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A review of the function, efficacy and value of biostimulant products available for UK cereals and oilseeds

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1. Abstract

A wide range of biostimulant products are available for use on cereal and oilseed rape (OSR) crops. The term 'biostimulant' covers everything that can be added to the plant or soil to stimulate natural processes to benefit the plant, beyond fertilisation or pesticidal action alone. The aim was to review the mode of action, efficacy and value of commercially available biostimulant products and determine priority areas for research. A list of products currently available for UK cereal and OSR crops is included. In this review, biostimulants were classified into 11 distinct 'product type' categories as; seaweed extracts, humic substances (HS), phosphite and other inorganic salts, chitin and chitosan derivatives, anti-transpirants, protein hydrolysates and free amino acids, non-essential chemical elements, complex organic materials, plant growth promoting bacteria (PGPB), arbuscular mycorrhizal fungi, non-pathogenic fungi and protozoa and nematodes. The review is divided into sections by these 'product types'. Each section reviews the effects and modes of action for effects on different plant species and was not restricted to cereals and OSR as these crop species have been the subject of relatively few studies for some types of biostimulants. The level of evidence available for effects on plants is summarised to enable readers to quickly assess this information.

For all product type groups, there was at least some evidence that biostimulants can positively affect plant growth. Overall, there was evidence for 9 out of 11 of the product type categories to increase crop yields, although in many cases this was from experiments in either controlled conditions (e.g. glasshouse) or non-UK field conditions. Across the 11 product types, there was also evidence for increased nutrient uptake (N, P or other), plant function (hormone effects, anti-transpirant effects, delayed senescence or improved photosynthesis), abiotic stress tolerance (salt, alkaline, drought or cold stress), and biotic stress tolerance (induced or physical against pathogens or pests). The majority of product type groups had at least some evidence available for these effects on cereal crops, but consistently less information available for OSR crops. Many product types also had some level of a plant protectant role against pathogens or pests.

There is limited information available on the most appropriate rates, timings and management for UK cereal and oilseed rape crops. Microbial products in particular will require careful management, as a wide range of factors can affect inoculant success (e.g. inoculum storage, indigenous soil microbes, climate). Recommendations for managing these factors are also included in the review. Finally, key research gaps that should be targeted to enable exploitation of biostimulant products for the benefit of UK cereal and OSR crops are described.

2. Introduction

2.1. Aims and Objectives

In 2012, over 6.2 million ha were treated with biostimulant products in Europe, making Europe the largest market globally (Calvo *et al.*, 2014). The Agrow Biostimulants 2015 Report quotes EU sales of over £450 million and states that the market is growing rapidly (Agrow Biostimulants, 2015). There are many biostimulant products on the market, which manufacturers claim can facilitate nutrient uptake, increase plant tolerance to and recovery from abiotic stress, improve efficiency of plant metabolism, enhance produce quality, improve efficiency of other agricultural inputs (nutrient and plant protection products), improve physiochemical properties of the soil, improve water use efficiency, increase yield, and benefit complementary soil micro-organisms. The term 'biostimulant' covers everything that can be added to the plant or soil to enhance plant growth beyond fertilisation alone, except those products that have a definite 'pesticidal' action. It is very difficult for growers and agronomists to understand which products work and which don't, or which situations the products work best in, as there is very limited independent information available.

The academic literature on biostimulants has increased markedly in parallel with the growth of the biostimulant market (du Jardin, 2012). A bibliographic analysis by du Jardin (2012) found that the number of peer-reviewed articles has increased almost four-fold from 10 in 2006 to 40 in 2010. Furthermore, a web of science search in July 2015 found 136 peer reviewed articles on the topic 'plant biostimulant' and a further 70 on the topic 'plant bioinoculant'. This reflects the increasing academic interest in this area, in response to the increasing availability of biostimulant products. Calvo *et al.* (2014) reviewed the academic literature on five categories of biostimulants, but they focused on reviewing the academic research and high level gaps in current understanding, with little relevance for farmers. These reviews, whilst helpful to the academic community, are not freely available and did not cover biostimulant products that are commercially available. There was also an EU-commissioned review by du Jardin published in 2012 which had a more market-oriented approach, but the main aim was to define the term 'biostimulant' and it focused only on a few biostimulant product types. One notable omission in the aforementioned review was microbial biostimulants, which make up a significant proportion of the market and are covered in detail in this review. The fundamental biology of some of these microbial inoculants have been described elsewhere (Lugtenberg and Kamilova, 2009, Owen *et al.*, 2015, Kurepin *et al.*, 2014, Verbruggen *et al.*, 2013). However, the difficulty of ensuring these products work on a field scale was recognised over twenty years ago (Killham, 1994), and, whilst these individual reviews refer to the complexity of these issues, very few consider how growers can reliably exploit these products in the field. This review aims to build on these key papers to help growers understand the principles of biostimulants, compare them, and determine the key questions that need to be asked to ensure that research in this area focuses on the priority areas for growers.

The aim of this project was therefore to "*review the mode of action, efficacy and value of commercially available biostimulants products and determine priority areas for research".* We addressed this through the following objectives:

1. Introduce and define the term 'biostimulant', summarise availability, regulation and use
2. Summarise and group biostimulant products in terms of mode of action
3. Summarise scientific understanding about how the different modes of action affect plant growth, soil micro-organisms and/or pathogens/pests
4. Review experimental data from both publically available sources (academic and grey literature) and commercial companies to compare product efficacy.
5. Collate and summarise information from objectives 1-4 in a table, distilling the key facts into an Information Sheet
6. Draw conclusions about which biostimulant products may be most beneficial and the strength, or otherwise, of the evidence
7. Recommendations for further research

2.2. Biostimulants definition, current use and regulation

2.2.1. Defining the term 'biostimulant'

Given the complexity of this research area, it is not surprising that there is a range of complex and relatively new terminology. In order to ensure a consistent interpretation and relevant comparison between studies, it is important to have a clear understanding of the definition of a number of key terms. There is also a much wider range of related terms defined in the glossary (Appendix 1).

Defining the term 'biostimulant' is complex due to the diversity of terminology associated with these types of products, the range of different effects and modes of action, and the varied origins and nature of the substances in question. The term 'biostimulant' itself attracts a certain level of ambiguity and in some cases is avoided to escape the negative connotations that can be associated with it. Faessel *et al.* (2014) highlighted that the terminology associated with stimulation products is varied and complex due to the abundance of definitions and lack of harmonisation. Some raise issues with the prefix “bio” as this suggests that the product may be linked to biocontrol or organic farming, which for a synthetic product may be misleading. Alternative terms include stimulators of growth/development, phytostimulants, or agronomic additives. In some cases it can be difficult to distinguish between a biostimulant and a fertiliser because the two are often linked; it is common for biostimulant products to be combined with conventional fertilisers. Some associate the term with ‘fake’ products due to historical misuse of the term, however it is becoming more common for the term ‘biostimulant’ to be used to describe certain product types and these negative connotations seem to be declining.

A plant biostimulant is defined by the European Biostimulants Industry Council (EBIC) as “*a material that contains substance(s) and/or microorganisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality, independent of its nutrient content.”* The EBIC was set up in 2011 with the aim of promoting biostimulant products by identifying them as different from mineral fertilisers. The EBIC advocates that the biostimulant definition must address a number of issues. Firstly, system effects arising from combining biostimulant components must be acknowledged. The definition must also highlight that a biostimulant may act indirectly on a plant by acting on the soil microbiome. The definition also clarifies that a biostimulant may positively affect crop development which results in an increase in yield or improvement in quality. Traon *et al*. (2014) highlight a number of issues with this definition and propose the following alternative definition for a biostimulant: “*any substance or microorganism, in the form in which it is supplied to the user, applied to plants, seeds or the root environment with the intention to stimulate natural processes of plants to benefit their nutrient use efficiency and/or their tolerance to abiotic stress, regardless of its nutrient content, or any combination of such substances and/or microorganisms intended for this use*”.

du Jardin (2012) provides a detailed analysis of the range of definitions for plant biostimulants which are offered by scientific papers. It is noted that the first biostimulant definition refers to “*materials that, in minute quantities, promote plant growth*” (Zhang and Schmidt, 1997) and clearly separates a biostimulant from nutrient and soil amendments which are applied in larger quantities. A later definition provided by Kauffman *et al*., (2007) again focuses on the requirement for the material to be applied in a low quantity. This definition also stipulates that a biostimulant is a material other than a fertiliser. Thus, the finer points of biostimulant definitions are still very much under debate, but the most commonly used definition appears to be the EBIC definition. However, this does not clearly acknowledge effects on plant growth. We will therefore use the EBIC definition with the addition that biostimulants may also affect plant growth as defined by other sources.

2.2.2. The biostimulant market, current use and focus of the review

The biostimulants market is rapidly expanding, and includes both emerging technologies and products which have been on the market for several decades, such as seaweed extracts and humic acids. Until recently there has been a lack of interest in biostimulants in the UK, partly due to low farmer confidence in the available products. This was historically driven by a lack of evidence of product efficacy. Additionally, historically biostimulant products were often expensive to buy and fake products emerged onto the market. However, the industry is now evolving, with major agrochemical companies such as BASF, Bayer CropScience and Monsanto BioAg, having joined the EBIC in 2015.

Several studies on the growth of the biostimulants market in recent years have been carried out by market research firms and agricultural industry organisations. A large range of estimates for the market size and value have been suggested, with differences in the criteria used to define the market explaining the contrasting figures. The global biostimulant market is estimated at US$1 billion (based on New Ag International database and communication with industry) and expected to reach over US$ 2 billion by 2020 (New Ag International, 2015). However, this prediction of the future market value may be exaggerated due to the inclusion of organic fertilisers. Other sources estimate the global market value at around €1 billion (Cox & Wong, 2013). Irrespective of how the market is defined, there is no doubt that the biostimulant industry is growing rapidly.

Europe is the largest market for biostimulants (New Ag International, 2015). In 2012 the EBIC estimated that the EU market value of biostimulants was €400-500 million and that the EU market is growing by 10% or more per year, with a forecast value of €800 million in 2018. The EBIC have reported that more than three million hectares in the EU are treated with biostimulants, and with an average of two applications per year.

Market demand trends indicate that biostimulants are of significant interest in North America, Brazil, China, India, Spain, France, Italy and a number of other EU countries (New Ag International, 2015). Future growth in the biostimulant market is expected to occur predominantly in Latin America, Europe, China, India and North America (New Ag International, 2015).

A number of key market drivers for the biostimulant industry have been identified by the EBIC:

i) European agricultural and food safety policies have integrated environmental considerations and advocate the safe use of agricultural inputs,
ii) consumer demand for healthy food products with minimal environmental impacts,
iii) high and volatile prices for agricultural inputs such as fertilisers incentivise efficient input use,
iv) biostimulant companies are expanding their connections with global distributors to target previously inaccessible markets, and
v) innovative biostimulant products have been developed to target specific agronomic needs.

Historically, the largest demand for biostimulant products was for use on high value crops: protected cultivars in greenhouses, orchards (grapes, citrus, stone fruits, apples, pears), open-field vegetables (tomatoes, salads etc.) and horticultural products (flowers and ornamentals) where quality is the main target. Biostimulants were initially used in organic production but are now being introduced into conventional crop production. The volatility in prices for conventional crops has transformed some low-value crops into high-value crops and results from a survey carried out by EBIC members in 2013 indicated that the use of biostimulants on extensive field crops like cereals was increasing.

The current review was funded by AHDB Cereals and Oilseeds to focus on the use and role of biostimulants on UK cereal and OSR crops. Where effects on plants are discussed, the wider literature has been reviewed to ensure that all possible effects are captured, including effects on non-cereal and OSR crops. However, the conclusions, summaries and discussion points are focused on cereal and OSR crops in the UK.

2.2.3. Regulation of the biostimulants market

Due to the lack of consensus on the definition of the term ‘biostimulant’, there are currently no specific frameworks for regulating biostimulants in the EU, United States and other countries.

Currently in the UK, regulatory processes allow free access to the market for biostimulant products, whereby efficacy and safety data is not required. This is in contrast with most other EU countries where a registration scheme based on pre-market approval is in place. Across the EU there is considerable variation in the regulatory processes required for placing a biostimulant on the market: in France, Italy and Hungary the time to authorisation is often greater than a year, with rigorous data requirements including toxicity, ecotoxicity, environmental fate, efficacy data, and labelling requirements, whereas Germany and Spain have more relaxed regulations in which a simple notification providing efficacy data and label information is sufficient. In all instances, efficacy data from field trials in preferable but data from lab studies or other assays may be accepted.

The European Commission is intending to revise Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertiliser (the Fertiliser Regulation) pertaining to inorgan ic fertilisers and to extend its scope to include plant biostimulants (among other materials). The Fertiliser Regulation co-exists with national regulatory frameworks which relate to the placing of fertilisers on the market. Currently, only mineral products are classified as ‘EC fertilisers’, meaning that organic and organo-mineral products, soil improvers, growing media and biostimulants are governed only by national legislations.

Traon *et al*. (2014) have suggested that the revised EU Regulation should aim to i) harmonise legislation for all fertilisers and related products, ii) guarantee the safety of the material placed on the market with regard to human health and the environment, iii) ensure efficacy/utility and the ability of farmers to rely on the quality of the products bought, iv) facilitate the access to the market of innovative products and v) to reduce the administrative burden for authorities and for industry. Several policy options have been developed by the Commission and extensive stakeholders’ consultations have been carried out. The draft regulations as part of the EU Circular Economy package have now been published and the regulations are expected to come into force in January 2018 (European Commission, 2016). The draft regulations include organic and inorganic fertilisers, liming materials, soil improvers, growing media, agronomic additives (e.g. nitrification inhibitors and chelating agents), organic and inorganic biostimulants and fertiliser product blends. There will be specific compliance specifications and associated testing requirements, which may include defined limits on heavy metal and microorganism contaminants (European Commission, 2016). This will mean that all biostimulant products will require evidence of efficacy for any claims made. Even after the UK leaves the European Union, these regulations will apply to products that are sold elsewhere in the EU, thus it is likely that most products produced and sold in the UK will need to fulfil them, regardless of whether the UK retains this legislation or not.

3. Categorising biostimulant products

3.1. Biostimulant product types

The term 'biostimulant' covers a very wide range of products. These can be split into two key groups, microbial and non-microbial products, and further broken down into product types, as defined in the literature (du Jardin, 2012, Calvo *et al.*, 2014) and commonly used by the industry. The current review will focus on these product type categories (Table 1) to enable growers to better compare products based on product contents, rather than specific product names which can change. However, difficulties of categorisation arise for products which contain multiple product types, the implications of which are explored further in Section 4.3.

The aim of this review is to help growers navigate and understand the biostimulants available for use on cereal and OSR crops, both now and in the future. There are three key steps to this which will be covered in the review: understanding the source of the biostimulant product, the evidence for its effectiveness and whether it is likely to produce beneficial results on farm.

Table 1. The major biostimulant product groups and types available in the UK and EU at present can be categorised into 'product type' groups, as defined by Calvo *et al.* (2014) and du Jardin (2012).

Group	Product type
Non-microbial	Seaweed extracts
	Humic substances
	Phosphite and other inorganic salts
	Chitin and chitosan derivatives
	Anti-transpirants
	Protein hydrolysates and free amino-acids
	Non-essential chemical elements
	Complex organic materials
Microbial	Plant growth promoting bacteria and rhizobacteria (PGPR)
	Non-pathogenic fungi
	Arbuscular mycorrhizal fungi (AMF)
	Protozoa and nematodes

3.2. Product effects and modes of action – Key definitions

Terminology is important when describing products and there are many unique terms that are commonly used in the biostimulant market, either to describe product types, modes of action or interactions. The key terms applicable to all biostimulant products are the 'mode of action’ and ‘effect’. A recent review commissioned by the Ministry of Agriculture, Agri-Food, and Forestry (MAAF) in France (Faessel *et al.*, 2014) defined these key terms as follows:

Mode of action: "A mechanism that helps to explain the effect of a product. This can be split into a mode of biological action describing physiological, histological or cellular phenomena; and a biochemical mode of action which describes chemical or enzymatic"

Effect: "Result or effect of modes of action. A claim refers to one or more effect(s) put forward by the company to categorize the product. Some effects may therefore exist, but may not be being claimed"

For example, a product’s effects may be improved nutrition and disease tolerance, whereas the modes of action might be P solubilisation and induced systemic resistance.

In addition to these key terms, there are a range of more specific terms that are used to describe biostimulant products. These are included in the glossary in Appendix 1.

3.3. Biostimulant products available for the UK Cereals and Oilseeds market

There are an increasing number of biostimulant products available on the market in the UK; whilst the exact number is unclear, a non-exhaustive list of products currently available in the UK and marketed for use on cereals and OSR crops is given in Table 2. The information in Table 2 was gathered either directly from suppliers or via supplier marketing materials, and is subject to change as the market evolves. The list of products available for horticultural and amenity crops is likely to vastly exceed this, but these products were out of scope of the current review. As a guide, the number of biostimulant products available in France was reported to be *ca*. 300 (Faessel *et al.*, 2014).

Table 2. Biostimulant products currently available for use on cereal and/or OSR crops in the UK. This list was produced in consultation with the companies listed. The product aims are the intended benefits of the products and have not been assessed by ADAS. The product type column is intended to provide the reader with a link to the relevant section of the review which discusses the evidence available for the effects of each product type on plants. The table has been split into three sub-tables; 2a. Non-microbial products containing a single active ingredient; 2b. Microbial products; 2c, Non-microbial products containing multiple active ingredients. This list is not exhaustive.

Table 2a. Non-microbial products containing a single active ingredient.

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
Atonik	Arysta	OSR	Synthetic nitrophenols - sodium 5-nitroguaiacolate, sodium o-nitrophenolate, sodium p-nitrophenolate	Higher yields, improved quality, reduce pod shatter, frost tolerance	Other (nitrophenols)	Foliar spray
ALGAFlex	Biotechnica	Cereals & OSR	Concentrated seaweed extract, principally derived from *Ascophyllum nodosum*	Increase yield, strengthen root system development. Improve tolerance to environmental stresses and diseases and increase activity of beneficial microbes	Seaweed extract	Soil drench or foliar spray
BIaminoAM3	Biotechnica	Cereals & OSR	L-amino acids	Strong and sustainable vegetative growth, increase crop yield and quality, improve resistance to environmental stresses, enhanced disease resistance.	Amino acids	Foliar spray
SAPONite	Biotechnica	Cereals & OSR	Plant extract containing active plant saponins	Improve water and nutrient intake, and speed and success of germination.	Other (plant extract)	Seed dressing
BioSilicate	Biotechnica	Cereals	Biologically available silicon	Stronger stalks and stems, reducing lodging, better photosynthesis from extended leaves and extra chlorophyll, reduced heat and drought stress, better resistance to fungal pathogens and sucking insects such as aphids, improved resistance to high salts or toxins	Non-essential chemical elements	Foliar spray

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
Seamac PCT	Headland Crop Nutrition (FMC)	Cereals & OSR	*Ascophyllum nodosum* extract	Promotes crop growth	Seaweed extracts	
Seamac Lion	Headland Crop Nutrition (FMC)	Cereals	*Ascophyllum nodosum* extract	Promotes crop growth via improved vigour and nutrition	Seaweed extracts	
Pow Humus^R Growth stimulant and soil conditioner	Neotech-Agri Ltd.	Cereals & OSR	Water soluble potassium humate granules (Potassium humate 85%; K_2O - 12%; K - 10%)	Improves germination, rooting and nutrient uptake. Increases yield and quality; increases fertiliser efficiency & reduces nutrient leaching; improves soil structure, health & water holding capacity; decreases stress and reduces toxic residues.	Humic substances	Soluble powder applied before and after sowing; use undissolved as seed treatment
Kelpak	OMEX	Cereals & OSR	Kelp species *Ecklonia maxima*	Enhances root growth, establishment, yield and quality also improves tolerance to abiotic stress, pollen germination and fruit set	Seaweed extracts	Foliar Spray
Kelpland	OMEX	Cereals & OSR	Kelp species *Ecklonia maxima*	Stimulates root growth and improves establishment	Seaweed extracts	Foliar Spray
Kelpomex	OMEX	Cereals & OSR	Extract of kelp species *Ecklonia maxima* (consists of a range of hormones, nutrients, amino acids and vitamins)	Enhances root growth, establishment, yield and quality also improves tolerance to abiotic stress. Approved for organic crops	Seaweed extracts	Foliar Spray
Symbio 50% Seaweed	Symbio	Cereals & OSR	Concentrated seaweed extract	High organic carbon level, contains full complement of micronutrients, soil conditioner and biostimulant	Seaweed extract	Foliar spray or soil drench
Symbio fulvic 30 liquid	Symbio	Cereals & OSR	30% natural fulvic acid	Improves low light and cold temperature growth, reduces plant water loss via transpiration, improves uptake of nutrients, stimulates beneficial soil bacteria and fungi	Fulvic acid	Foliar spray or soil drench

Product	**Company**	**Target Crop**	**Product Contents**	**Product aim (as described on product label)**	**Product type category**	**Application type**
Symbio prosilicon	Symbio	Cereals & OSR	Silicon	Increases photosynthesis and promotes growth in cereal crops, promotes nutrient absorption and improves nutrient balance in plants, enhances growth and strength of roots and stems and increases mechanical strength of cereals to increase lodging resistance. Reduces transpiration and increases water use efficiency, Increases plant drought stress tolerance.	Non-essential chemical element	Foliar spray
Symbio Chitogro	Symbio	Cereals & OSR	Chitosan	Stimulates healthy root growth, increases recovery rate after pathogen attack, improves germination and seedling survival rates, stimulates beneficial soil biology	Chitosan	Foliar spray
Symbio supa yucca	Symbio	Cereals & OSR	Concentrated form of *Yuccah schidigera* based wetting agent	Stimulates beneficial soil microorganisms	Other - plant extract	Foliar spray or soil drench
Symbio biobooster fush hydrolysate	Symbio	Cereals & OSR	Fish hydrolysate	Promotes low light and cool season plant growth, and plant growth in conditions of water logging and heat and moisture stress. Improves soil structure and root growth, promotes mycorrhizal fungal growth	Other	Foliar spray

Table 2b. Microbial products.

