benefits of kelp for enhancing food quality.

There is a need to better clarify the timing and technique of the application of kelp extracts to plants. In my opinion, the application instructions for each of the five kelp products in this research work were too general, yet timing of application was crucial to the response in the field broccoli work. Better definition of timing and technique would give more consistent and better response to kelp products and generate more interest in adoption in horticulture and viticulture.

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Appendices 1 - 4. Inconclusive experiments

A number of experiments were embarked upon but discontinued because they proved to be inconclusive. These experiments were in the main conducted as preliminary experiments prior to settling upon broccoli and tomato as the species to be studied. Working with kelp had been a green-fields area for the University, and it was necessary to conduct exploratory experiments to find the species that gave the best fit. A brief summary of each experiment, outlining its contribution to my work, is presented here.

Appendix 1. Wheat development in soil treated with kelp

Wheat (*Triticum aestivum*) is an extremely important crop in the Australian economy and there have been reports of wheat responding to applications of kelp extract application (Calvo et al, 2014; Nelson and van Staden, 1984; Nelson and van Staden, 1986). The experiment described briefly here was conducted as part of the planning phase for my project, and to evaluate the potential to study wheat further. This experiment was designed to test the hypothesis that a soil drench of the kelp extract would stimulate early root growth of the wheat plant.

The experiment was conducted in the glasshouse, in 12 cm pots, using unfertilised slaking soil from the Waite arboretum, mixed with sand (ratio 4:1); 1200 g of this soil mix was added to each pot and drenched with Kelp Extract C (described in Section 2.0 of the main body of the thesis) at application rates of either 0, 2.65, 26.5, 132 or 267 litres per hectare (L ha^{-1}). The recommended soil application rate for turf is 12 L ha^{-1}. The design of the experiment was a simple randomised block with five replications. One wheat plant (*T. aestivum* var. Axe) per pot was pre-germinated and propagated in each pot.

While harvest results for this experiment were inconclusive, with no significance differences in the development of roots and shoots and no difference in water use for the different treatments, the differences in tillering of the plants may be of interest. Tillering occurred for some of the plants receiving either 2.65, 132 or 267 L ha^{-1} of kelp extract, but not for the untreated pots or for the mid-range treatment of 26.5 L ha^{-1}. After 25 days (18.7.2019) all plants were at the five-leaf stage (Zadok score of 1.5). Tiller emergence commenced at 31

days (24.7.19). If tillering had been stimulated by the addition of kelp then this was most likely caused by plant growth hormones from the kelp. Kelp extracts have been implicated in plant growth responses through both nutrient uptake and hormone responses in plants (Calvo et al, 2014). Tillering is at least in part controlled by auxins and cytokinins and there is strong evidence that the application of kelp extracts will result in cytokinin- and auxin-like activity in plants (Stirk and van Staden, 1997). Although the lack of a response to kelp addition precluded further experiments on wheat in this project, the effect of kelp extracts on tillering in wheat could be the basis for further work.

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Appendix 2. Germination Index to assess response of various plant species to seed treatment with commercial kelp extracts.

Senn (1987) stated that "seed treated with seaweed extract will germinate sooner than non-treated seed". There is a substantial amount of work indicating that kelp extract from numerous species can improve the germination of seeds of a number of plant species, including tomato (*Solanum lycopersicum*) (Moller, 1996; Finnie and van Staden, 1985; Hernández-Herrera et al, 2014).

The "Germination Index" (Krader, 2005) provides a method for rating treated seed based upon germination and root growth. This method was adapted to study the effect of seed from a range of plant species treated with various kelp extracts. The Germination Index is determined as:

100 x (germination in treatment/germination in control) x (root length in treatment)/ (root length in control).

This experiment compared the effect of pre-soaking seeds of wheat (*Triticum aestivum* var. Axe), broccoli (*Brassica oleracea* var. italica), tomato (*Solanum lycopersicum L*.), canola (*Brassica napus*) and medic (*Medicago trunculata*) with five commercial kelp extracts at six different strengths to study the effect of kelp extracts on seed germination rate and early root growth. The commercial kelp extracts A, B, C, D, E, described in Section 2.0 of the main body of the thesis, were tested. Application rates of the kelp treatments were 0; 0.01%; 0.1%; 1%; 10%; 50%; 100% and ten seeds for each replicate were added to each dish. Seeds were placed upon filter paper in Petri dishes, 90 mm in diameter, to soak over-night in 2 mL

of the respective kelp solution. The Petri dishes were placed into an incubator at 25°C. Seed germination was recorded daily for a week, and root lengths measured after 48 hours for all species other than tomato, which was measured after 96 hours. The dishes were photographed, and root lengths were determined using the program ImageJ. The Germination indices were calculated from this data.