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
Mycortex	Biotechnica	Cereals	Mycorrhizal fungi, *Trichoderma* fungi, beneficial bacteria, humates, saponins	Improve root growth, plant nutrition, N fixation, disease resistance, stress resistance, increase soil microbes, improve soil quality and structure	AMF, PGPB, humic acids, fulvic acids	Granular or liquid - apply at sowing
BACTOLifeAZ	Biotechnica	Cereals & OSR	Nitrogen fixing and other supportive microbes.	Increase N use efficiency, and increase N levels in plants and soil. Improve soil structure, produce plant growth stimulants, solubilise key nutrients (phosphates and potassium), digest organic matter and promote germination and root development.	PGPB	Soil drench or foliar spray
BACTOLife DP104	Biotechnica	Cereals & OSR	Range of beneficial bacterial and fungal species.	N fixation, phosphorus and sulphur solubilisation, improved water capture and breakdown of organic matter	PGPB and non-pathogenic fungi	Soil drench or foliar spray
RGPRO Ag-Grow 3	PlantWorks Ltd.	OSR	6 species of plant growth promoting rhizobacteria at CFU/Ml 10^8. Bacterial species: *Gluconacetobacter diazotrophicus*, *Agrobacterium* spp., *Bacillus amyloliquifaciens,Bacillus megaterium,Azosprillum brasilens, Rhizobium species*	Improves nutrient uptake, plant health and development and stress tolerance. Improves soil health and biological status.	PGPR	Granular soil application, seed drilling or broadcasting

Product	**Company**	**Target Crop**	**Product Contents**	**Product aim (as described on product label)**	**Product type category**	**Application type**
RGPRO Ag-Grow 4	PlantWorks Ltd.	Cereals	5 species of arbsucular mycorrhizal fungi at 500k Propagules per litre, 4 species of plant growth promoting rhizobacteria at CFU/Ml 10^7. Mycorrhizal fungi: *Funneliformis mosseae, Funneliformis geosporus, Claroideoglomus claroideum, Rhizophagus irregularis,Rhizophagus microaggregatum Bacterial species: Gluconacetobacter diazotrophicus, Bacillus megaterium, Azosprillum brasilense, Rhizobium species*	Improves nutrient uptake, plant health and development and stress tolerance. Improves soil health and biological status.	Arbuscular mycorrhizal fungi and PGPR	Granular soil application, seed drilling or broadcasting
Symbio liquid endo mycorrhizal inoculant	Symbio	Cereals	Arbuscular mycorrhizal fungal inoculant	Increases plant growth in poor soils, increases yield, healthy plants are more resistant to stress and disease, reduces need for fertiliser and water	Arbuscular mycorrhizal fungi	Seed coat, soil drench or mixed with compost teas
Symbio Endo Mycorrhizal Transplanter	Symbio	Cereals	9 species Endo mycorrhizae, *Trichoderma* Spp. *Bacillus* Spp.	Increases germination and early plant growth, may reduce fertiliser and water inputs	Arbuscular mycorrhizal fungi	Seed coat, or applied with seed drill
Symbio Granular Mycorrhizal Inoculant	Symbio	Cereals	4 species endo mycorrhizae	Improves plant growth may reduce fertiliser and water inputs, improves nutrient and water uptake	Arbuscular mycorrhizal fungi	Apply with seed drill
Symbio Bacillus Booster	Symbio	Cereals & OSR	5 x *Bacillus* Spp.	Increases germination, nutrient uptake and stress resistance	PGPR	Seed coat or soil drench.

Product	**Company**	**Target Crop**	**Product Contents**	**Product aim (as described on product label)**	**Product type category**	**Application type**
Symbio Tricho Booster	Symbio	Cereals & OSR	5 x *Trichoderma* Sp.	Increases germination and fungal dominance in soil	Non-pathogenic fungi	Seed coat or soil drench.
Symbio Microbial Growing Media StarterSymbio	Symbio	Cereals & OSR	*Bacillus* Spp., *Trichoderma* Spp. and *Phanerchaete* Spp.	Mix with green and brown waste for rapid aerobic compost production	PGPR & Non-pathogenic fungi	Mix with green waste to make biologically active compost
Fungal Additive for compost teas	Symbio	Cereals & OSR	*Bacillus* Spp. *Trichoderma* Spp. and *Phanerchaete* Spp.	Add to compost teas to ensure fungal dominant compost tea and add fungi from mature soils to the mix	PGPR & Non-pathogenic fungi	Soil or foliar drench with compost tea
Bacterial Additive for compost teas	Symbio	OSR	*Bacillus* Spp.	Add to compost teas to ensure bacterial dominant compost tea	PGPR	Soil or foliar drench with compost tea
Compost for compost teas	Symbio	Cereals & OSR	Bacteria, fungi, protozoa and beneficial nematodes	Restores microbial populations in damaged soils, improves nutrient uptake yield and stress resistance	PGPR	Soil and foliar drench

Table 2c. Non-microbial products containing multiple active ingredients.

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
Multoleo	Arysta	OSR	GA 142 (*Ascophyllum nodosum* filtrate) (physio activator technology with B)	Improves pod-setting and limits pod-abortion, yield, activates plant nutrition pathways improves nutrient uptake efficiency, boron source for crop	Seaweed extracts	Foliar spray
Rooter	Arysta	Cereals & OSR	GA 142 (*Ascophyllum nodosum* filtrate), (physio activator technology with P & K)	Improves growth and activity of root system, increases tolerance of unfavourable growing conditions, increases root length and biomass, activates plant nutrition pathways, more effective uptake of nutrients and water from soil, increased yield and quality	Seaweed extracts	Foliar spray (2-4 leaf)
BIOHumate	Biotechnica	Cereals & OSR	Biologically active natural source ingredients	Increase cation exchange capacity, and nutrient absorption, reduce nutrient leaching and increase stress tolerance and plant vigour.	Humic substances	Soil drench
Radiate	De Sangosse	Cereals & OSR	7.0% N, 8.5% Zn, biostimulant	Improves root development, nutrient uptake, photosynthetic efficiency and stress tolerance	Other - Micronutrient complexes	Seed treatment
Seamac Gold	Headland Crop Nutrition (FMC)	Cereals & OSR	*Ascophyllum nodosum* extract, plus N, P and K	Plant growth stimulant	Seaweed extracts	
Seamaxx	Headland Crop Nutrition (FMC)	Cereals & OSR	*Ascophyllum based* seaweed extract with N, P, K, B, Cu, Fe, Mn, Mo, and Zn	Plant growth stimulant & fertiliser	Seaweed extracts	

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
C Weed 50	Micromix	Cereals & OSR	50% w/v seaweed concentrate produced at lower temperatures utilising only *Ascophyllum nodosum* - formulated with Humic acids and harvested only during selected periods of growth	Earlier establishment, increases early rooting, photosynthetic area, leaf and shoot growth and plant carbohydrate production, improves sugar content in treated crops, resistance to disease and pests, storability of treated crops, improves shelf-life of plants and flowers	Seaweed extract	
Matrix/Radical	Micromix	Cereals & OSR	Undisclosed, 'synthhormone', mix of components that have a syngergistic effect when put together	Supports growth and root development, drought amelioration strategy	Other - Growth hormone pre-cursors and analogues	
Optiphite GP	Micromix	Cereals & OSR	N, Phosphite, K, Phosphate, amino acids, humate-lignate active-uptake formulation technology	Reinforces plant disease defence and enhances root development	Phosphite, amino acids, humic substances	
Patron Z	Micromix	Cereals & OSR	N, Zn, Ammonium Acetates, Amino acids (wide range) with alkyl polyglucoside surfactant (with humic acids)	Enhances root and seedling development, increases root mass and length, improves seedling disease resistance, improves nutrient uptake efficiency	Amino acids, humic acids	
Prodigy	Micromix	Cereals & OSR	N,P,K, trace elements, plant extract amino acids, *A. nodosum* extract, extract of immature citrus, Zn, Mn and ammonium acetate, humic acids, and Phosphorus acid as phosphites/phosphonates plus seed coating agent	Promotes germination, early root development, maximises seedling health and survival and improves speed of growth	Seaweed extract, humic acids, amino acids, phosphite, other - citrus extracts	

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
ProPlex Liquid	Micromix	Cereals & OSR	N, Zn, Fe, B, Cu, S, Mg, C, Mn, Humic & fulvic acids, *A. nodosum* extract, amino acids, vitamin B1+D14	Improves speed of growth, rooting, health, low-stress yield and quality	Seaweed extract, amino acids, humic substances	
VitAmix	Micromix	Cereals & OSR	K, phosphite, humic and fulvic acids, chelated Cu, Mn, Zn, Fe, + Bo, Mo	Improves seedling establishment, promotes root development, reduces disease, corrects deficiencies and prevents physiological disorders	Phosphite, humic substances	
C-Weed AAA	Micromix	Cereals & OSR	*A. nodosum* concentrate produced from a cool extraction process, plus a wide range of L-amino acids from fermentation of plant extracts.	Earlier establishment, increases early rooting, photosynthetic area, leaf and shoot growth, plant carbohydrate production, improves sugar content in treated crops, improves resistance to disease and pests, storability of treated crops	Seaweed extract with L-amino acids	
AMIX Micronutrients	Micromix	Cereals & OSR	Humic-lignate complexed Cu, Mn, Zn, Fe, Ca, Mg and combinations	The AMIX range are all biostimulants and all produce yield increases in the absence of deficiency and are capable of increasing plant health levels	Humic substances with non-essential chemical elements	
Sinergy	Micromix	Cereals & OSR	an NPK liquid based on Phosphite with Silicon and amino acids	Improve quality and plant health	Phosphite, non-essential chemical elements and amino acids	
ProAlexin PNS	Micromix/ Phyto Innovation Ltd	Cereals & OSR	Blend of Citrus Bioflavonoids, Fruit Acids (Citric Acid, Lactic Acid, Malic Acid), Essential Fatty Acids (Caprylic Acid), Palm Kernal Oil Extract	Synergistic blend promotes health and survival, improves speed of growth, yield and crop quality	Other - Citrus extracts, Natural Acids	

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
ProAlexin PEL	Micromix/Phyto Innovation Ltd	Cereals & OSR	Blend of Citrus Bioflavonoids, Fruit Acids (Citric Acid, Lactic Acid, Malic Acid), Essential Fatty Acids (Caprylic Acid), Palm Kernal Oil Extract	Synergistic blend promotes health and survival, improves speed of growth, yield and crop quality	Other - Citrus extracts, Natural Acids	
Fulvital^R Plus Liquid trace elements	Neotech-Agri Ltd.	Cereals & OSR	Liquid fulvic acid & trace elements (Fe 1.2%; Zn 0.8%; Mn 0.6%; Cu 0.4%)	Provides natural source of chelated Fe, Zn, Mn and Cu in plant accessible form. Improves germination, faster root and shoot growth; reduces stress and increases soil CEC. Sequestering agent that unblocks nutrients in soil.	Low molecular weight fulvate substances	Foliar spray
Humicraft^R Liquid Growth stimulant and soil conditioner	Neotech-Agri Ltd.	Cereals & OSR	Water soluble suspension of humates and seaweed (potassium humate 10%; potassium alginate 10%; amino acids 10%; K_2O 3%; Fe 0.3%)	Improves germination, rooting and nutrient uptake. Increases yield and quality; increases fertiliser efficiency & reduces nutrient leaching; improves soil structure, health & water holding capacity; decreases stress and reduces toxic residues.	Seaweed extract & humic substances	Foliar spray
Bio 20	OMEX	Cereals & OSR	Biostimulant, N, P K	Fertiliser, stress relief and plant health promotion	Seaweed extracts	Foliar Spray
DP98	OMEX	Cereals & OSR	Phosphite (PO_3) with N and K	Stimulates root growth, improves establishment and improves the uptake and systemic movement of nutrient cations within the plant	Phosphite	Foliar Spray
Kickstart	OMEX	Cereals & OSR	Phosphite (PO_3) with N and K	Improves root growth and crop establishment	Phosphite	Foliar Spray

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
Superphite Plus	OMEX	Cereals & OSR	Phosphate (PO_4) and Phosphite (PO_3) plus K, Mg, Mn, Zn, and organic plant growth stimulants	Boosts growth and provides essential nutrients in a single application	Phosphite and seaweed extracts	Foliar Spray
Vitomex	OMEX	Cereals & OSR	Phosphite (PO_3) with K, Mg, Cu and Zn	Improves plant health and tolerance of abiotic stress	Phosphite	Foliar Spray
Symbio Humic 80 Soluble Granular	Symbio	Cereals & OSR	Potassium humate (10% K_2O)	Stimulates plant growth and metabolism and soil microbiology.	Humic acid	Foliar spray or soil drench
Symbio Humic 30 Liquid	Symbio	Cereals & OSR	Potassium humate (10% K_2O)	Stimulates plant growth and metabolism and soil microbiology.	Humic acid	Foliar spray or soil drench
Symbio CMS Shoot 5.0.2	Symbio	Cereals & OSR	Complex carbs, amino acids, fulvic acid	Biostimulant and fertiliser, promotes low light growth and carbohydrates and protein for young plants	Other, amino acids, fulvic acids	Soil Drench

4. Biostimulant effects and mode of action

For each of the biostimulant product types, the available literature describing effects on plants have been summarised below, together with details on the modes of action if known. Each section also includes a data summary table; these are not exhaustive but are intended to provide an indication of the level and types of data available for cereal, maize and/or OSR crops. In many cases, there was very limited yield information available, therefore controlled environment (e.g. glasshouse, growth cabinet) studies have also been included, as have other crops such as maize.

4.1. Non-microbial biostimulants – efficacy and mode of action

4.1.1. Seaweed extracts

The use of seaweed in food and agriculture around the world dates back thousands of years (Dillehay *et al*., 2008), but it wasn't until the 1950s that a procedure was developed to produce seaweed extracts (reviewed by Craigie, 2011; Khan *et al*., 2009). Historically, seaweed, in its solid form, was applied to the soil as a fertiliser and/or organic amendment (Khan *et al*., 2009). More recently, properties beyond fertilisation effects have been recognised (reviewed by Khan *et al*., 2009), and are the reason that seaweed extracts today are classed as biostimulants.

There are three main categories of seaweed, or macroalgae, which together contain over 9000 species: the brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta) algae. The most common group used in agriculture are the brown algae, which are found in temperate zones around the world (Khan *et al*., 2009). The species most widely studied and commonly used in biostimulants is *Ascophyllum nodosum* (L.), although some seaweed extract products do not state the species of seaweed used.

Seaweed extracts are usually sold in liquid form (although they can sometimes be dried) and the colour can range from brown/black through to colourless, depending on the starting material and method of manufacture (Craigie, 2011). The method of extraction is usually not disclosed, but common methods include the use of water, alkalis or acids; physical disruption by milling at low temperature to produce a 'micronized' suspension; liquifying at ambient pressure; or heating with alkaline solutions and pressurizing (Craigie, 2011). The latter method is reported to be one of the most widely used processes (Craigie, 2011).

Thus, seaweed extracts are inherently variable as they are derived from different species and by different extraction processes, hence will have different extract stability properties (Stirk *et al*., 2014; Rayorath *et al*., 2008). Seaweeds can be applied by different methods including seed treatments, soil application, soil drench, foliar spray, post-harvest treatment. The different methods of application may affect the efficacy of the product (Battacharyya *et al*., 2015). For example seed treatments and soil applications may have a greater effect on soil borne pathogens, mycorrhizal associations and rooting, whereas foliar sprays may have a greater effect on abiotic stress tolerance (Battacharyya *et al*., 2015). Additionally, the effect of dose rate, application frequency and timing will have an impact on the effectiveness of the products (Arioli *et al*., 2015; Battacharyya *et al*., 2015) and studies to elucidate these effects are still needed. Exactly how the various components (plant growth regulators; PGRs), nutrients, betaines, polymers) of seaweeds act on plants to enhance growth, vigour and health are not fully understood (Sharma *et al*., 2014). However, more detailed analysis of the composition of extracts and the effects on plant growth and gene expression are starting to reveal some of the modes of action (Sharma *et al*., 2014).

Seaweed extracts have been reported to improve crop yield, root structures, flowering and leaf development, fruit set, plant disease tolerance, tolerance of abiotic stresses such as cold and drought, soil structure, soil water holding capacity, and soil microbiology (Arioli *et al*., 2015). However modes of action for these effects are not well understood (Arioli *et al*., 2015).

The seaweed components which are reported to elicit these plant responses include PGRs such as cytokinins, auxins, and abscisic acid (ABA) (Crouch *et al*., 1992; Crouch & van Staden 1993; Reitz and Trumble 1996; Durand *et al*., 2003; Stirk *et al*., 2003; Ordog *et al*., 2004); gibberellic acids (Stirk *et al*., 2013; Stirk *et al*., 2014); molecules such as betaine and proline which buffer against osmotic changes; alginate and diverse polysaccharides which promote root growth and induce defence mechanisms; and minerals and trace elements (Craigie, 2011).

Brown seaweeds such as *Ascophyllum nodsum, Fucus vesiculosus* and *Saccharina longicruris* contain the cell wall polysaccharide alginate and storage carbohydrates such as laminaran, mannitol and fucans (Painter, 1983; Lane *et al*., 2006; Sharma *et al*., 2014). Laminaran and fucoidan exhibit a wide range of biological activities (Rioux *et al*., 2007). Laminarin has been reported to stimulate natural defence responses in plants and the induction of genes encoding pathogenesis-related proteins with antimicrobial properties (Fritig *et al*., 1998; van Loon & van Strien, 1999). Most polysaccharides activate defence responses of plants and protection against pathogens by activating salicylic acid, jasmonic acid and ethylene signalling pathways (Vera *et al*., 2011).

Cytokinins have been detected in fresh seaweeds (Hussein & Boney, 1969) and seaweed extracts (Brain *et al*., 1973; Tay *et al*., 1985; Featonby-Smith & Van Staden, 1984). Cytokinins in vegetative organs are associated with nutrient partitioning, whereas in reproductive organs, high levels of cytokinins may be associated with nutrient mobilization (Khan et al., 2009). Stirk & Van Staden (1996) tested six commercially used seaweed extracts for cytokinin-like and auxin-like activity using two bioassays (soybean callus and mung bean rooting). All of the extracts showed cytokinin-like activity and improved mung bean rooting. The products tested included Kelpak (*Eckionia maxima*), Marinure, Maxicrop, Redicrop, Seamae (*Ascophyllum nodosum*) and SM3 (*Laminariaceae* and *Fucaceae* species; Stirk & Van Staden, 1996).

Eris *et al*., (1995) investigated the effects of Maxicrop (*A. nodosum*) on peppers in the field, with the seaweed extract applied in three different concentrations and at five different stages of growth. Maxicrop increased fruit yield (5 – 43%), increased the length, diameter and internal wall diameter of the fruit, and also resulted in a ten day earlier fruit harvest. Other assessments of the treated fruit showed that the seaweed extracts increased fruit quality and chlorophyll content (Eris *et al*., 1995). The authors note that it is probable that the increased yields can be attributed to the cytokinin-like substances present in the seaweed extract. Similarly, Khan *et al*. (2011) demonstrated that the extract of *A. nodosum*, induced cytokinin-like activity in the model plant *Arabidopsis thaliana* when applied as a liquid culture or foliar spray. However, Wally *et al*., (2012) reported that the levels of phytohormone present in commercial seaweed extracts are often insufficient to account for observed enhanced growth and development in *Arabidopsis*; they found that *A. nodosum* extracts increased levels of endogenous cytokinins and abscisic acid, while auxin levels were depressed. The addition of a similar extract, coded as AZAL5, in a nutrient solution increased shoot and root growth and the uptake of nitrogen and sulphate in OSR seedlings (Jannin *et al*., 2013). Transcriptomic analysis indicated that a plasmid division regulator was responsible for an increase of chloroplast number, but did not increase net photosynthesis (Jannin *et al*., 2013). A glasshouse pot experiment testing the effects of AZAL5 on wheat found that the seaweed extract increased yield and grain potassium uptake, but did not affect shoot biomass or shoot nutrient content, suggesting that the main site of action was the reproductive organs (Stamatiadis *et al*., 2014).

Brassinosteroids have been found in the commercial seaweed product Kelpak™ which is made from *E. maxima* (Stirk *et al*., 2014). Arioli *et al.* (2015) also state that brassinosteroids and strigolactones have been found in the commercial product Seasol™ (unpublished data), which is a mixture of two seaweed species, *Durvillaea potatorum* and *A. nodosum*. In two field studies on broccoli in Australia, the extract (Seasol) increased leaf number, stem diameter and leaf area by 6, 10 and 9% respectively (Mattner *et al*., 2013).

Betaines have been reported in several brown algae genera such as *Ascophyllum, Fucus, Laminaria* (Craigie 2011). *A. nodosum* extracts contain various betaines and betaine-like compounds (Blunden *et al*., 1986). Betaines act as an osmolyte by protecting cells against osmotic stress (Khan *et al*., 2009; Sharma *et al*., 2014) and can enhance chlorophyll content (Whapham *et al*., 1993; Blunden *et al*., 1997) by inhibiting chlorophyll degradation (Gernard *et al*., 1991). They have also been found to elicit physiological responses (Blunden 1977; Blunden *et al*., 1996b). Blunden *et al*., (1986) compared the effects of *A. nodosum* extracts and a betaine mixture in the same concentrations as those present in the seaweed extract. Both treatments resulted in similarly increased leaf chlorophyll levels compared to the control treatments on 63 and 69 days after application. Blunden *et al*. (1996a) reported that chlorophyll content of dwarf french bean, barley, maize and wheat increased when treated with *A. nodosum* extracts as a soil drench. It is suggested that the enhanced leaf chlorophyll content may be a result of betaines present in the extract which are slowing down the degradation of leaf chlorophyll (Blunden *et al*., 1996a).

Marine algae are also reportedly rich in auxins and auxin-like compounds (Crouch & van Staden, 1993). Seaweed products applied to maize promoted root growth and development, in a similar way to auxin (Jeannin *et al*., 1991). Rayorath *et al*., (2008) found that in *Arabidopisis A. nodosum* extracts promoted root and shoot growth compared to controls. Using a reporter gene construct these authors also found evidence that the seaweed extracts modulate the concentration and localisation of auxins (Rayorath *et al*., 2008). Seaweed extract concentrate (SWC) stimulated root growth in tomato seedlings which led to an increase in root:shoot ratio and biomass accumulation (Crouch & van Staden, 1992), and increased root:shoot ratio in wheat (Nelson & van Staden 1986). Crouch & van Staden (1991) report that treating the cuttings of some flowering plants, such as marigold (*Tagetus patula*) with Kelpak (a product derived from *E. maxima*) increased root number and dry weight. Another study reported that treatment with Kelpak increased the number of rooted cuttings and root vigour in *Pinus pinea* (Atzmon & van Staden, 1994). These effects are attributed to the presence of auxins in the extracts.

Extracts of Tasco (*A. nodosum*) in turf grasses and tall fescue (*Festuca arundinacea*) increased the activity of antioxidant enzymes, including superoxide dismutase (SOD) (Fike *et al*., 2001; Zhang, 1997), glutathione reductase (GR) and ascorbate peroxidase (AsPX) (Ayad, 1998). This increased antioxidant capacity could alleviate abiotic stresses which result in the production of reactive oxygen species (ROS), such as drought, extremes of temperature and salinity (Hodges, 2001).

A number of studies and reviews have mentioned the effects of seaweed extracts on diseases and pests. Treating the plant with a systemic inducer or elicitor by means of a seaweed extract could increase pathogenesis-related proteins, which result in protection from diseases (Craigie, 2011, Moon & Anderson 2003, 2006). Laminarin, present in some seaweed extracts, can stimulate natural defence responses in plants and the induction of genes encoding pathogenesis-related proteins with antimicrobial properties (Fritig *et al*., 1998; van Loon & van Strien, 1999, Klarzynski *et al*., 2000, 2003, Kobayashi *et al*., 1993; Mercier *et al*., 2001).

Using a sand culture technique, Wite *et al*. (2015) found that clubroot (*Plasmodiophora brassicae*) primary and secondary infections in broccoli were reduced by up to 55% and 84%, respectively, 45 days after treatments with the commercial seaweed extract Seasol™ (mixture of *D. potatorum* and *A. nodosum*). The reason for this suppression is not known, but the authors suggest that it may be due to the activation of natural plant resistance mechanisms and or the presence of natural plant growth regulators. Mattner *et al*., 2013 also reported that Seasol could supress the growth of *Sclerotinina minor* in lettuce and white blister (*Albugo candida*) in broccoli.

Glasshouse grown carrots treated with an extract of *A. nodosum* showed significantly reduced disease severity compared to the control after inoculation with the fungi *Alternaria radicina* and *Botrytis cinerea* (Jayaraj *et al*., 2008). Plants which had been treated with the seaweed extract or salicylic acid had significantly increased activity of defence enzymes (including peroxidase, polyphenoloxidase, and chitinase among others) compared to the control plants 12hr after treatment. The study also reported that treated carrots had higher transcript levels of a number of defence related genes compared to the control plants. Stephenson (1966) reported a reduction in black bean aphid (*Aphis fabae*) infestations on broad beans which had been treated with Maxicrop spray, compared to the control.

Stephenson (1966) also noted that fewer winged adults landed on the seaweed treated leaves of sugar beet than on controls, which suggests an aversion response rather than an insecticidal effect. Maxicrop treatment has also been reported to reduce the population of red spider mites (*Tetranychus telarius*) in apple orchards and glasshouse chrysanthemums (Stephenson, 1966). Hankins & Hockey (1990) reported a reduction in two spotted red spider mite (*Tetranychus urticae*) on strawberries grown in glasshouses following Maxicrop treatment. The mechanism behind this response is unknown (Craigie, 2011) however, the increased levels of anthocyanins and phenolic constituents in leaves may alter the palatability of leaves to insect predators (Craigie, 2011). Seaweeds and seaweed extracts may also improve moisture-holding capacity and promote the growth of beneficial soil microbes (Khan *et al*., 2009).

Foliar applications of *E. maxima* extracts to glasshouse-grown maize increased shoot weight by 37-42% and root weight by 34-45% (Matysiak *et al*., 2011). Similarly, applications of the brown alga *Sargassum* spp. increased maize shoot weight by 48-50% and root weight by 54-57% (Matysiak *et al*., 2011). These authors also reported that seeds soaked (primed) in the extracts had improved germination rates, which could have significant effects on crop establishment (Sharma *et al*., 2014). Foliar applications of *Kappaphycus alvarezii* and *Gracilaria edulis* sap to field grown wheat in India increased wheat yield by up to 20% and 13% respectively, increased nutrient uptake, and improved grain quality (Shah *et al*., 2013). Foliar applications of *K. alvarezii* extracts to field grown soybean in India increased grain yield by 57%, and also increased straw yield and nutrient uptake (Rathore *et al*., 2009).

A range of experiments from both peer reviewed papers and data provided by OMEX are summarised in Table 3. The majority of research on seaweed extracts has been on plants other than cereals and OSR. For cereals, significant increases in above-ground biomass, below-ground biomass and yield were found in 7/11, 6/6 and 3/7 experiments respectively with significant yield responses of cereal crops ranging from 73-134% of the untreated control, no treatments in these studies had a significant negative effect (Table 3). Fewer data were available for effects on OSR crops (2/2, 3/5 and 0 respectively), with biomass (above- and below-ground) responses ranging from 89-173% of the untreated control, of which no effects were significantly negative. Apart from three OSR experiments, these data were from either controlled conditions (e.g. glasshouse) or field studies outside of the UK. Whilst the UK based evidence is limited, and it is difficult to extrapolate from pot based research to the field, the summarised experiments provide evidence that seaweed extracts can affect the growth of wheat, maize and OSR rape. Further work is required to determine the field based effects on UK cereal and OSR crops.

Table 3. Effect of seaweed extracts on above-ground growth, below-ground growth or yield of cereal, maize and/or OSR crops.

Product type & species included	Year	Crop	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above ground biomass	Below ground biomass	Yield	Above ground	Below ground	Yield	
Seaweed extracts (*Kappaphycus alvarezii* & *Gracilaria edulis)*	2013	Wheat	-	Field, India	1/1	-	1/1	101-111	-	101-120	Shah *et al.*, 2013
Seaweed extracts (*Ecklonia maxima)*	1986	Wheat	Kelpak 66	Pot	1/1	1/1	1/1	106-265	113-222	98-116	Nelson and Van Staden 1986
Seaweed extracts (*Kappaphycus alvarezii)*	2004-2005	Wheat	-	Pot	1/1	1/1	1/1	119-154	123-172	113-134	Zodape *et al.*, 2009
Seaweed extracts *(Ascophyllum nodosum)*	1986-1987	Barley	Nitrozyme	Field, Canada	0/2	-	0/2	Not available†	-	73-131	Taylor *et al., 1990*
Seaweed extracts (*A. nodosum*)	1988-1989	Barley	Nitrozyme	Field, Canada	0/2	-	0/2	Not available†	-	85-109	Taylor *et al., 1990*
Seaweed extracts (*A. nodosum)*	2013	Oilseed rape	-	Hydroponics	1/1	1/1	-	115-132	89-115	-	Billard *et al.*, 2013
Seaweed extracts (*A. nodosum)*	2012	Oilseed rape	-	Hydroponics	1/1	1/1	-	123	102	-	Jannin *et al.*, 2013
Seaweed extracts (*E.maxima* & *Saragassum spp*)	2009-2011	Maize	Kelpak SL and Algamino Plant	Glasshouse	2/2	2/2	-	111-125	134-157	-	Matysiak, *et al.*, 2011
Seaweed extracts (*A. nodosum)*	1991	Maize	-	Growth Chamber	2/2	2/2	-	103-124	116-133	-	Jeannin *et al.*, 1991

Table 3 continued.

Product type & species included	Year	Crop	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above ground biomass	Below ground biomass	Yield	Above ground	Below ground	Yield	
Seaweed extract	2014	Oilseed rape	Kelpak	Field, Norfolk, UK	-	0/1	-	-	136-139	-	Omex 2014a*
Seaweed extract	2014	Oilseed rape	Kelpak	Field, Norfolk, UK	-	1/1	-	-	106-173	-	Omex 2014b*
Seaweed extract	2014	Oilseed rape	Kelpak	Field, Norfolk, UK	-	0/1	-	-	107	-	Omex 2014c*

*Data analysed by ADAS

†Numbers not reported

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by ‘-‘.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.1.2. Humic substances

Humic substances (HS) are the product of natural decomposition of plant and microbial remains, and comprise up to 80% of soil organic matter. HS are complex mixtures of polydispersed materials, which can be split into three main categories: humic acids (HA), fulvic acids (FA) or humin. Both humic and fulvic acids can be extracted, but humin cannot (Killham, 1994). Humic acids include the following major functional groups: carboxyls, phenolic hydroxyls, alcoholic hydroxyls, ketones and quinones (Russo and Berlyn, 1991). Fulvic acids are a subset of HA, with lower molecular weights and higher oxygen contents. Both HA and FA can be extracted from soil and other organic materials using a strong base (e.g. sodium hydroxide or potassium hydroxide), and then precipitated using a strong acid (e.g. hydrochloric acid) (Schlesinger, 1997). The extracted HA or FA are relatively pure, since the procedure will separate HS from other non-humic substances. The International Humic Substances Society state that the properties of these products are surprisingly consistent, despite the chemical differences (IHSS, 2007).

These substances have beneficial effects on the physical, chemical and biological properties of soil, therefore their role in sustaining plant growth has been recognised for some time and there has been increasing interest in adding HS as a soil amendment in agriculture. Additionally, HS regulate soil carbon and nitrogen cycling, the fate and transport of anthropogenic-derived compounds and heavy metals as well as stabilising soil structure (Piccolo, 1996). As biostimulants are products that affect plants either directly or via indirect effects on the rhizosphere, this review will focus on biological interactions; effects on physical or chemical soil properties are considered out of scope.

The use of soluble HS as plant growth promoters is not novel, however they are often applied with other fertiliser products and/or in situations of nutrient deficiency, which makes it difficult to discern any biostimulant effects. HS which have a low molecular mass easily reach the cell membrane and may be taken up by plant cells (Vaughan & Malcom, 1985; Muscolo & Nardi, 1999). The effects of HS are mainly exerted on cell membrane functions, promoting nutrient uptake (Visser, 1986; Varanini & Pinton, 1995), or plant growth and development, by acting as hormone-like substances (Vaughan & Malcom, 1985; Nardi *et al*., 1996). The biostimulant effects of HS are characterised by both structural and physiological changes in roots and shoots related to nutrient uptake, assimilation and distribution (nutrient use efficiency traits) (Canellas *et al*., 2015).

Further, HS have been found to affect the emergence of lateral roots (Canellas *et al*., 2002; Canellas & Olivares, 2014; Zandonadi *et al*., 2007), increase root hair length and density and cell proliferation in the root ground tissue in maize (Canellas *et al*., 2010), and increase the number, thickness and fresh weight of secondary roots in cucumber (Mora *et al*., 2012).

There is substantial evidence to support effects of HS on primary metabolism. HS have been shown to impact glycolysis and respiratory enzymatic activities (Nardi *et al*., 2007), photosynthetic metabolism (Ertani *et al*., 2011), carbohydrate metabolism (Canellas *et al*., 2013), and chlorophyll content, which in turn could affect photosynthesis (Sladky, 1959). Additionally, there is evidence to support HS effects on photosynthesis through stimulation of enzymatic activities related to the photosynthetic sulphate reduction pathway (Ferretti *et al*., 1991).

It is also well documented that HS have hormonal-like activities. In particular, the effects of HS have been likened to those of auxin, with a number of authors showing that physiologically active indoleacetic acid (IAA) concentrations are present in HS (Dobbs *et al*., 2010; Trevisan *et al*., 2009, 2011; Jindo *et al*., 2012). Other signalling molecules are also important: Zandonadi *et al*. (2010) showed that root development stimulation and the H^+-ATPase activation elicited by HS depends on mechanisms that use NO (nitrous oxide) as a messenger, which is induced in the early stages of lateral root development. There is also evidence to support gibberellin-like (Nardi *et al*., 2000; Pizzeghello *et al*., 2002) and cytokinin-like activities of HS (Nardi *et al*., 1988; Piccolo *et al*., 1992; Muscolo *et al*., 1996).

The enhancement of N uptake/assimilation and N metabolism in plates treated with HS has been documented in barley (Piccolo *et al*., 1992, Albuzio *et al*., 1986). Humic acid might also benefit plant growth by chelating unavailable nutrients and buffering pH (Mackowiak *et al*., 2001). In addition, HS have been documented to have a role in alleviating salinity stress in beans and maize (Aydin *et al*., 2012; Mohamed *et al*., 2012). There are also indications that HS may improve drought-tolerance in rice (Garcia *et al*., 2012).

Calvo *et al*., (2014) reviewed a large number of studies assessing the impact of humic substances on growth and nutrient uptake of 16 plant species including cucumber, wheat, maize, pepper, tomato, beans, but one notable exception was OSR. In the majority of cases positive growth responses were reported, although most studies were growth chamber or hydroponic based. Calvo *et al*., (2014) reported that root system development was most commonly reported as an initial effect of humic acids on plant growth. However, high doses of HS can have negative effects on plant growth (Asli and Neumann 2010; Ayuso *et al*., 1996). Tahir *et al*., (2011) found that application of lignite-derived humic acid at a high dose had a negative effect on the growth and nutrient uptake of wheat, as well as nutrient accumulation in the soil in comparison to lower doses. Table 4 summarises a subset of studies from peer-reviewed literature on responses of cereal, maize and OSR crops to application of humic substances. Three out of four cereal and maize experiments showed a significant yield increase to humic substances with reported ranges from 78 to 139% of the untreated control. One of these experiments detected a significant decrease in yield under deficit irrigation conditions, but significant increases in yield under adequate water conditions. Available data more strongly supports increases in shoot and root dry weight increases in wheat, maize and barley from laboratory and glasshouse studies, with 9/11 and 6/7 reported experiments showing significant increases and 1/11 and 0/7 reported experiments showed a significant negative effect respectively. However, most of these studies were non-UK based and only 3 of the 12 reported were field based. Furthermore, no evidence could be found for OSR. Therefore, it would be interesting to understand whether similar effects are possible in conventional UK cereal and OSR cropping, under both standard fertiliser and chemical inputs.

Table 4. Effect of humic substances (HA = humic acid, FA = fulvic acid) on above-ground growth, below-ground growth or yield of cereal, maize and/or oilseed rape crops.

Product type	Year	Crop	Product or application Name	Location	Proportion of experiments showing a significant effect			Range of responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
HA	2009	Wheat	HA dervied from lignite	Glasshouse, Pakistan	1/1	-	-	100-114	-	-	Tahir *et al.*, 2011
HA	1976	Maize	Sodium humate	Laboratory, Glasshouse	3/4	3/4	-	97-276	94-182	-	Lee and Bartlett 1976
FA	1988	Wheat	FA derived from Chinese or Australian coal	Glasshouse, Australia	1/1	-	-	122-130	-	-	Dunstone *et al*., 1988
FA	1988	Wheat	FA derived from Chinese or Australian coal	Field study, Australia[a]	-	-	0/1	-	-	101	Dunstone *et al.*, 1988
FA	1982	Wheat	FA derived from coal	Shade house, China[a]	-	-	NSA	-	-	128-139	Xudan 1986
FA	1982	Wheat	FA derived from coal	Field study, China[a]	-	-	NSA	-	-	107-118	Xudan 1986
HA & FA	2006-2008	Barley	Humistar	Laboratory, Poland	1/1	1/1	-	105	209	-	Szcepanet and Wilczewski 2011
FA	2009	Maize	FA	Nethouse, China[a]	1/1	-	1/1	118-130	-	109-119	Anjum *et al*., 2011
HA & FA	2008	Maize	HA & FA derived from Poplar sawdust	Laboratory, France	1/1	1/1	-	200	134	-	Eyheraguibel *et al*., 2008
HA	2010	Maize	HA derived from coal[b]	Laboratory, Israel	1/1†	-	-	75	-	-	Asli and Neumann 2010
FA	2013	Wheat	FA	Pot study, China	1/1	1/1	1/1[c]	91-112	110-156	78-126	Zhang *et al*., 2016
FA	2013-2014	Wheat	FA	Field study, China	-	-	1/1	-	-	110-111	Zhang *et al*., 2016

Table 4 continued.