Despite a large amount of recorded data (2,100 Petri dishes), the results did not show any clear effect of kelp treatments. Higher rates of kelp extracts were inhibitory. As a result of the experiments conducted here, numerous adjustments should be made to the protocol of any future experiments involving kelp and the Germination Index. It is recommended that for further experimentation the protocol be modified to a 90-minute soak rather than overnight, with treatments limited to 0.1% and 1% kelp solutions and more replications. The seed washing process should be changed. The bleaching process requires aeration to remove the fungicide seed coatings most effectively. Reduced times for the seed soak have been suggested and seeds should not be kept in kelp solution in the incubator.

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Appendix 3. Seedling emergence in glasshouse

This experiment was designed to compare the effect of the range of soil applied kelp products (A, B, C, D, E) on seedling establishment for the range of plant species used in Appendix 2. Published research has reported enhanced seedling establishment of broccoli with a kelp product (Mattner et al, 2013). No effects of kelp were detected in this experiment, but several improvements have been identified for future experiments of this type.

The experiment was designed with ten replications (single seeds) of each treatment to each plant species (wheat (var. Axe), broccoli, tomato, canola, medic, *Kennedia nigricans*) There were 24 treatments: four rates (dilutions of 0, 0.01%, 0.1%, and 1%) for each of the six kelp applications (Kelp extract A, B, C, D, E and no kelp). The soil was 3:1 of coarse sand to fine sand. Seeds were soaked overnight, in respective solutions, and germinated in trays with one individual seed per germination segment (4 cm x 4 cm x 4cm). The fungicide seed coatings were not removed first, and no bleach treatment was given. Seedling emergence and first true leaf emergence were recorded each day for 37 days.

The final counts for the emergence experiment showed no significant differences in plant emergence. Results were erratic and did not replicate those of Mattner et al (2013).

In retrospect, the design of this experiment was too large and unwieldy. Overall, the results were inconsistent. Things to consider in planning a future experiment include the effect of (i) spatial arrangement of the plants in the greenhouse; (ii) potential interaction between

the kelp extract and fungicide seed coating; (iii) timing of the seed soaking; (iv) depth of pot; and (v) soil type.

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Appendix 4. Metabolomics

Broccoli is an abundant source of phytochemicals associated with health benefits; these include glucosinolates, carotenoids, tocopherols, and flavonoids (Sanlier and Guler Saban 2018). This experiment, to evaluate the impact of kelp treatments upon the concentration of critical phytochemicals in broccoli, was an ambitious exploratory study which was interrupted and ultimately terminated, due to the restrictions caused by COVID 19. The phenolic phytochemicals found in broccoli are activated by molecules that stimulate the plant defence mechanism (Flores et al, 2021). Seaweed extracts have been shown to act as biostimulants for plant defence mechanism and may lead to the accumulation of these health stimulating phytochemicals in the broccoli head.

Preliminary studies were undertaken to develop a procedure to detect whether there were differences in the phytochemical content of samples retained from the alginate experiment. Mass spectrometry analysis of broccoli extracts using LC-MS can be used to identify a range of compounds found in brassicas and reported to have nutritional benefits for humans (Olsen et al, 2009). An initial calibration experiment was conducted in which the broccoli curds were frozen in liquid nitrogen, extracted and analysed using a non-targeted technique to measure the metabolites in a qualitative manner. This initial experiment was conducted to calibrate the equipment and to streamline the extraction process for broccoli. A further experiment was conducted to devise a method to analyse for target phytochemicals. A method was developed to detect the glucosinolates (glucoraphanin, glucobrassicin, neoglucobrassin), but not tocopherols (d-, c-, a-tocopherol) or carotenoids (lutein, zeaxanthin, b-carotene). Metabolomics could have been used to determine whether there

were any detectable differences between the treated and untreated samples retained from Broccoli Field Experiment 4; Comparing kelp with alginate (Section 2.4).

The decision not to proceed was influenced by time constraints, the impact of COVID-19 and the nature of the retained samples. These samples would have been frozen for over 12 months before analyses could be conducted and it is unknown whether the phytochemicals would still be intact. In addition, the retained curd samples, which were processed in line with requirements for retail sale requiring 6 cm of stem below the lowest floret, were composed of both stem and florets and the percentage of each would have varied between samples. Because the chemical composition of florets likely differs from that of the stems, it would be better to separate the florets from the stems. The method developed does provide a starting point for study of phytochemicals in broccoli treated with kelp extracts.

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