Product type	Year	Crop	Product or application Name	Location	Proportion of experiments showing a significant effect			Range of responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
HS	1996	Barley	HS derived from organic materials[b]	Laboratory, Spain	NSA	NSA	-	67-207	47-163	-	Ayuso *et al.*, 1996

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

[a] Droughted conditions

[†] negative significant result

[b] High rates used[c] Significant negative result under deficit irrigation conditions, significant positive result under moderate water deficit, and under full irrigation.

NSA = No statistics available

Significance level *P* < 0.05

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by ‘-‘.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.1.3. Phosphite and other inorganic salts

Phosphite (Phi) is a reduced form of phosphate with the chemical formula $H_2PO_2^-$ (Phi). It is often applied in the form of phosphorus acid (H_3PO_3) to soils. Alternatively, Phi can be applied as phosphite salts containing a metal cation (e.g. K^+, Na^+, NH_4^+) and a non-metallic anion (phosphite (PO_3^{3-}), hydrogen phosphite (HPO_3^{2-}), or dihydrogen phosphite ($H_2PO_3^-$)). Potassium dihydrogen phosphite (KH_2PO_3) and dipotassium hydrogen phosphite (K_2HPO_3) are among the most common components of phosphite products (Deliopoulos *et al.*, 2010). In comparison to other biostimulant product types, phosphite and other inorganic salts should be relatively easily compared since they are known chemical structures. The most common application method for phosphites is as a foliar spray (Deliopoulos *et al.*, 2010). There is evidence for biostimulant and fungicidal effects of Phi products, but there may be a risk of phytotoxicity if phosphite is applied at a rate exceeding 5 g/l or 36 kg/ha (Hardy *et al.*, 2001; Barrett *et al.*, 2003; Deliopoulos *et al.*, 2010).

There are a range of other inorganic salts which have shown fungicidal effects, including bicarbonates, phosphates, silicates and chlorides (Deliopoulos *et al.*, 2010). However, there is comparatively less evidence for biostimulant activity of these inorganic salts. Inorganic salts are generally produced via inorganic chemistry methods or through mining of geological deposits (du Jardin, 2012).

Phosphite as a biostimulant

Phosphite has been applied to soils as a pesticide, supplemental fertiliser or biostimulant (Gómez-Merino and Trejo-Téllez, 2015), although there is debate over the fertiliser and growth benefits of this salt (Thao and Yamakawa, 2009). A previous AHDB funded review on micronutrients concluded that Phi is unlikely to act as a P fertiliser, since a meta-analysis of available field experiments showed no correlation between yield response and soil or tissue P status (Roques *et al.*, 2013). Other studies have also concluded that the fertiliser benefit of Phi to plants is limited, if any; it is recognised that microbes can convert Phi to phosphate (Pi) in the soil, but rarely in significant quantities (Gómez-Merino and Trejo-Téllez, 2015, Thao and Yamakawa, 2009). Nonetheless, Roques *et al*. (2013) did find some significant yield responses to Phi (Table 5), suggesting therefore that Phi can have positive effects, but that these are principally fungicidal or biostimulant in origin.

Data submitted by OMEX for the current review and by Frontier for the earlier micronutrient review (Roques *et al.*, 2013) have indicated growth responses and improvements in crop quality in response to Phi application, but a series of field experiments by Teagasc found no significant yield responses (Table 5). The OMEX experiments were predominantly glasshouse-based and there were no records of high disease or pest incidence, suggesting that Phi was acting as a biostimulant; the Frontier and Teagasc experiments were field based, but disease and pest pressures are unknown.

Positive responses of plants to application of Phi reported in the literature have been reviewed by Gómez-Merino and Trejo-Téllez (2015). However, there is also evidence for a negative effect of Phi application on plant growth and yield, which appears to be linked to the Pi status of the plant; i.e. plants with insufficient Pi appear have suffered leaf chlorosis, stunted growth, reduction in primary root growth, and decreased respiration rates (reviewed by Gómez-Merino and Trejo-Téllez, 2015 and Thao and Yamakawa, 2009). In contrast, there may be a synergistic effect of increased Pi and Phi levels (Bertsch *et al.*, 2009, Thao & Yamakawa, 2009). Consequently Gómez-Merino and Trejo-Téllez (2015) concluded that in the presence of sufficient Pi, phosphite can be successfully used as a biostimulant. The evidence for phosphite biostimulant effects and modes of action is reviewed below.

Ávila *et al.* (2011) reported a decrease in biomass of maize under low Pi conditions when Phi was applied, with no effect found under adequate Pi supply, although additional positive biochemical responses were observed. There are few published papers on biostimulant interactions of phosphite and cereals and/or OSR crops. Consequently, the evidence from the literature for a response (or not) to phosphite addition in cereals and OSR is limited. Nonetheless, there have been a range of positive responses found in other crops including lettuce, celery, onion, potato, pepper, tomato and fruit crops, such as increased yield, biomass, P content, quality, mycorrhizal colonisation and chlorophyll content (reviewed by Gómez-Merino and Trejo-Téllez (2015)). There is also evidence for biomass and yield responses to phosphite applications in UK cereal and OSR experiments (Table 5). There are a number of UK studies that have been carried out by industry on phosphite, with most looking at yield benefits and 4/17 demonstrating a significant yield increase. None of the studies in Table 5 reported a significant decrease in yield. Yield responses ranged from 95-112% of the untreated control. There have also been significant above- and below- ground biomass increases found in cereals and maize plants (3/5 and 4/4 respectively), with one study on maize reporting a decrease in above- and below- ground biomass under low P conditions. Although negative responses have been reported with root and shoot biomass being reduced in maize, thought to be a result of the Phi replacing part of the P supply at low P.

It has been suggested that Phi may act as a biostimulant by influencing sugar metabolism, causing internal hormonal and chemical changes (Lovatt & Mikkelsen, 2006, Ávila *et al.*, 2011), stimulating defence responses (Olivieri *et al.*, 2012), and/or altering plant P nutrition (Varadarajan *et al.*, 2002). However, these are largely hypotheses; there is limited evidence for how Phi causes these effects, most of which is not from cereal and OSR crops. Whilst the evidence does indicate that plants can respond to Phi addition, the mechanism is poorly understood and needs more research at both the laboratory and field scale. In particular, there is a need for a better understanding the mode of action, more research in cereal and OSR crops, and an improved understanding of management interactions to allow the development of best practice guidelines.

Phosphite as a disease or pest control agent

Deliopoulos *et al.* (2010) reviewed the evidence for fungal disease suppression by inorganic salts and concluded that Phi salts can have positive effects. Phosphites are generally applied to reduce susceptibility to oomycetes (predominantly downy mildews and *Phytophthora* spp.); Deliopoulos *et al*. (2010) concluded that the likely mode of action is inhibition of fungal sporulation and stimulation of plant defence mechanisms. However, they did acknowledge that there were a wide range of responses to Phi application.

There is also evidence for a soil drench phosphite application inhibiting the development of the endoparasitic nematodes *Heterodera avenae* and *Melooidogyne marylandi* in wheat and bristle oat crops (Oka *et al.*, 2007). The number of nematodes that penetrated the plant roots were not affected, but the development of the nematodes was severely impaired. The authors hypothesised that the mode of action could be by induced resistance, but there was no clear evidence of this.

Thus there is evidence in the literature for the effect of Phi as a disease or pest control agent, but the mode of action is not fully understood at present. The majority of this research has been carried out under controlled conditions on horticultural or fruit crops. However, whilst it appears likely to exhibit disease control responses in cereal and OSR crops, more work is required in the field to determine the scale and consistency of any potential benefits.

Table 5. Effect of phosphite on above-ground growth, below-ground growth or yield of cereal, maize and/or oilseed rape crops.

Product type	Year	Crop	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
Phosphite	2015	Maize	DP98, Vitomex	Glasshouse, UK	1/1	-	-	105-115	-	-	Omex, 2015a
Phosphite	2013	Spring barley	Superphite Plus, DP98	Field, UK	-	1/1	0/1	-	110-117	100-108	Omex, 2013*
Phosphite	2013	Winter OSR	DP98	Glasshouse & Growth cabinet, UK	1/1	2/2	-	109-133	109-143	-	Omex, 2013a*; Omex, 2015b
Phosphite	2015	Spring wheat	DP98	Field, UK	0/1	-	0/1	109-125	-	105-106	Omex, 2015c*
Phosphite	2012, 2013	Winter wheat	DP98, Vigga, 0-28-19	Glasshouse, UK	2/2	2/2	-	104-133	113-142	-	Omex, 2013a*; Omex, 2013b*; Omex, 2013c*
Phosphite	2011*	Maize	Potassium phosphite	Glasshouse	1/1†	1/1†	-	83-91	68-92	-	Ávila *et al*., 2011
Phosphite	2008-2011	Winter wheat & winter barley		Field, UK			4/9			97-106	Frontier trials cited by Roques *et al*., 2013
Phosphite	2011-2012	Winter wheat & spring barley		Field, Ireland			0/6			95-112	Teagasc trials cited by Roques *et al*., 2013

Significant responses include both positive and negative responses, however, any negative responses are indicated in the footnotes.

†Significant negative effects of phosphite on root and shoot biomass, where phosphite is replacing part of the P supply at low P.

*Data analysed by ADAS

[a]Year is year of publication.

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by ‘-‘.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

Other inorganic salts

Other inorganic salts are used as biostimulants much more rarely than Phi, hence there is minimal evidence cereal and OSR crop responses. Phosphates, chlorides and silicates are also known for their interactions with pests and diseases, but less so as biostimulants. There is evidence for interactions with fungal disease suppression, as reviewed by Deliopoulos *et al.* (2010) (Table 6).

Table 6. Example studies that have found significant effects of inorganic salt application on to reduce various diseases in cereal crops. Adapted from Deliopoulos *et al.* (2010).

Inorganic salt	Crop	Disease	Reference
Bicarbonate	Wheat	Leaf rust	Karabulut *et al*., 2006
Phosphate	Barley	Powdery mildew	Reuveni *et al*., 1998b
Phosphate	Maize	Common rust	Reuveni *et al*., 1996b
Phosphate	Maize	Northern leaf blight	Reuveni *et al*., 1996b
Silicate	Wheat	Glume blotch	Leusch & Buchenauer, 1989
Silicate	Wheat	Powdery mildew	Rémus-Borel *et al*., 2005
Chloride	Barley	Crown and root rot	Elmer, 2003a
Chloride	Wheat	Leaf rust	Melgar *et al*., 2001
Chloride	Wheat	Lglume blotch	Kettlewell *et al*., 1990
Chloride	Wheat	Powdery mildew	Kettlewell *et al*., 2000
Chloride	Wheat	Septoria blotch	Mann *et al*., 2004
Chloride	Wheat	Tan spot	Melgar *et al*., 2001
Chloride	Wheat	Yellow rust	Russell, 1978
Phosphite	Maize	Downy mildew	Panicker & Gangadharan, 1999

4.1.4. Chitin and chitosan derivatives

Chitin poly (β-(1-4)-N-acetyl-D-glucosamine) is an abundant natural polysaccharide which can be found in a wide range of organisms, most notably exoskeletons of arthropods (e.g. crustaceans and insects) and the cell walls of fungi (Hayes *et al*., 2008) and is the second most abundant polymer after cellulose (Rinaudo, 2006). The annual worldwide production of chitin was estimated at 10^{10}-10^{12} ton in 2013 (Gortari & Hours, 2013). Chitin and chitosan (the deacetylated counterpart of chitin) are used in various applications, including agricultural applications and biomedical uses such as tissue engineering and drug delivery vehicles (Khor & Lim, 2003; Sharp, 2013). It is most often the waste products of marine shellfisheries that form the basis of chitin-based biostimulant products in agriculture (Rinaudo, 2006; Sharp, 2013). Crustacean production worldwide in 2013 exceeded 10.2 million tonnes, therefore there is a large potential source of shell waste that could feed into chitin production (Hayes *et al*. 2008; FAOSTAT, 2015).

A range of methods are available to extract chitin, but the most commonly used is a chemical procedure (Gortari & Hours, 2013). An acid treatment is used to extract chitin from crustaceans by dissolving the calcium carbonate, and is followed by an alkaline treatment to solubilise protein; this process is adapted to suit the source (Rinaudo, 2006). This is an environmentally hazardous process, hence there has been research into biological processes such as microbiological fermentation and enzymatic methods, which are reviewed in detail by Gortari & Hours (2013).

Partial deacetylation of chitin under alkaline conditions leads to the production of chitosan (poly(D-glucosamine), which is a collective name for a group of compounds that can also be used in agriculture (Kong *et al.*, 2010; Rinaudo, 2006). It is also possible to produce oligochitins by acid degradation of chitin, which can similarly be used in agriculture (Rinaudo, 2006). Chitin and chitosan often contain impurities, which may vary depending on the method of extraction. Products can also contain different chain lengths of chitin or chitosan, which may affect their properties (Sharp, 2013). It is also possible to produce oligochitins by acid degradation of chitin, which can also be used in agriculture (Rinaudo, 2006). Thus, whilst these products can be defined to a chemical level, the potential for variation in plant and/or microbial response to differing chain lengths and impurities means that caution should be used when comparing products. Chitosan is insoluble except in dilute organic acids (e.g. acetic acid, formic acid, lactic acid etc.), has a high viscosity and can coagulate with proteins at high pH, which has resulted in some chemical modifications to improve these characteristics (Rabea *et al.*, 2003). Chitosan can also be combined with other substances; these products are out of scope of the current review, but have recently been reviewed by Das *et al.* (2015) and Badawy & Rabea (2011).

In a recent review on the use of chitosan in horticulture, Pichyangkura & Chadchawan (2015) reported that over 20 vegetable crops had been assessed for response to chitosan. A wide variety of chitosan products were used, and the methods of application included seed coating, root coating, soil supplements, or plant sprays during the growing season. The review demonstrated that the source, form, chain length and degree of polymerisation of chitin and chitosan products can all affect crop responses, as can the growth stage of the crop at application hence these should all be considered and understood prior to application (Pichyangkura & Chadchawan, 2015). It is also possible that chitin may cause phytotoxic effects if supplied in too high concentrations, or if the soil water content is too low (reviewed by Sharp, 2013). Sharp (2013) therefore suggested that these may be avoided or mitigated by ‘wetting in’ after application.

Chitin & chitosan interactions with plants

Chitin & chitosan derivatives have a range of potential effects and interactions with plants. The main activities of chitin and chitosan are described below; other hypothesised effects of chitin-based product applications have limited evidence and are therefore out of scope of this review, but they are described in detail by Sharp (2013).

Chitosan has been shown to interact with plants in various ways including having a direct anti-microbial action against a range of bacteria & fungi, inducing plant defence responses and/or improving tolerance of plants to abiotic stress (Sharp, 2013; Pichyangkura & Chadchawan, 2015; Bautista-Baños *et al*., 2006); and stimulating beneficial microorganisms, which indirectly benefit the plant (Kishore *et al.*, 2005; Badawy & Rabea, 2011). Both the bio-pesticidal and biostimulant effects have been reviewed, as it is important to understand all potential effects of a product to avoid unwanted side-effects.

Chitin-derived products have been found to have significant anti-microbial effects. Whilst still unclear, the hypothesised modes of action as outlined by Sharp (2013) and Badawy & Rabea (2011) include:

i) interactions between the chitosan molecules and the target organism cell membrane, resulting in leaking of intracellular components;
ii) chelation of mineral nutrients/toxic elements, to prevent the production of mycotoxins by the pathogens and limit microbial growth;
iii) activation of plant defences;
iv) binding with DNA and therefore interfering with the synthesis of mRNA and proteins;
v) formation of barrier films on the surface of the cell, leading to reduced cell permeability and nutrient uptake;
vi) adsorption of electronegative substances, leading to microorganism death.

These are described in detail in Badawy and Rabea (2011), but the current conclusion is that no potential mode of action is more likely than any other, with evidence for the majority occurring in a range of scenarios.

Whilst chitosan can have anti-microbial effects on both gram positive and gram negative bacteria, it is thought that bacteria are generally less sensitive to chitosan than fungi (Kong *et al.*, 2010). There is evidence for chitosan anti-microbial activity against a range of key cereal and OSR fungal diseases including grey mould (*Botrytis cinerea*), Fusarium wilt (*Fusarium oxysporum*), common root rot of barley and wheat (*Drechstera sorokiana*), as well as diseases of other key crops including *Rhizoctonia solani* and *Piricularia oryzae* (Rabea *et al.*, 2003). In contrast to chitosan, chitin does not appear to have a significant direct anti-microbial effect (Ramírez *et al.*, 2010). This may be because chitin is insoluble and uncharged whereas chitosan is a cationic polymer (Sharp, 2013).

The application of chitosan induces a range of defence genes in plants, including glucanase and chitinase, as well as reactive oxygen scavengers such as superoxide dismutase, catalayse and peroxidase (Pichyangkura & Chadchawan, 2015). Induced plant defences have been demonstrated in both cereal and OSR crops. Yin *et al.* (2006) analysed the gene expression changes in OSR in response to oligochitosan application, and found that plant defence mechanisms were stimulated. Chitosan application was also found to elicit a defence response in OSR (Płażek *et al.*, 2003). Pre-treating with oligochitosan reduced the frequency and size of Sclerotinia rot (*Sclerotinia sclerotiorum*) in OSR (Badawy & Rabea, 2011, Lu *et al.*, 2003).

A laboratory study by Bhaskara Reddy *et al.* (1999) found that chitosan treatment of spring wheat seeds resulted in improved germination and vigour, which was similar to the effect of the fungicide benomyl. There was also a reduction in the levels of seed-borne *Fusarium graminearum* in the chitosan treatments. The chitosan treatments had greater concentrations of phenolic acid and lignin in the leaves of 10 day old seedlings, which the authors attributed to the chitosan treatment acting as an elicitor for plant defence mechanisms. Thus the chitosan appeared to be acting both directly on the pathogen but also eliciting plant defence mechanisms from an early stage (Bhaskara Reddy *et al.*, 1999). Similarly, improvement in germination has been reported for maize seedlings when primed with chitosan (Guan *et al.*, 2009). Again, physiological changes in the plant were identified.

There is some evidence for virus control in tobacco (Zhao *et al.*, 2007) and potato (Ozeretskovskaya *et al.*, 2006) following oligochitosan application, which is thought to be a result of induced plant responses (Sharp, 2013). Finally, there have also been reported effects on insect pests with one study finding 80% mortality of lepidopterous and homopterous insects, and aphid mortality ranging from 60-80% (Zhang *et al.*, 2003). Rabea *et al.* (2005) reported insecticidal activity of a range of synthesised chitosan derivatives against *Spodoptera littoralis* (cotton leaf worm). However, it is not known whether these insecticidal effects are applicable to insect pests of cereal and OSR.

Chitin can also cause a reduction in plant pests and diseases via the enhancement of beneficial microbes (Badawy & Rabea, 2011). This is hypothesised to be a result of chitin application stimulating the production of chitinase enzymes by other 'beneficial' soil microorganisms since the production of these enzymes by supplementary beneficial microorganisms has been found to increase following chitin application (Kishore *et al.*, 2005). Chitinases can break down the cell walls of pathogens, and therefore have a beneficial effect for the plant by reducing the pathogen load in the soil. Chitin has also been found to improve biocontrol and plant growth when applied with the bio-pesticide *Bacillus subtilis* AF1 (Manjula and Podile, 2001). Consequently, commercial products have been developed that take advantage of the synergistic effect of applying chitin along with chitinolytic biological control agents, such as *Trichoderma* species (López-Cervantes & Reiner, 2012).

Chitin may also exhibit a biocidal effect on nematodes. In a series of three glasshouse experiments on wheat infected with a nematode (*Heterodera avenae*), Spiegel *et al.* (1989) found that straw, grain and ear dry weights were all increased in the chitin (as ClandoSan) treatments, compared to the untreated controls. Chitin applications consistently increased grain yield by 1.5 times, and significantly reduced nematode numbers by between 51-60% in two of the three experiments (Table 7). Furthermore, in the absence of nematodes, chitin application still increased yield compared to the untreated control, suggesting that it had a beneficial effect on wheat growth beyond nematode control.

Other uses of chitin include encapsulation of bio-pesticidal organisms for more controlled application and storage, and as a carbon source for other beneficial and bio-pesticidal organisms, such as the bio-insecticide *Bacillus thurigensis* (Sharp, 2013). There is also limited evidence that chitin may stimulate nodulation in leguminous crops, since it has a similar structure to the lipochitooligosaccharide 'Nod factors' produced by rhizobia bacteria prior to the development of root nodules (Staehelin *et al.*, 2000). However, there are conflicting results for interactions between chitin and arbuscular mycorrhizal fungi. Arbuscular mycorrhizal fungal growth and sporulation were stimulated when chitin was applied to *Allium amppelloprasum*, *Plantago lanceolata* and *Lactuca sativa* plants (Gryndler *et al.*, 2003), but chitin application reduced mycorrhizal colonisation, nitrogenase activity and growth of *Vicia faba* (faba bean) plants (El-Sayed *et al.*, 2002). Thus, the interactions of chitin with key beneficial microorganisms are still poorly understood.

Chitosan has been proposed to improve crop tolerance to abiotic stresses. Production of both NO (nitric oxide) and H_2O_2 (hydrogen peroxide) was increased in OSR epidermal cells in response to chitosan application, which occurred at the same time as stomatal closure and LEA protein gene expression of leaves, suggesting that chitosan may improve resistance to water stress (Li *et al.*, 2009). This is in agreement with previous studies that have shown chitosan application can reduce drought stress symptoms and transpiration rates, and induce stomatal closure (Bittelli *et al.*, 2001, Boonlertnirun *et al.*, 2007), leading to suggestions that chitosan may have potential to be developed into an anti-transpirant (du Jardin, 2015). For example, chitosan has been shown to be effective in reducing water use by field-grown peppers (Bittelli *et al.*, 2001) and beans (Iriti *et al.*, 2009). It is thought to affect stomatal opening via abscisic acid (ABA) signalling (Iriti *et al.*, 2009).
In experiments comparing chitosan with a film-forming anti-transpirant in beans, it was concluded that the film-forming anti-transpirant had a longer-lasting effect and hence was more effective in reducing water loss and maintaining yield in severe droughts, but chitosan could be more suitable for mitigating episodic droughts in temperate conditions, besides having additional benefits in disease control (Iriti *et al.*, 2009). Since reducing water loss by reducing stomatal opening also reduces photosynthesis, Khan *et al.* (2002) found that foliar application of chitosan reduced photosynthetic rates on the day after the application, but then the rate of photosynthesis increased three days after application to up to 18% of the control rate. This was correlated with the increased stomatal conductance and transpiration rate observed. There were also no effects on maize or soybean plant growth parameters including height, root length, leaf area or shoot, root or total dry mass (Khan *et al.*, 2002). At the time of publication, no information could be found on stomatal responses in wheat and OSR.

Other studies have suggested that chitosan can induce tolerance to salt and extreme temperature (Pichyangkura & Chadchawan, 2015). For example, priming of maize seeds with chitosan resulted in improved germination rates under low temperature conditions (Guan *et al.*, 2009). Chitin and chitosan derivatives also have a nitrogen content in the range of 6.1-8.3% (Ramírez *et al.*, 2010), thus chitin-derived products may be a source of slow release nitrogen fertiliser. This should be considered when assessing biostimulant effects on plants, to ensure that any response is not simply a consequence of increased N availability.

Implications for crop yields

All of the above interactions between chitin-derived products and plant responses have the potential to lead to yield responses of the crop. Studies are available for wheat crops, with significant yield increases reported in 9/12 experiments and no significantly negative effects reported, ranging from 94-134% of the control (Table 7). Above-ground growth increases have also been reported in 4/7 studies on wheat and maize, none of these studies reported significant negative effects. Furthermore, as described above, in the absence of nematodes, chitin application still increased yield compared to the untreated control, suggesting that it had a beneficial effect on wheat growth beyond nematode control (Spiegel *et al*,.1989), potentially as a biostimulant. However, the available data from field based studies is limited, therefore it is unclear whether positive effects of chitin and chitosan products are likely to occur in UK crops. Nonetheless, the yield increases can be significant under the correct conditions, therefore this warrants further investigation. Field based trials are required to determine the applicability of chitin-based products in the UK.

Table 7. Effect of chitin and chitosan on above-ground growth, below-ground growth or yield of cereal, maize and/or oilseed rape crops.

Product type	Year	Crop	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
Chitosan	1996*	Dryland wheat	Not available[d]	California	-	-	1/1[†]	-	-	134	Freepons, 1996
Chitosan	1996*	Irrigated wheat	Not available[d]	California	-	-	1/1[†]	-	-	110	Freepons, 1996
Chitin	1985-1987	Winter wheat	ClandoSan	Screenhouse, pot trial	2/3	-	2/3	Not available[a]	-	-	Spiegel *et al.*, 1989
Chitin	1985-1987	Winter wheat	ClandoSan + nematode	Screenhouse, pot trial	2/3	-	3/3	Not available[a]	-	-	Spiegel *et al.*, 1989
Chitosan	2011-2013	Winter wheat	Chitin oligosaccharide	Field, China	-	-	2/4	-	-	94-111[b]	Wang *et al.*, 2015
Chitosan & chitin	2002*	Maize	CH5, CHIT5	Greenhouse, Canada	0/1	0/1	-	Not available[c]	Not available[c]	-	Khan *et al.*, 2002

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

[†] No statistics available

[a] Above-ground biomass data presented in graphs therefore not possible to calculate percentage responses

[b] Significant responses all positive

[c] Information not shown in paper

*Year of publication

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by ‘-‘.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.1.5. Anti-transpirants

Anti-transpirants are chemicals applied to plant leaves to reduce transpiration (water loss). There are two types: film anti-transpirants, such as oils, waxes or phenyl mercuric acetate, which form a colourless film over the leaf surface; and metabolic inhibitors such as abscisic acid and chitosan, which reduce stomatal opening.

Anti-transpirants were extensively studied in the 1960s and 1970s, but it was concluded that the inevitable reduction in carbon dioxide uptake caused by reducing stomatal apertures had too great an impact on photosynthesis, and so outweighed the benefits of the reduced water loss in most circumstances (Gale & Hagan, 1966; Das & Raghavendra, 1979; Solarova *et al.*, 1981). Anti-transpirants were therefore used principally in situations where reducing water loss is important but photosynthesis is not, such as prolonging the life of cut Christmas trees.

More recently, a deepening understanding of crop physiology led to the hypothesis that at key growth stages where drought sensitivity is highest, the benefits of anti-transpirants may outweigh the costs, in drought conditions (Kettlewell *et al.*, 2010). This idea was tested in a series of experiments on wheat at Harper Adams University, in which wheat was either sheltered from the rain or uncovered from GS37-39 until harvest, and either untreated or sprayed with a film anti-transpirant (di-1-*p*-menthene) at a range of growth stages (Kettlewell *et al.*, 2010). It was already known that wheat is most sensitive to drought when meiosis occurs in the pollen mother cells (Saini & Westgate, 1999), which fits with the results of the experiments: that the anti-transpirant increased yield if applied before GS45 (flag leaf sheath swollen) but reduced yield if applied after GS51 (start of ear emergence). The yield benefit of the anti-transpirant also depended on the soil moisture deficit (SMD) at application. Kettlewell *et al*. (2010) suggested that in the UK, applications to wheat before boot stage would be beneficial in the isolated years when there is drought stress at this growth stage, which could be determined using a threshold for SMD. For the soil type on which these experiments were done, the threshold SMD was calculated to be 64 mm, or one third of available water capacity (Kettlewell, 2011), although this threshold will vary with grain price and the cost of anti-transpirant application.

Follow-on work by Weerasinghe *et al*. (2016) concluded that pollen viability was the key mechanism for the yield benefit shown by Kettlewell *et al*. (2010). Meiosis occurred at the boot stage, 11-16 days after anti-transpirant application, and drought conditions reduced pollen viability by 15.2% in untreated plots or 6.7% in anti-transpirant treated plots, relative to well-watered controls. The mean yield benefit of the anti-transpirant in drought conditions was 0.66 t/ha. Controlled environment experiments by Abdullah *et al*. (2015) also confirmed the conclusion of Kettlewell *et al*. (2010), that application timing is key to the yield benefit of film anti-transpirants in wheat. They showed that after a few days of drought, the photosynthetic reduction caused by anti-transiprant application is outweighed by the photosynthesis reduction caused by drought stress in untreated plants. In untreated plants, yield loss due to drought was mainly due to reductions in grains per ear and mature ears per plant, rather than to changes in grain weight. Further work is needed to confirm the mechanism of anti-transpirant action and the benefits of carefully-timed film anti-transpirants in a wider range of field conditions.

Film anti-transpirants have also been investigated for disease control, with products including Vapor Gard (di-1-*p*-menthene) and Ethokem (polyethanoxy amine) giving significant control of *Blumeria graminis* (powdery mildew) on barley (Sutherland & Walter, 2003), and *Pyrenophora Avenae* (oat leaf blotch) and *Pyricularia Oryzae* (rice blast) *in vitro*l (Sutherland & Walters, 2008).

The use of abscisic acid (ABA) as an anti-transpirant has been shown to be effective in protecting seedlings of pepper, tomato and artichoke prior to transplanting, whereas film anti-transpirants were less effective in reducing drought stress in the same situations (Goreta *et al*., 2007; Leskovar *et al*., 2008; Shinohara & Leskovar, 2014). Exogenous ABA applications have also been used to maintain yield in drought conditions in crops such as soybean (Travaglia *et al*., 2009), sunflowers (Hussain *et al*., 2012), and peas (Latif, 2014). As a plant hormone, ABA has been extensively studied in wheat, including its role in drought tolerance. However, ABA has been less widely tested as an applied anti-transpirant for cereal crops; one of the few examples was a recent study in China (Zhang *et al*., 2016), in which ABA (applied at stem extension, ear emergence and grain filling) increased root mass and grain yield relative to an untreated control, under drought conditions in a pot experiment. Significant yield benefits of ABA were also recorded in two field experiments in which drought conditions occurred. In a series of three field experiments on wheat in Argentina, exogenous ABA gave significant yield benefits in the two years with moderate droughts, but no benefit in the year with the most severe drought, when the yields of all treatments were less than 1 t/ha (Travaglia *et al*., 2010).

The experiments by Zhang *et al*. (2016) described above also included fulvic acid (FA) (Table 4), but although there were positive yield effects of FA, these were largest in well-watered conditions, suggesting that the main mode of action of FA is not as an anti-transpirant (see Section 4.1.2). The anti-transpirant action of FA has also been studied in wheat by Li *et al*. (2005), but the experimental details are unavailable. Chitosan has also shown some anti-transpirant effects in horticultural crops (see Section 4.1.4).

Given the high cost of ABA treatments, compared to film anti-transpirants, and the lack of positive results in field conditions similar to those experienced in the UK, existing data does not support the use of ABA on UK cereal and OSR crops. As a by-product of the shellfish industry, chitosan is less expensive than ABA, but has not been tested as an anti-transpirant on cereals or OSR under UK field conditions. Film anti-transpirants do show promise for protecting the yield of UK cereal crops in the occasional years when drought conditions occur before booting stage, in April/May, but should be used with care as they can reduce yield in crops not suffering drought stress.

Table 8 summarises experiments testing the use of anti-transpirants on wheat crops; no data was found on other cereal or OSR crops. Every study involved testing the crop response to anti-transpirants under drought conditions, and some included a comparison under well-watered conditions. There is evidence for significant yield increases of wheat under drought stress in response to film anti-transpirants (13/14 experiments), but also a number of negative responses have been reported in 5/5 of studies under well-watered conditions (Kettlewell *et al*., 2011; Abdullah *et al*., 2015), highlighting the need for anti-transpirants to be correctly targeted to crop conditions.

Table 8. Effect of anti-transpirants (AT) on above-ground growth, below-ground growth or yield of cereal, maize and/or oilseed rape crops.

Product type	Year	Crop	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
Film AT (di-1-p-menthene 96%)	2003-2005	Winter wheat	Emerald	Field with rain shelters, UK	-	-	3/3*	-	-	88-110*	Kettlewell *et al*., 2010
Film AT (di-1-p-menthene 96%)	2009-2011	Winter wheat	Emerald	Field with rain shelters, UK	-	-	4/4	-	-	107-112	Weerasinghe *et al*., 2016
Film AT (di-1-p-menthene 96%)	2014	Wheat	Vapour Gard®	Controlled temperature glasshouse	-	-	2/2*	-	-	83-145*	Abdullah *et al*., 2015
Metabolic AT (Abscisic acid)	2003-2005	Wheat	ABA - not a commercial product	Field with natural drought conditions, Argentina	-	-	2/3	-	-	79-132	Travaglia *et al*., 2010
Metabolic AT (Abscisic acid)	2013	Winter wheat	ABA - not a commercial product	Pot experiment with rain shelters, China	1/1	1/1	1/1	101-112	115-132	103-125	Zhang *et al*., 2016
Metabolic AT (Abscisic acid)	2013-2014	Winter wheat	ABA - not a commercial product	Field with rain shelters, China	-	-	1/1	-	-	106-138	Zhang *et al*., 2016

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

*In every experiment in these studies, significant negative yield responses to anti-transpirants occurred in well-watered conditions and significant positive yield responses in drought-stressed conditions.

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by ‘-‘.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.1.6. Protein hydrolysates and free amino acids

Protein-based products can be split into two main categories: protein hydrolysates, which consist of a mixture of peptides and amino acids of animal or plant origin, and individual amino acids such as glutamate and proline.

Protein hydrolysates are produced through enzymatic, chemical or thermal hydrolysis of a variety of animal and plant residues. These residues include animal epithelial or connective tissues (Cavani *et al*., 2006; Ertani *et al*., 2009, 2013a), animal collagen and elastine (Cavani *et al*., 2006), carobgerm protein (Parrado *et al*., 2008) and alfalfa plants (Schiavon *et al*., 2008; Ertani *et al*., 2009, 2013b). Individual amino acids include the twenty structural amino acids involved in the synthesis of proteins, and non-protein amino acids which are found in abundance in specific plant species (Vranova *et al*., 2011).

Protein hydrolysates have been shown to stimulate production of root and leaf biomass (Zhang et al., 2003; Schiavon *et al*., 2008; Ertani *et al*., 2009). Short-term application of protein hydrolysates increased the root dry weight of maize plants compared to the untreated plants (Ertani *et al*., 2009).

The mode of action of protein-based biostimulants is not fully known, however, recent studies have identified their target metabolic pathways and some of the mechanisms through which they exert their effects on plants (Schiavon *et al*., 2008; Ertani *et al*., 2009; Ertani *et al*., 2011a; Ertani *et al*., 2013). There is evidence to suggest that protein hydrolysates may promote nitrogen assimilation in plants via a coordinated regulation of C and N metabolism (Nardi *et al*., 2015). Schiavon *et al*. (2008) showed that a protein hydrolysate derived from alfalfa plants enhanced shoot biomass production, soluble sugar accumulation and N assimilation of hydroponically-grown maize plants. The protein hydrolysates increased the activity of enzymes functioning in the tricarboxylic acid cycle and enzymes involved in N reduction and assimilation.

An abundance of evidence supports a role for protein hydrolysates and specific amino acids in tolerance of abiotic stresses including salinity, drought, temperature and oxidative conditions (Ashraf & Foolad 2007; Chen & Murata 2008; Kauffman *et al*., 2007; Apone *et al*., 2010; Ertani *et al*., 2013a). Under salinity stress, a protein hydrolysate derived from alfalfa plants was found to improve the growth of maize plants by increasing the ratio of Na^+ and K^+ in the leaves and synthesising flavonoids (Ertani *et al*., 2013b). Kramer (1980) reported that perennial ryegrass plants exposed to prolonged high air temperature stress and treated with a protein-based product showed improved photochemical efficiency and membrane thermostability relative to untreated plants (Kauffman *et al*., 2007). The accumulation of glycine betaine and proline is generally correlated with increased stress tolerance, and exogenous application of these compounds can enhance tolerance to abiotic stresses in a variety of crops including maize, barley, soybean, alfalfa and rice (Chen & Murata 2008; dos Reis *et al*., 2012; Ahmad *et al*., 2013). Additionally, Arginine, which plays a role in the storage and transport of nitrogen, has been shown to accumulate under abiotic and biotic stress (Lea *et al*., 2006).

Application of protein hydrolysates to plant leaves and roots has been shown to increase Fe and N metabolism, nutrient uptake, as well as water and nutrient use efficiencies for both macro and microelements (Cerdán *et al*., 2009; Ertani *et al*., 2009; Halpern *et al*., 2015). The higher nutrient uptake in plants treated with protein hydrolysates has been attributed to

i) an increase in soil microbial activity and soil enzymatic activities,
ii) improvement of micronutrient mobility and solubility,
iii) modifications in the root architecture of plants and,
iv) an increase in nitrate reductase, glutamine synthetase and Fe(III)-chelate reductase activities

(Cerdán *et al*., 2009; Ertani *et al*., 2009; García-Martínez *et al*., 2010; Colla *et al*., 2014; Lucini *et al*., 2015, Colla *et al*., 2015).

A meat hydrolysate derived from tanning residues has shown similar effects to those of the alfalfa protein hydrolysate in maize seedlings (Ertani *et al*., 2013b). These effects included increased short-term growth and macro-element content along with decreased nitrate, phosphate and sulphate content. Additionally, Vernieri *et al*., (2006) demonstrated that the application of a protein hydrolysate influenced nitrogen metabolism in plants, speeding up the incorporation of nitrate into proteins, through the activation of N assimilation-related enzymes. The increased nitrogen use efficiency was supported by the higher leaf chlorophyll content in treated plants.

Exogenous application of glycine betaine significantly increased the net photosynthetic rate and the activities of two key C4 photosynthetic enzymes of maize seedlings grown under nitrogen stress (Zhang *et al*., 2014).

Studies using individual amino acids indicate that they may play a signalling role in regulating nitrogen acquisition by roots. Decreased nitrate, ammonium influx and transporter transcript were found in response to exogenously applied glutamine in barley roots (Fan *et al*., 2006; Miller *et al*., 2007).

Recently, there are growing food safety concerns about the use of animal-derived protein hydrolysates, as demonstrated by the ban on animal-derived protein hydrolysate application to the edible parts of crops in organic farming (European Regulation no. 354/2014) (Colla *et al*., 2014). Additional limitations may be imposed on animal derived-protein hydrolysate application in the production of food for vegetarians or people with religious dietary restrictions. Notably, more than 90% of the protein hydrolysate market in horticulture comprises products obtained through the chemical hydrolysis of proteins from animal origin, while the enzymatically produced protein hydrolysates from plant biomass are less common as they have been more recently introduced to the biostimulant market (Colla *et al*., 2015).

In a number of cases, detrimental effects of some animal derived protein hydrolysates on plant growth have been noted and attributed to an unbalanced amino acid composition (Oaks *et al*., 1977), higher concentration of free amino acids (Moe, 2013) and high salinity (Colla *et al*., 2014). However, Corte *et al*. (2014), conclude that protein hydrolysates did not negatively affect eukaryotic cells and soil ecosystems, and can be used in conventional and organic farming without posing harm to human health and the environment.

Table 9 summarises experiments testing the use of protein hydrolysates on maize crops; no data was found for field experiments or experiments testing the effects on wheat, barley or OSR crops. Application of protein hydrolysates significantly increased above-ground biomass in 3 experiments on maize. In 2/3 of these experiments there were also increases in below-ground biomass, but a significant decrease in below-ground biomass was observed in one study (Colla *et al.,* 2013, Ertani *et al.,* 2009, Ertani *et al.,* 2013a).

Table 9. Effect of protein hydrolysates on above-ground growth, below-ground growth or yield of cereal, maize and/or oilseed rape crops.

Product type	Year	Crop	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
Protein hydrolysate	2009†	Maize	Alfalfa hydrolysate & meat flour hydrolysate	Laboratory, Italy	1/1	1/1	-	104-115	105-142	-	Ertani *et al*., 2009
Protein hydrolysate	2013†	Maize	Trainer	Laboratory, Italy	1/1	1/1[a]	-	107-111	95-96	-	Colla *et al*., 2013
Protein hydrolysate	2012†	Maize	Alfalfa hydrolysate	Laboratory, Italy	1/1	1/1	-	177	119	-	Ertani *et al*., 2013a

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

†Dates are date of publication

[a] significant negative response; dry root biomass

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by ‘-‘.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.1.7. Non-essential chemical elements

Non-essential chemical elements are elements that are not required by cereals and OSR crops, and they do not provide nutrition to cereal or OSR crops, therefore cannot be considered as nutrients. However, they can promote plant growth and may be important for certain plant species (e.g. sugar beet). Non-essential elements include aluminium (Al), cobalt (Co), sodium (Na), selenium (Se) and silicon (Si) (Pilon-Smits *et al.*, 2009). Whilst it is relatively unusual to see these elements proposed as the sole component of a biostimulant product, they are sometimes found in biostimulant product mixes, and are considered to play a role in more complex biostimulants including some compost teas and other complex organic materials (du Jardin, 2012).

These elements are present in all soils. The availability of Al is greatest under low pH conditions, and can cause issues of toxicity in areas exposed to mining and acid deposition (Pilon-Smits *et al.*, 2009). Si and Na are relatively common elements in soils. In contrast, Co and Se are present in lower quantities with soil concentrations reportedly 15-25 ppm and <1 ppm (mg/kg soil) respectively (Pilon-Smits *et al.*, 2009).

Sources of these elements can include industrial by-products, geological deposits, complex biostimulants, irrigation waters, and commercial fertiliser mixes (du Jardin, 2012). For example, common methods of application for silicon (Si) include foliar spraying, soil incorporation or fertigation (Savvas and Ntatsi, 2015). It may be worth considering the potential application of these beneficial elements that may already be occurring before applying more since they are often required in low quantities. Evidence for effects on plants and modes of action for each of the key beneficial elements are outlined below. Each of these has also been reviewed in more detail by Pilon-Smits *et al.* (2009).

Aluminium

Al and other 'non-essential chemical elements' can be detrimental to crop growth in crop plants that are not thought to be hyper-accumulators. In low pH soils for example, Al can become toxic for wheat and other cereal crops. Despite this, Al has been demonstrated to improve growth or pest tolerance for a range of plant species including *Miscanthus sinensis* (maiden grass), and *Festuca arundinacea* (tall fescue) (reviewed by Pilon-Smits *et al.,* (2009)). The modes of action are hypothesised to be increased antioxidant activity, decreased Fe toxicity or increased P availability (Pilon-Smits *et al.*, 2009). Increases in root and shoot biomass have been found in triticale in response to Al addition (Dinev and Stancheva, 1993). Whilst beneficial growth in response to Al has been reported for a range of plant species (Pilon-Smits *et al.*, 2009), most studies on cereal crops relate to Al tolerance rather than beneficial growth responses (Niedziela *et al.*, 2012). Those plants that do respond positively to Al are usually thought to be hyper-accumulators, or are acid tolerant species which have developed a tolerance to high Al levels (Pilon-Smits *et al.*, 2009). Al can also be toxic for OSR crops, and can depress growth (Ligaba *et al.*, 2004). Crops can produce organic acids which improve tolerance of high Al levels (Ligaba *et al.*, 2004; Ma *et al.*, 2000; Stass *et al.*, 2008), but it seems unlikely that Al will be beneficial for most cereal and OSR crops.

Cobalt

Whilst Co is present in very low levels in most soils, it can be toxic to plants in high concentrations. Nonetheless, Co is an essential element for rhizobacterial symbionts and in low levels it can be beneficial to certain plants, particularly legumes (Pilon-Smits *et al.*, 2009, Gad, 2006). Bacterial nitrogenase requires Co as it is necessary for the formation of vitamin B12, which is an essential part of enzyme production for these organisms (Pilon-Smits *et al.*, 2009). Low Co concentrations have also been found to increase wheat growth, but an inverse relationship was found with Co concentration and yield, indicating that higher levels are toxic to wheat growth (Aery and Jagetiya, 2000). Co is thought to delay leaf senescence by inhibiting ethylene biosynthesis, improve drought resistance of seeds and, in hyper-accumulators, Co may improve resistance to herbivory (Pilon-Smits *et al.*, 2009). However, there is very limited evidence to support application to cereal or OSR crops. Since Co is known to aid N-fixing microbes, it is possible that it may provide additional benefits when included in microbial biostimulant products, particularly those that include N-fixing bacteria. Still, the dosage should be carefully considered, since there is clear evidence that higher levels can be phytotoxic.

Sodium

Sodium is toxic to plants at high levels. Nonetheless, Na is required by plants that use C4 photosynthesis (Ohnishi *et al.*, 1990) and is used as a fertiliser for sugarbeet (Defra, 2010). Some plants can also replace K^+ with Na^+ under low K conditions, and Na application may aid with drought tolerance (Pilon-Smits *et al.*, 2009). Nonetheless, since K is unlikely to be limiting under commercial agricultural conditions, it seems unlikely that Na will have a significant positive effect on cereal or OSR crop growth in the UK.

Selenium

Selenium (Se) is very similar to sulphur in composition and therefore can be taken up via the same pathways. In general, it is not thought that Se is essential for crop growth, but there is some evidence for plant growth responses to small quantities of selenium fertilisation. (Pilon-Smits *et al.*, 2009). Application of Se is common in Finland, with the aim of increasing Se content of wheat grain since Se is an essential trace element for humans and livestock (Kieliszek and Błażejak, 2013). This approach has also been considered and may have potential in the UK (Broadley *et al.*, 2009). There is also some evidence that Se may improve resistance to pests and plant growth. However, current evidence suggests that the biggest benefit of Se addition at low levels would be improving the nutritional content of grain for livestock and human consumption.

Silicon

There is some evidence that silicates and silicon can induce biostimulant responses, in addition to pest and disease tolerance. Effects include alleviation of salt stress, adverse climate conditions, drought stress and nutrient deficiency. A recent study found that seed-priming with Si resulted in maize plants that were more tolerant of alkaline stress (Latef & Tran, 2016), and delay of plant senescence processes has also been demonstrated. Again, the modes of action for these effects are poorly understood, with suggestions including anatomical changes in plant tissues, enhancement of the antioxidant defence system in plants, immobilisation of complex metals, or modulation of gene expression and signalling via phytohormones (Savvas & Ntatsi, 2015).

A lot of research indicates that Si is linked to improved pest and disease tolerance of crops. Wheat plants with applied Si had a greater resistance to green-aphids (*Schizaphis graminum* (Rond.)). Si addition was also found to reduce powdery mildew severity in wheat (Guével *et al.*, 2007), with root applications being more effective than foliar applications, but this did not translate to any improvement in plant growth. In addition, applications of Si resulted in significant reductions in grazing of wheat plants by rabbits (Cotterill *et al.*, 2007) and slug feeding on wheat seedlings (Griffin *et al.*, 2015). There is also evidence of mechanical benefits: Si can accumulate in cell walls forming phytoliths and strengthen stems, resulting in reduced lodging (Ma, 2004; Liang *et al.*, 1994). Phytoliths are formed in cell walls when silica is deposited into polymerised SiO_2 (Savvas & Ntatsi, 2015). Hypothesised modes of action include Si acting as a physical barrier, preventing penetration by pests and stimulation of natural defence mechanisms (Pilon-Smits *et al.*, 2009, Savvas and Ntatsi, 2015).

Barley and wheat are both thought to be accumulators of silicon with shoot dry weight concentrations of 1.8% and 2.5% respectively (Guntzer *et al.*, 2012). Whilst not an essential nutrient, improved plant growth in the presence of Si has been demonstrated for a range of plant species (Liang *et al.*, 2007). Since most crop species can either accumulate or tolerate increases in Si availability, it is potentially less risky to apply Si to crops than other beneficial elements. There is limited evidence for Si interaction with OSR, with most literature reporting on interactions with cereal crops. This area therefore warrants further investigation.

Implications for crop growth and yields

The growth and/or yield responses of cereal and OSR crops to various non-essential chemical elements are shown in Table 10. There was a significant negative effect of Al application on root growth of OSR, and high doses of silicic acid resulted in a significantly negative yield response in wheat. However, improved plant growth has been demonstrated for Si, although there are limited studies available to report on, with one study reporting a significant increases and decreases in yield, and another reporting a significant increase in above-ground biomass. These demonstrate the variation in possible responses to non-essential chemical elements and emphasise the need to understand the environmental conditions and dosage applied.

Table 10. Effect of non-essential chemical elements on above- or below-ground growth, or yield of cereal, maize and/or oilseed rape crops.

Product type	Year	Crop	Application name & rate	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
Al	2003	Oilseed rape	50-100 µM Aluminium as $AlCl_3$	Phytotron & controlled growth chamber, Japan	-	1/1[a]	-	-	13-25	-	Ligaba *et al.*, 2004
Se	1999-2001	Winter wheat	Selenium 0.5-20 g/ha as Na_2SeO_3	Field trial, Slovakia	-	-	0/3	-	-	Not available	Ducsay and Lozek, 2006
Si	2005-2006	Wheat	Silicic acid (2.5-7.5g silicic acid per Kg soil)	Greenhouse, pot study, Pakistan	-	-	1/1[c]	-	-	85-157	Abro *et al.*, 2009
Si	2003	Winter wheat	Sodium silicate (7.14 mmol)	Greenhouse, China including drought treatment	1/1	0/1	-	132-174	82-88	-	Gong *et al.*, 2003

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

[a]significant negative response; rate of root growth

[b]drought treatment included

[c]Significant negative and positive responses (negative at high dose)

[d]Year of publication

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by '-'.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.1.8. Complex organic materials

There are a wide range of organic material additions that have been demonstrated to have additional 'biostimulant' effects, beyond fertilisation alone. However, of all the product type categories listed here, complex organic materials is the least well defined, and there is some debate over whether they should be grouped into a standalone section. These product types include materials that are derived from any organic material including, but not limited to, composts, manures, sewage sludge extracts, agro-industrial and urban waste products (du Jardin, 2012). The category therefore includes amendments which are traditionally not considered as biostimulants, due to their use as fertilisers. Nonetheless, they warrant a description since they are proposed to have additional effects on plants including promoting rhizobacterial activity, nutrient cycling and nutrient use efficiency, control of soil borne pathogens and enhance degradation of pesticide residues and xenobiotics (du Jardin, 2012). These proposed effects are difficult to attribute to a specific component as complex organic materials can contain other biostimulant product types, including humic and fulvic acids and seaweed extracts, amino acids and others, and may host a range of potential plant growth promoting bacteria and fungi (du Jardin, 2012).

Compost teas could be classified as complex organic materials as they are produced by ‘brewing’ composted material in water under set conditions and then applying the liquid product, the ‘compost tea’ to the crop (Scheuerell & Mahaffee, 2002). Compost teas are generally ‘brewed’ by one of two methods; nonaerated compost tea (NCT) and aerated compost tea (ACT), but within these broad methods there are variations in compost feedstock, compost age, water ratio, fermentation time, addition of nutrients, as well as physical and chemical factors including temperature and pH (Scheuerell & Mahaffee, 2002); hence comparison between products is very difficult. Nonetheless, they may provide a simple way to enhance benefits from compost sources and there is interest in better understanding compost teas with farmer-led trials on ‘Using compost teas on crops’ being conducted as part of the Innovative Farmers Programme. These are arable trials investigating whether spraying with compost tea has an effect on yield and disease. After the first year of trials yields were increased by 1.3-50%. However, these were not replicated trials and two of the three farms were organic, therefore whilst encouraging more work is needed to understand the consistency of these effects under varying conditions (Innovative Farmers, 2016).

This variation is not limited to compost teas but applies to a range of complex organic materials. It is likely that the majority of complex organic materials will be relatively unique, and entirely dependent on the source of the material. Caution is therefore advised when trying to compare different complex organic materials since the nutrient contents and characteristics may vary significantly between products. For this reason we have not covered this topic in detail here as the level of research required exceeds the scope of this review.

4.2. Microbial biostimulants – Effects and modes of action

4.2.1. Plant growth promoting bacteria and rhizobacteria

The rhizosphere is the volume of soil that is immediately surrounding and is influenced by plant roots. Roots deposit between 5-21% of photosynthetically fixed carbon into the rhizosphere as root exudates (Lugtenberg & Kamilova, 2009). Root exudates are commonly low molecular weight compounds which are easily decomposable, and so act as a key carbon source for rhizosphere microorganisms (Marschner, 2012). The rhizosphere is therefore a unique environment in which microorganisms, including bacteria, survive in association with plant roots, and the density of microorganisms greatly exceeds that found in the bulk soil (Marschner, 2012). However, despite the increased availability of carbon in the rhizosphere, it is a relatively nutrient-limited zone, and is therefore a highly competitive environment. In addition, microorganisms only colonise a fraction of the root surface, predominantly the apical, root hair and basal zones. This is thought to be because these are the zones from which root exudates are produced as the root grows; fewer microbes are associated with older root structures, from which exudation is reduced and limited to higher molecular weight and more recalcitrant products (Marschner, 2012).

Plant growth promoting rhizobacteria (PGPR) are organisms that can be found either within the rhizosphere or plant roots. They are a subset of plant growth promoting bacteria (PGPB), which also include bacteria that are found in aerial parts of the plant. There is a large body of evidence to support positive interactions of PGPB with plant growth and development, either by direct or indirect effects (Lugtenberg & Kamilova, 2009). PGPB are found in all natural environments, but the specific species mix and plant-soil interactions depend on the environment and the plant species present (Compant *et al.*, 2010). PGPR include free-living nitrogen-fixing bacteria such as *Azospirillum* and symbiotic rhizobacteria such as *Rhizobium* and *Bradyrhizobium* (Lugtenberg & Kamilova, 2009). Many others can be found in the phyla Actinobacteria, Proteobacteria, and Fimicutes, including species in the genera *Bacillus, Pseudomonas, Azospirillum, Azobacter, Alcaligens, Arthobacter, Agrobacterium, Burkholderia, Comamonas, Pantoea, Rhizobium, Serratia,* and *Variovorax* (Kloepper *et al.*, 1989, Ahmad *et al.*, 2008).

Plant growth promoting bacteria have been studied since the early 20th Century, and there has been interest in inoculating soils with PGPB and PGPB for decades (Ruzzi & Aroca, 2015). A number of reviews have been published (Calvo *et al.*, 2014; Compant *et al.*, 2005; Compant *et al.*, 2010; Ruzzi & Aroca, 2015; Spaepen *et al.*, 2009), indicating that PGPB and PGPR can stimulate plant growth, increase yield, improve nutrient availability, reduce pathogen infection, and improve tolerance to biotic and abiotic stresses. The modes of action for these key effects are discussed in more detail below.

Improved plant nutrition

Improved assimilation of key nutrients including N, P and K has been found in the presence of PGPB, which may be caused by a range of mechanisms. Certain PGPB species can fix atmospheric nitrogen, including *Azotobacter* and *Azospirillum*, reviewed by Calvo *et al.* (2014). There is also evidence for nutrient solubilisation by some PGPB, which improves nutrient availability for plants. This is particularly relevant for nutrients such as P that readily bind to soil particles making them unavailable to plants. Again, there are a range of PGPB that can solubilise P, via two main modes of action: production of organic acids, or production of phosphatases (reviewed by Calvo *et al.*, 2014; RodríGuez & Fraga, 1999). Organic acids solubilise insoluble phosphate forms by chelating the bound cations, making the phosphate readily available for plant uptake. In contrast, acid phosphatases and phytases can dephosphorylate organic phosphorus forms such as phytate, converting them to forms that are available for plant uptake. Some microorganisms can also solubilise potassium (K) from rock K minerals, either using organic acids to dissolve rock K or solubilising K by chelating silicon ions (Parmar and Sindhu, 2013). Thus, when applied and managed well, PGPR may enable reductions in fertiliser application rates (N and P) (Adesemoye & Kloepper, 2009); this has been demonstrated when PGPR were applied along with an AMF inoculant to tomato plants (Adesemoye *et al.*, 2009). There are also examples of increases in P uptake in wheat when PGPR are applied (Afzal & Bano, 2008; Shaharoona *et al*., 2008). Following seed inoculation of barley with *Bacillus megaterium* RCO1 and *Bacillus* M-13, available phosphate in soil was found to increase, as were root and shoot weight and total biomass (Çakmakçi *et al*., 2007). Although, responses can be variable, for example P-solubilising rhizobacteria resulted in increased growth and yield of OSR, but not P uptake in one study (May, 1997).

Some PGPB may also improve uptake of micronutrients including Zn, Cu, Mn, Ca, Mg and S (reviewed by Calvo *et al*., 2014). However, the mode of action for this is less well understood, with improved root lengths and biomass proposed as one possible mechanism

(Calvo *et al.*, 2014). In contrast, there is a relatively well understood mode of action for the improved uptake of Fe in the presence of PGPB: PGPB produce siderophores, which are Fe-binding chelators that enable the capture and uptake of Fe into cells (Sharma and Johri, 2003).

Plant growth promotion

PGPB can promote plant growth via interactions with hormones. A range of phyto-hormones including auxins, cytokinins, giberellins and ethylene are known to be produced by PGPB (Dodd *et al.*, 2010), which can increase above and below-ground plant growth (Lugtenberg & Kamilova, 2009). PGPB can also degrade plant hormones including the stress hormone, ethylene, or modify the plant hormone status (Dodd *et al.*, 2010). The complexity of the interactions between specific PGPR strains and various phytohormones are out of scope of the current review, but have been reviewed in detail by Dodd *et al.* (2010).

Reduced pathogen infection

There is evidence for a wide range of PGPR interacting with and reducing the damage caused by plant diseases. (Lugtenberg & Kamilova, 2009; Ramamoorthy *et al.*, 2001; Compant *et al.*, 2005). For example, *Pseudomonas* spp. can reduce take-all disease in wheat, and mixtures of *Pseudomonas* spp. were found to be more effective than single species (Duffy and Weller, 1995; Pierson and Weller, 1994). *Pseudomonas putida* (strain BK8661) has been found to suppress growth of *Septoria tritici* and *Puccinia recondite* in vitro and on wheat leaves (Flaishman *et al*., 1996). There is also a good range of evidence for induction of systemic resistance by PGPR in various plant species reviewed by Ramamoorthy *et al*. (2001). Given the vast range of potential PGPR, it is perhaps unsurprising that a number of modes of action have been identified that result in reduced pathogen effects. These are reviewed in detail by Lugtenberg & Kamilova (2009) and Compant *et al.* (2005), and include antagonism, signal interference, induced systemic resistance, siderophore production, competition for nutrients and niches, and interference with pathogen activity and survival. A short description is included in

Table 11, summarised from Lugtenberg & Kamilova (2009).

Table 11. Common modes of action for biocontrol of plant diseases by plant growth promoting rhizobacteria (PGPR). Summarised from Lugtenberg & Kamilova (2009).

Mode of action	Description
Antagonism	Bacteria produce antibiotics that are delivered in sufficient quantities to the correct areas of the rhizosphere and therefore directly kill pathogens. A range of antibiotics can be produced, including hydrogen cyanide (HCN).
Signal interference	Some PGPR can degrade homoserine lactones (AHL) which are thought to be used as signalling molecules by certain pathogenic bacteria, and may also be involved in biofilm production. Thus by degrading AHL, certain PGPRs can affect the ability of bacteria to act as pathogens.
Induced systemic resistance	Presence of certain PGPR in the rhizosphere may result in the plant developing resistance to certain pests and pathogens. In contrast to antagonism, induced systemic resistance doesn’t require the PGPR to colonise a large proportion of the root system, and therefore may have a faster and more consistent action against pests.
Siderophore production	In soils where Fe^{3+} is low, the production of siderophores may mean that the PGPR make Fe unavailable for phytopathogens resulting in improved plant health.
Competition for nutrients and niches	It is possible that certain PGPR strains can colonise the entire plant root and outcompete pathogens for both nutrients and space. Whether this is consistent under field conditions is unclear.
Interference with pathogen activity and competition	By growing on or in the pathogen, it interferes with its fundamental growth and activity.

Improved tolerance to biotic and abiotic stresses

Certain strains of PGPR produce an enzyme that converts the precursor for ethylene into 2-oxybutanoate and NH_3. This can reduce the effect of stresses including pathogenic bacteria, heavy metals, salt and drought (Ahmad *et al.*, 2008; Lugtenberg & Kamilova, 2009). Certain strains of PGPR may also aid in the degradation of soil pollutants, which is likely to be less relevant for UK cereals and OSR cropping, but further information is available in Lugtenberg & Kamilova (2009). Some PGPR have been found to be active not only against bacterial pathogens, but also other pests including fungi (possibly a result of HCN and/or siderophore production) (Ahmad *et al.*, 2008), root knot nematodes, and even against some insects although this is less well documented (Péchy-Tarr *et al.*, 2008; Siddiqui *et al.*, 2005; Ahmad *et al.*, 2008). There is also some evidence for biofortification via improved protein and micronutrient content of wheat when PGPR and cyanobacteria were applied to field trials in New Delhi (Rana *et al.*, 2012).

In order to have a positive effect on plants, any applied PGPB or PGPR need to be ‘rhizosphere competent’, meaning they must be able to survive in the highly competitive rhizosphere environment (Compant *et al.*, 2005). There are a wide range of traits associated with both rhizosphere and endosphere (within plant cells) colonisation by bacteria; these vary significantly between species which may go some way to explaining the variable results seen in field studies (Compant *et al.*, 2010). This issue has been reviewed by Compant *et al.* (2010), who concluded that a better understanding of specific plant-soil-PGPB interactions is required. A review by Lugtenberg & Kamilova (2009) concluded that for a soil bacterial inoculant to be effective, it must maintain its efficacy under a wide range of environmental conditions, including pH, temperature and ion concentrations. Whilst initial versions of products often fail in this regard (Lugtenberg & Kamilova, 2009), improved product efficacy may be achieved following further research.

Implications for crop yields

There is a large amount of literature available summarising the use of PGPR on cereal crops. A subset of this is included in Table 12. For cereals and maize, 13/15 experiments reported showing significant yield increases. However, 7/15 of these were glasshouse or pot based, and no field studies were carried out in the UK. In addition, Lucy *et al*., 2004 reviewed 34 studies of PGPR interactions with cereal and OSR crops including wheat, barley, maize and OSR, with the majority of studies reporting either a positive growth or yield response. However, some were only significant under low N conditions, suggesting that background fertiliser availability can be important in these effects (Lucy *et al*., 2004). A recent European consortium study on 'Rhizobacteria for reduced fertiliser inputs in wheat' (RHIBAC) carried out a range of glasshouse (at least 14) and field trials (at least 19) across Europe (von Wirén, 2010). The responses were variable, with some negative responses at full fertilisation whereas a few reported similar yield responses to those of N fertilised plots. This information was available in a draft report and may be subject to changes (von Wirén, 2010). Nonetheless, it demonstrates the complexity of these interactions.

As for most other product types, the studies in Table 12 are not UK based and are often carried out in stressed environments, where wheat yields are around 3 t/ha. It is therefore difficult to extrapolate to a UK setting. Nonetheless, there is a reasonable body of evidence that shows that PGPR can have a beneficial effect on both plant growth and yield. Many studies first screen a wide range of bacterial strains before using the most promising strains in the field experiments. This may partly explain why there are limited negative results found. These conditions also varied from sterilised soil to soil with varying nutrient contents. Thus, the specific conditions in which these species are being used is important and UK based work is required to determine the best species/environment/crop combinations.

Table 12. Effect of plant growth promoting rhizobacteria on above-ground growth, below-ground growth or yield of cereal, maize and/or oilseed rape crops.

Product type	Year	Crop	Organism Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
PGPR	2007-2008	Spring wheat	*Bacillus* spp., *Azospirillum brasilense* Sp.245, *Paenibacillus polymyxa* and PGPR species mixes	Glasshouse, non-sterilised soil, Turkey	1/1[†]	1/1	1/1	114-131	111-118	104-122	Çakmakçi *et al*., 2014; Turan and Sahin, 2014; Turan *et al*., 2012
PGPR	2007-2008	Spring wheat	*Bacillus* spp., *A. brasilense* Sp.245, *P. polymyxa* and PGPR species mixes	Field, Turkey	3/4[e]	-	4/4	73-121	-	103-150	Çakmakçi *et al*., 2014
PGPR	2007-2008	Spring barley	*Bacillus* spp., *A. brasilense* Sp.245, *P. polymyxa* and PGPR species mixes	Glasshouse, non-sterilised soil, Turkey	1/1	1/1	1/1	114-124	110-121	108-126	Çakmakçi *et al*., 2014
PGPR	2007-2008	Spring barley	*Bacillus* spp., *A. brasilense* Sp.245, *P. polymyxa* and PGPR species mixes	Field, Turkey	2/4	-	2/4	101-133	-	93-167	Çakmakçi *et al*., 2014
PGPR	2006[d]	Barley	*Bacillus* spp.	Glasshouse, sterilised soil, Turkey	1/1	1/1	-	99-108	108-117	-	Canbolat *et al*., 2006
PGPR	2010[d]	Wheat	*Bacillus simplex, Bacillus megaterium, Bacillus cereus, Paenibacillus alvei*	Glasshouse, South Africa	1/1	1/1	-	94-226	107-173	-	Hassen & Labuschagne, 2010
PGPR	2009	Wheat	N fixing and P solubilising bacteria	Field, Iran	-	-	1/1	-	-	104-115	Saber *et al*., 2012

Table 12 continued.

Product type	Year	Crop	Organism Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
PGPR	2011-2012	Wheat	Mix of *Bacillus* spp.	Field, Sicily, Italy	1/1	-	-	99-120	-	-	Saia *et al*., 2015
PGPR	2007	Oilseed rape	*Pseudomonas fluorescens* and *Enterobacter radicincitans*	Pot study, 'semi-field', Germany	0/1	-	-	96-112	-	-	Krey *et al*., 2011
PGPR	2007	Maize	*P. fluorescens* and *E. radicincitans*	Pot study, 'semi-field', Germany	0/1	-	-	97-104	-	-	Krey *et al*., 2011
PGPR	2013[d]	Oilseed rape	Wide range of PGPR, mixtures and single inoculants	Greenhouse, Ireland	1/1	-	-	82-136[a]	-	-	Oteino *et al*., 2013
PGPR	1985	Oilseed rape	*Pseudomonas putida, P. fluorescens, Arthrobacter citreus, Serratia liquefaciens*	Field, Canada	-	-	4/4[c]	-	-	Up to 157[b]	Kloepper *et al*. 1988

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

[†] Two experiments in study but only mean values over the two experiments reported therefore treated as a single experiment

[a] Total biomass

[b] Lowest results not reported, none significantly lower than control

[c] More experiments included in study but insufficient data/information to report besides the four included.

[d] Year of publication

[e] 1/4 experiments had a significantly negative response, 2/4 experiments had a significantly positive response

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by '-'.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.2.2. Non-pathogenic fungi

Non-pathogenic fungi, or 'root-associated fungi' are species of fungi that interact with plants in a positive way, thus excluding pathogens. They are different to mycorrhizal fungi as they do not rely on their host plant for survival, although they do benefit from being present in the carbon-rich rhizosphere. Thus, they can be produced more easily on a commercial scale than mycorrhizal fungi, and therefore may be preferable for development as inoculants. These non-pathogenic fungi can be found in the rhizosphere, on the rhizoplane (the surface of the root), and within root tissues (endophytes) (reviewed by Owen *et al.* (2015)).

Potential non-pathogenic fungi that associate with roots include *Aspergillus*, *Trichoderma*, *Penicillium*, *Saccharomycetes*, *Mortierella* and *Mucor* species (Owen *et al.*, 2015). However, the most common species found in inoculants or proposed for use are *Trichoderma*, *Penicillium*, *Piriformospora* and yeast species. Non-pathogenic fungi have been demonstrated to stimulate plant growth, improve plant nutrition, protect against plant diseases, increase tolerance to abiotic stress, and contribute to bio-remediation via the sequestration of harmful substances (Owen *et al.*, 2015). However, not all fungi can provide all of these benefits. Three of the more well-known groups are discussed below.

Trichoderma species

Trichoderma species of fungi inhabit the outer cortical layers of the root epidermis, and are well known for their ability to act against plant pathogens (Harman, 2006). There is evidence for a range of modes of action for this activity including mycoparasitism (the parasitism of one fungus by another fungus), antibiosis, direct competition for resources and space, and induction of systemic or localised resistance in plant hosts (Harman *et al.*, 2004a). *Trichoderma* can locate and then attack other fungi, such as *Rhizoctonia solani*, via the production of cell-wall-degrading antifungal enzymes (Harman *et al.*, 2004a). There is also evidence for increased growth in plants colonised by *Trichoderma*, thought to be driven by auxin production (Contreras-Cornejo *et al.*, 2009).

Harman *et al.* (2004a) reported that over 500 commercial and academic trials have assessed the effect of *Trichoderma* species T-22 on maize, and that the average increase in yield was ~5% (using 'typical' agricultural practices). They stated that the yield increases were generally larger under stressed conditions, whereas under optimal conditions there was minimal scope for yield improvement, although there was some evidence of yield improvements following seed treatment of maize plants (Harman *et al.*, 2004b). As with many microbial inoculants, the effects are often species specific, with some strains having a negative effect on yield and crop responses. *Trichoderma* are also resistant to cyanide, which is produced by some pathogenic fungi, and they can produce enzymes to degrade it (Ezzi and Lynch, 2002). Maize plants that received a seed treatment of *Trichoderma* have been shown to require less fertiliser nitrogen to produce the same yield (Harman, 2000), thus enhancing N use efficiency. Furthermore, there is evidence that under certain conditions, *Trichoderma* can also increase the uptake of a range of other elements including arsenic, cobalt, cadmium, chromium, nickel, lead, vanadium, magnesium, manganese, copper, boron, zinc, aluminium and sodium (Harman *et al.*, 2004a), although the extent of the improved uptake is relatively limited in most cases. *Trichoderma* spp. are also capable of solubilising other plant nutrients including rock phosphate, iron, copper, manganese and zinc via the reduction of metallic ions to increase solubility and production of siderophores (which can chelate iron) (Altomare *et al.*, 1999).

Penicillium species

Penicillium species inhabit the rhizosphere of a wide range of agricultural plants including cereal and OSR crops (Richardson *et al.*, 2011). The main benefit of *Penicillium* and *Aspergillus* species for plant growth is thought to be via the provision of P that would otherwise be inaccessible to the plant; for instance, inoculation of wheat plants with *Penicillium bilaiae* has been shown to increase P uptake (Asea *et al*., 1988). *Penicillium* species can mobilise inorganic phosphate from rock phosphate, via the production of organic anions (e.g. gluconate, oxalate, citrate) (Whitelaw *et al.*, 1999). Their ability to mineralise organic P has also been demonstrated in laboratory media, but the extent to which this can occur in soils is relatively poorly understood. The role of *Penicillium* in mobilisation of P is reviewed in more detail by (Richardson *et al*., 2011). Other benefits include increased plant and root growth (Wakelin *et al*., 2007), which may to be a consequence of phytohormone production, such as auxins and gibberellins by the fungus (Anstis, 2004; Richardson *et al*., 2011). The enhanced root growth is also thought to increase nutrient capture.

However, as for most microbial inoculants the results in the laboratory do not always translate to the field. In a series of experiments on *Penicillium bilaii*, over 26 field sites a positive yield response occurred in only five out of 47 trials, whereas nine showed a decrease in yield, yet 33 trials showed a response to P fertiliser (Karamanos *et al.*, 2010). However, these experiments were conducted from 1989 to 1995, and there have been significant improvements in application methods, storage and management understanding of non-pathogenic fungi since then. There were variable interactions found between *Penicillium radicum* and take-all disease, with no effect, or even an increase, of *P. radicum* inoculation on take-all severity of wheat plants grown under controlled conditions (Wakelin *et al.*, 2006).

However, in the same study, the highest reduction in take all was found (11% reduction in root infection compared to the untreated control) when a fluquinconazole fungicide (Jockey) was applied along with the *P. radicum* inoculant, whilst also increasing shoot dry weight. Thus, the reasons for variable responses under field conditions can sometime be explained by interactions with native organisms.

Despite some variable evidence, there have been commercial inoculants containing *Penicillium* available in the US for over 20 years. Jumpstart® is a commercial *Penicillium* inoculant marketed by Monsanto BioAg that is already available in Canada, though not yet in the UK. Furthermore, a strain of *P. radicum* has been commercialised in Australia for cereal crops (Wakelin *et al.*, 2007). There is also some interest in manipulating plant fungal associations by making use of molecular biotechnology techniques (Behie & Bidochka, 2013)

Piriformospora indica

Piriformospora indica is an endophytic fungus that associates with a range of plant hosts including wheat, barley and rice (Waller *et al.*, 2005, Varma *et al.*, 2012). It is often referred to as a 'mycorrhizal' inoculant, but it is important to not confuse this with arbuscular mycorrhizal fungi, since AMF cannot be cultured without a host plant whereas *P. indica* can easily be grown in culture (Varma *et al.*, 1999). It has been found to promote growth of maize, barley, wheat and field mustard (Franken, 2012), possibly by interactions with a range of phytohormones (Franken, 2012, Schäfer *et al.*, 2009, Sirrenberg *et al.*, 2007). There is also some evidence for improved germination and seed production in plants colonised by *P. indica* (Varma *et al.*, 2012). *P. indica* can induce systemic resistance against leaf pathogens, and exhibits some control over various pathogens of wheat, barley and maize (Serfling *et al.*, 2007; Waller *et al.*, 2005; Kumar *et al.*, 2009). There is evidence for improved plant tolerance of stresses including drought, salt, high and low temperature, and systemic resistance to toxins and heavy metals ions (Varma *et al.*, 2012). These effects may be partially controlled by changes in plant proline and/or reactive oxygen species levels (Zarea *et al.*, 2012; Baltruschat *et al.*, 2008; Sherameti *et al.*, 2008). There is some evidence for increased N and P uptake in the presence of *P. indica* (Yadav *et al.*, 2010, Kumar *et al.*, 2011), although it is difficult to distinguish between enhanced root growth and N/P uptake by *P.indica.* A range of both positive and negative effects of rhizobacteria on the growth of *P.indica* have been found, from stimulatory to neutral or inhibitory (Varma *et al.*, 2012), the response of axenically grown barley roots was also found to vary depending upon the species of co-inoculated microorgansims (Varma *et al.*, 2012).

The limiting factor for use of *P. indica* in commercial inoculants in Europe is that it was isolated in India and it is not thought to be well suited for growth at lower temperatures, thus the search for a related species with similar characteristics may be of greater interest (Serfling *et al.*, 2007). Franken (2012) proposed that before *P. indica* can be readily used in commercial inoculants, further research is required into alternatives including related fungal isolates, methods of inoculant production, and inoculant formulation and stability. It may also be possible to use a filtrate from the inoculant which may avoid application of the fungus, but still stimulate plant growth.

There is minimal evidence of the effects of non-pathogenic fungi on yields of cereal and OSR crops, and no data were available at the time of writing on UK cereal or OSR crops (Table 13). In 2/7 studies on cereal crops, non-pathogenic fungi were found to significantly increase yield, ranging from 106-111% of the control. No evidence for effects on OSR growth could be found. The yield benefits are variable, probably because the main benefits of ‘non-pathogenic fungi’ are to increase the uptake of nutrients such as P and N, or to reduce the effect of pathogens, which would only be expected to increase yield in situations of limited nutrient availability or when pathogen levels were above treatment threshold level. This would be a key area of future research for any products that contain non-pathogenic fungi to prove their effectiveness in a commercial market.

Table 13. Effect of non-pathogenic fungi on above-ground growth, below-ground growth or yield of cereal, maize and/or oilseed rape crops.

Product type	Year	Crop	Product and/or organism name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
Non-pathogenic fungi	2004	Wheat	*Trichoderma harzianum*	Field, Turkey	-	-	0/1	-	-	107	Öğüt *et al.*, 2005
Non-pathogenic fungi	2005†	Barley	*Piriformospora indica*	Open-air pot study, Germany	0/1	-	1/1	102-103	-	106-111	Waller *et al.*, 2005
Non-pathogenic fungi	2006	Wheat	*Penicillium* sp. Strain KC6-W2, *P. bilaiae, P. radicum*	Pot trial, Australia	1/1	-	-	93-119[a]	-	-	Wakelin *et al.*, 2007
Non-pathogenic fungi	2013†	Spring wheat	JumpStart® (*Penicillium bilaiae*)	Field, Alberta, Canada	-	-	1/2	-	-	Not available[b]	Zhang *et al.*, 2013
Non-pathogenic fungi	2003-2004	Wheat	*P. indica*	Field, Germany	1/3[a]	-	0/3	Not available[b]	-	Not available[b]	Serfling *et al.*, 2007

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

[a]significant response was positive, no significant negative responses

[b]results only available in graph form, therefore not possible to calculate percentage range

†Year of publication

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by '-'.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.2.3. Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) can form a mutualistic symbiosis with over two thirds of all land plants and can be found across all major terrestrial biomes (Treseder and Cross, 2006). Intraradical structures, typical of the AMF symbiosis, are formed when the AMF penetrate the cell walls of plant roots (Parniske, 2008). External to the plant root, AMF also produce an extraradical mycelium (ERM), which is made up of hyphae that extend into the soil beyond the plant root system. This ERM is used to significantly extend the volume of soil available for nutrient uptake, but is also used by AMF to search for new plant hosts (Friese & Allen, 1991, Smith & Smith, 2011).

Hyphal turnover can be very rapid, as quick as a few days (Staddon *et al.*, 2003), therefore spores are usually used in AMF inoculum. These take time to colonise and grow into the plant, but can ultimately provide a whole season or multi-season benefit for plants. The germination triggers of AMF spores differ between species and environments, and can be controlled by the origin of the inoculant (Kapulnik & Douds, 2013).

Given the wide range of plant hosts for AMF, it is almost certain that there is a community of AMF in most UK soils, including agricultural soils (Daniell *et al.*, 2001). Despite the large body of research now available on AMF, the vast majority of studies suggest that AMF are most common in natural systems, and it is in these systems that they are most valuable (Smith & Smith, 2011). Yet, AMF can, and frequently do, colonise a wide range of agricultural plants, including most UK cereal crops, with the notable exception of Brassicas (Table 14).

Table 14. The ability of cereal and oilseed rape crops to form the arbuscular mycorrhizal (AM) association.

Crop	Able to form AM association?	References
Wheat	Yes	Hetrick *et al*., 1993
Barley	Yes	Harley & Harley, 1987
Oilseed rape	No; most brassica species are not capable of forming the AM association.	Harley & Harley, 1987
Oats	Yes	Harley & Harley, 1987

The diversity of AMF populations in arable fields is lower than in neighbouring woodlands (Daniell *et al.*, 2001); this is perhaps unsurprising, given most UK arable fields are grown as mono-cultures (Verbruggen & Toby Kiers, 2010). Whilst a reduction in diversity is often thought to reduce an environment's resilience to environmental changes, it does not necessarily mean that these AMF are not interacting with their host plants. As resources become more limited and growers are encouraged to think more strongly about the general health of their soils, there is a growing interest in understanding the role AMF have to play in UK cropping. This section will look at the evidence for the proposed effects and modes of action of AMF in cereal crops.

Historically, the main role of the arbuscular mycorrhizal (AM) symbiosis was thought to be in aiding plant phosphorus (P) nutrition, as the AMF can access P that is outside the depletion zone that builds up around the root surface (Fitter *et al.*, 2011). However, there are a range of AMF benefits to host plants that have proven the symbiosis to be more complex. AMF can improve soil structure (Rillig *et al*., 2002), improve the water status of their host plants (Augé, 2001; Ruiz-Lozano *et al*., 2001), increase host disease and pest resistance (Fritz *et al*., 2006; Jung *et al*., 2012), protect against heavy metal contamination (Guo *et al*., 1996; Göhre & Paszkowski, 2006), reduce nutrient leaching (Asghari & Cavagnaro, 2011; Asghari & Cavagnaro, 2012), and increase the uptake of additional nutrients including copper (Liu *et al*., 2000a), zinc (Thompson, 1996; Cavagnaro, 2008) and nitrogen (Leigh *et al*., 2009). AMF can also modify the soil environment, with evidence for changes in pH (Bago *et al*., 1996; Li *et al*., 1991; Villegas & Fortin, 2001) and nutrient availability, carbon exudation and release of glomalin (Purin & Rillig, 2007), which in turn may improve soil structure, plant water uptake and diffusion of gases through soils (Bronick & Lal, 2005; Horn & Smucker, 2005).

The modes of action of AMF are better defined for some effects than others; the best understood effect of AMF on plants is aiding in plant P acquisition, via extension of the zone of soil from which P uptake can occur (Smith & Read, 2010). Although AMF may not always increase the total P content of their host plant, they can sometimes be responsible for the entire P supply, i.e. P uptake is via the AMF rather than the plant roots alone (Smith *et al.*, 2004). Thus, AMF may be benefitting plants in a more subtle way than simply increasing total P uptake.

The interactions of AMF with plant N nutrition are less well understood than with P nutrition. The interactions between AMF and soil N cycling are greater than previously thought (Hodge & Storer, 2015); AMF can take up and transfer N to their host plant (Hodge & Fitter, 2010), and this ability can differ between AMF species (Leigh *et al*., 2009). Yet, since AMF have a high N requirement themselves, the extent to which they will provide the host with N is thought to be relatively limited (Hodge & Fitter, 2010). There is mixed evidence for the transfer of N to plants via AMF under field conditions (Hodge & Storer, 2015). AM tomato plants acquired more N on an organically managed Californian farm than non-AM mutant tomato plants (Cavagnaro *et al.*, 2012), whereas there have been mixed results found in grassland field systems (Blanke *et al.*, 2011; Karanika *et al.*, 2008). Thus, there is still a significant level of uncertainty over the interaction between AMF and crop N nutrition in the field, with very limited field-based evidence available. There is evidence for potential environmental benefits, with AMF presence reducing nitrate leaching (Asghari & Cavagnaro, 2012), reducing potential nitrification rates (Veresoglou *et al.*, 2011) and reducing N_2O production (Bender *et al.*, 2014), but none of these experiments were carried out in cereal crops.

Compatible AMF and N-fixing bacteria have been shown to increase the availability of N to plants in both glasshouse and field based studies (Toro *et al.*, 1998, Xavier & Germida, 2003; Tajini *et al.*, 2011; Wang *et al.*, 2011), although these studies were not on cereal or OSR crops. Under P limiting conditions, it is thought that the AMF release the N-fixing bacteria from P limitation, which in turn increases the N available for the plant (Artursson *et al.*, 2006; Tajini *et al.*, 2011; Wang *et al.*, 2011). Thus, there may be a synergistic, positive effect of dual symbiosis for N-fixing bacteria and AMF in nodulating plants such as peas and beans (Mortimer *et al.*, 2013). N-fixing genes have also been discovered in the endosymbionts of certain species of AMF, but their role is poorly understood (Minerdi *et al.*, 2001).

There is evidence for mycorrhizal induced systemic resistance against a range of pests and diseases including nematodes, biotrophic and necrotrophic pathogens and herbivorous arthropods (summarised by Cameron *et al.* (2013)). For example, take-all has been systemically reduced in the roots of barley plants when inoculated by AMF in a laboratory pot study (Khaosaad *et al.*, 2007), but this effect may vary between barley varieties (Castellanos-Morales *et al.*, 2011). Antibiotic production by *Pseudomonas fluorescens*, a rhizosphere bacteria proposed as a potential as a biocontrol agent of take-all, was also found to be stimulated by the presence of AMF (Siasou *et al.*, 2009) in a glasshouse study on wheat.

Glomalin is a glycoprotein produced by AMF that may positively affect soil structure, although it is unclear whether this effect is an intentional, or if it is only released upon hyphal senescence with its main function instead being physiological, as a chaperonin or pest deterrent within the fungal hyphae themselves (Klironomos & Kendrick, 1996; Purin & Rillig, 2007). Nonetheless, glomalin is persistant in soils and has been demonstrated to improve soil aggregate stability (Rillig & Mummey, 2006).

Thus, it is clear that AMF can have a wide range of interactions with their plant hosts. However, positive interactions do not occur in all situations and it is important to recognise that microbial inoculants require careful management, and successful inoculation can be difficult to achieve (Killham, 1994). The key practical issues with the use of AMF to improve crop yields in the field were summarised by Smith & Smith (2011) and include;

1. There are no ‘elite’ universal AMF which maximise growth of all plants
2. Production of high quality inoculum requires suitable host plants, as AMF are obligate symbionts, thus it is expensive to produce and quality testing is essential.
3. Large scale inoculation in the field is neither easy nor cheap (partly because of the cost of production, partly because of practicality of application) and survival of inoculants is problematic (as for most biological inoculants).
4. Where fields have received high P inputs, the likelihood of strong growth responses to AMF inoculation are slim, as most research has demonstrated that the majority of responses primarily occur under low P scenarios.

There are also particular management factors that are likely to specifically affect the success of an AMF inoculant in UK arable soils. These include: availability of N and P, tillage, presence of Brassica species, and fungicides (Gosling *et al*., 2006). Gosling *et al*., (2006) also summarised a range of examples where organic systems showed greater numbers of AMF propagules, diversity or root colonisation compared to conventional systems yet concluded that there was still a large amount of variation in response to native AMF in the field. A meta-analysis of publications on AMF inoculation in laboratory and field studies found that after plant functional group, N fertilisation had the greatest effect on the plant response to AMF inoculation (Hoeksema *et al.*, 2010), with higher N fertilisation resulting in lower AMF response. This is in contrast to work by Johnson *et al.* (2003), who found that nitrogen enrichment (mediated, in this case by ambient soil fertility) resulted in a decrease in allocation to AM structures under high P, but increased allocation to AM structures in P deficient soils. Since AMF have a high N requirement themselves (Hodge & Fitter, 2010), it is hypothesised that under conditions where P is limiting, but N is not, application of N may increase the plant response to AMF (Johnson *et al.*, 2003). However, in most conventional arable situations, the background P levels may be too high to see benefits from AMF inoculation and it may take time for the levels to fall to the point at which AMF become beneficial.

Since AMF obtain their C from their host plant, they can, under certain conditions, have a negative effect on plant growth. In fact, there have been calls in SE Australia to select wheat varieties for low AM colonisation in order to reduce the risk of the ‘parasitic’ effect (Ryan *et al.*, 2005), although this is an unusual suggestion.

There is a need to better understand the interactions of UK crop species with soil microorganisms including AMF, which are likely to be interacting with almost all UK cereal crops. At present we have no clear understanding as to whether this interaction is positive or negative, and, particularly whether inoculation will improve this interaction and ultimately improve yield and stress tolerance. It would be possible, with field-based research to complement the more fundamental laboratory and glasshouse results currently available, to better understand the effects of N and P rates, crop rotations and tillage on AMF presence and inoculation success.

There are a wide range of responses to AM inoculation reported in the literature, a subset of which are summarised in Table 15. Some of these studies include commercial inoculant products that are or have been available for purchase. Cereal and maize yield responses ranged from 93-233% of the control in experiments, with 5/7 reporting a significant yield increase in response to AMF application and no negative responses. However, only 2/11 studies were UK based, and 5/11 studies used sterilised soil. There was also a significantly negative response in above-ground biomass in 1/7 reported experiments.

However, it is important to recognise that varying P and N conditions and natural microbial populations may have affected the results, therefore caution should be used when drawing conclusions for UK scenarios. Furthermore, the UK studies did not show strong responses (Clarke and Mosse, 1981; Khaliq and Sanders, 2000). This was also raised by Khaliq and Sanders (2000), who found no response of UK field-grown barley to AMF inoculation. Pellegrino *et al*. (2015) carried out a meta-analysis of 38 experiments on the responses of wheat to AMF, 21 of which included an inoculation treatment; where the strain of AMF was specifically selected, inoculation with AMF increased grain yield by an average of 20%, and harvest index by an average of 25%. The authors commented that key drivers for the growth response were soil organic matter, pH, total N and available P concentration, soil texture, climate and inoculant species. However, there was only one UK-based study included. However, the studies were carried out under a wide range of field conditions in the USA, Iran, Mali, India, China, Australia, and Canada and the authors noted that Central Europe, which has the highest yields was not represented. Thus, whilst an impressive increase in yield, it is unlikely to be representative of UK conditions. Given the effect of climate and soil type on these types of interactions, it is difficult to translate research findings from non-UK experiments into a UK setting, but it does demonstrate the level of interaction that is possible between AMF and key crop species.

Table 15. Effect of arbuscular mycorrhizal fungi on above-ground growth, below-ground growth or yield of cereal, maize and/or oilseed rape crops.

Product type & species name(s)	Year	Crop	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
AMF (*Glomus mosseae*)	1981	Wheat	-	Sterilised soil, pot study, Spain	SNR	SNR	-	96-173	99-1033[†]	-	Azcón & Ocampo, 1981
AMF (*G. mosseae* and *Glomus etunicatum*)	2001 - 2002	Wheat	-	Field, Texas, USA	1/1	-	1/1	106-138	-	117-141	Al-Karaki *et al*., 2004
AMF (*Glomus*, *Gigaspora* and *Paraglomus* spp)	2009	Maize	AM 120, BEI	Controlled-environment glasshouse, sterilised soil, South Carolina, USA	2/3	-	-	78-246	-	-	Wiseman *et al*., 2009
AMF (*Glomus intraradices*)	2013	Maize	AEGIS®	Field, Campania, Italy	-	-	1/1	-	-	97-118	Cozzolino *et al*., 2013
AMF (*G. intraradices*)	1991 - 1992	Winter wheat	-	Field, Wash, USA	-	-	1/1	-	-	95-130[a]	Mohammad *et al*., 1998
AMF (*G. intraradices*, AMF mixture)	2003	Barley	-	Sterilised soil, glasshouse, Jordan	1/1	-	-	105-140	-	-	Mohammad *et al*., 2003
AMF (*G. intraradices*)	2005	Spring wheat	-	Sterilised soil, glasshouse, Australia	1/1*	1/1*	-	NA	NA	-	Li *et al*., 2006
AMF (*G. mosseae*)	2000	Barley	-	Sterilised and non-sterilised soil, Field based, Leeds, UK	-	-	0/1	-	-	96-97	Khaliq & Sanders, 2000
AMF (*G. mosseae*, *G. caledonius*, *G. fasciculatus*)	1981[c]	Barley	-	Field, Rothamsted, UK	-	-	0/1[d]	-	-	93-233	Clarke & Mosse, 1981

Table 15 continued.

Product type & species name(s)	Year	Crop	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
AMF (*G. intraradices*)	2013	Spring wheat	Mix MYKE® PRO PS3	Field, Alberta, Canada	-	-	2/2	-	-	NA	Zhang *et al.*, 2013
AMF (*Scutellospora calospora, Acaulospora laevis, Gigaspora margarita, Glomus aggregatum, Rhizophagus irregulare (syn G. intraradices), Funneliformis mosseae (syn G. mosseae), G. fasciculatum, G. etunicatum, G. deserticola.*	2011-2012	Wheat	Micronised Endo Mycorrhizae	Field, Sicily, Italy	1/1	-	-	109-122	-	-	Saia *et al.*, 2015

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

SNR = Statistics not reported

[†]Control was 12 g, treated was 124 g

*Significant negative response

[a]Significant positive response in absence of P addition

[c]Year of publication

[d]Large LSD value means this is not significant.

NA = Data only reported in graph format therefore no numbers available

Many AM species formally known as Glomus species have been renamed. Previous names used here to be consistent with cited literature

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by ‘-‘.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.2.4. Protozoa and nematodes

Protozoa are heterotrophic, unicellular, aquatic organisms that are commonly found in the soil food web. They are non-photosynthetic, and include groups such as ciliates, amoeba and flagellates. These organisms often consume bacteria, but certain species can also feed on fungi (Lawrence, 2005). They are relatively rare additions to commercial biostimulants, however, there have been demonstrated examples of protozoan interactions in soils, and therefore there is interest in including these organisms in commercial bioinocula. The key activity of protozoa that leads to biostimulant effects is selective grazing of soil bacteria. Thus, it follows that the specific effect of a protozoan addition to soil will depend entirely on the protozoan species and their feeding preferences. Nematodes are non-segmented worms, typically around 50 µm diameter and up to 1 mm long (USDA, 1999). Parasitic nematodes are most familiar in agriculture (e.g. potato cyst nematodes), but there are non-parasitic nematodes that feed on bacteria, fungi or other nematodes, some of which can be used as biocontrol agents (USDA, 1999). Furthermore, there is some evidence of biostimulant activity of certain nematodes (e.g. Jiang *et al*., 2012).

Protozoa and nematodes make up a substantial proportion of the rhizosphere microbial biomass, and play an important role in the soil food web (Crotty *et al.*, 2012), if not the greatest influence on soil nitrogen mineralisation (De Ruiter *et al.*, 1993). A model of a winter wheat food web proposed that the protozoa (amoebas) and nematodes contributed 18% and 5% to mineralisation in winter wheat respectively. However, if they are deleted from this food web model, their contributions become more apparent and these values increase to 28% and 12% of N mineralisation, since their grazing activity is thought to further stimulate microbial mineralisation processes (De Ruiter *et al.*, 1993). These organisms have both been found to have effects on plant growth besides nutrition, which are explored further below.

Protozoa have direct interactions with rhizosphere bacteria (Bonkowski, 2004). Clarholm (1985) was the first to outline the interactions between protozoa, bacteria and plants, using a glasshouse study in which wheat plants were grown in sterilised soil: plant N uptake was increased by 75% when protozoa were present, and sugar application (to mimic deposition of carbon by plant roots, 'rhizodeposition') further increased total shoot N content by 18% when protozoa were present. This led to the development of the 'microbial loop in soil' theory, whereby plant roots were thought to exude carbon via rhizodeposition, which encourages bacterial growth and the mineralisation of N from soil organic matter. The protozoa then selectively graze on the bacteria favouring nitrifiers and releasing NH_4.

Nitrifiers then convert NH_4 to NO_3 via nitrification, and thus make more NO_3 available for the plants. This theory was further updated in 2004 to acknowledge new research demonstrating additional effects of protozoa on plant growth, besides improving nutrient availability (Bonkowski, 2004; Jentschke *et al.*, 1995). Protozoa were found to stimulate the release of indole-3-acetic acid (IAA+) by bacteria. Both IAA and NO_3 can act as signalling molecules, which then induce lateral root growth of plants. With more roots, more root exudation can occur, and so the cycle continues. It has been proposed that the effects of soil bacteria and protozoa on root branching occur via effects on the auxin and cytokinin balance in plants (Krome *et al.*, 2010). There is also some evidence for synergistic interactions between arbuscular mycorrhizal fungi (AMF) and protozoa: Koller *et al.* (2013) demonstrated that when both protozoa and AMF hyphae were present in organic matter, the N uptake and transport by AMF to the host plant was increased. They proposed that the bacteria initially removed N from the system, which was then remobilised by the protozoa via the 'microbial loop'.

However, the 'microbial loop in soil' is not a fully concluded theory, and given the complex interactions, it is not easy to prove (Bonkowski & Clarholm, 2015). It has been suggested that the presence of protozoa simply increases N available to plants, regardless of root exudation and this is the source of the main benefit of protozoan presence for plants (Ekelund *et al.*, 2009). Ekelund *et al.* (2009) observed no changes in the proportion of IAA-producing bacteria or plant root morphology (Ekelund *et al.*, 2009), which is in contrast to previous studies (Krome *et al.*, 2010, Bonkowski & Brandt, 2002). Thus, there is still a lot of fundamental research needed in this area to fully understand these interactions and whether they could be beneficial for UK cereals and OSR.

Similar to protozoa, nematodes may feed off bacteria (bacterivores) or fungi (fungivores) in soils (Geisen *et al.*, 2016) as well as plants (herbivores), depending on the nematode species. Bacterivorous species are of interest as potential biostimulants, for the same reasons described for protozoa above. Mao *et al.* (2007) found that root system development in pot grown tomatoes was increased in the presence of enhanced bacterial-feeding nematode populations. This was accompanied by an increase in soil IAA levels, thus the authors proposed that the nematodes stimulated root growth via increased IAA production. Similarly, the roots of *Arabidopsis thaliana* were more highly branched, longer and thinner in the presence of bacterivorous nematodes, with increases in both mineral N and IAA contents (Jiang *et al.*, 2012). Whilst nematodes are not currently proposed for use in cereal and OSR crops, there is some interest in use of entomopathogenic nematodes

(EPNs) in integrated pest management (IPM) for controlling certain insect pests. These are used in hydroponics and greenhouses around the world (Grewal, 2012).

Despite there being some potential benefits of both protozoa and nematodes in soils, the story is far from complete. A recent study has shown that certain protozoa feed not only on soil bacteria, but also soil fungi, potentially including beneficial fungi such as arbuscular mycorrhizas and yeast (Geisen *et al.*, 2016). There are thought to be differences in the benefits for plants between protozoan and nematode species (Cheng *et al.*, 2011), thus such interactions should be considered carefully prior to applying to soils. Bjørnlund *et al.* (2012) also suggested that bacterivorous protozoa and nematodes generally increase plant performance if nutrients (particularly N) are limiting. However, since bacterivores (especially protozoa) may be selective (Rønn *et al.*, 2015), if the strains of bacteria not grazed by protozoa are detrimental to plant health, the application of protozoa and/or nematodes could have a negative effect on plant health, which may be further exaggerated under high N scenarios. Furthermore, in an experiment in which the presence of protozoa increased the N content and biomass of barley grown under controlled conditions, the number and biomass of aphids were also increased (Bonkowski *et al.*, 2001), possibly due to the crop having a higher N content.

The relative importance and proportions of protozoa, bacteria and nematodes in soil are affected by physical and chemical factors, which thus determine the soil community. For example, protozoa and nematodes are both aquatic organisms and therefore rely on adequate soil moisture content (Rønn *et al.*, 2015), and clay-rich soils are though to favour protozoa over nematodes (Rønn *et al.*, 2015). Thus, there are a number of key questions that still need addressing before protozoa and nematodes can be confidently and routinely used in UK cereals and OSR crops.

Table 16 includes evidence of growth responses to protozoa application in wheat and barley plants. However, these data should be considered with caution, these are fundamental studies looking for interactions between plants and protozoa, therefore the experimental conditions were established to increase the chances of this being identified, including using sterile and low nutrient content soils. Therefore, whilst they all demonstrate that protozoa can have positive effects on plant growth (Bonkowski *et al*., 2001; Clarholm, 1985; Kuikman *et al*., 1990), field interactions may be different. No evidence could be found for OSR crops, and the majority of work on nematodes is on tomatoes and other horticultural crops.

Table 16. Effect of protozoa on above-ground growth, below-ground growth or yield of cereal, maize and/or oilseed rape crops.

Product type	Year	Crop	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
Protozoa	1985[a]	Wheat	Protozoa (native)	Glasshouse, test tubes	1/1	1/1	-	185	166	-	Clarholm, 1985
Protozoa	1997	Barley	Protozoa (native)	Laboratory microrosm	1/1	1/1	-	138	147	-	Bonkowski *et al.*, 2001
Protozoa	1990[a]	Wheat	Protozoa	Glasshouse	1/1	0/1	-	162-169	109-114	-	Kuikman *et al.*, 1990

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

[a] Year of publication

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by ‘-‘.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.3. Product mixes and complexes

Whilst the majority of products can be categorised using the product types outlined in this review, some do not fall clearly into any of these categories, either because they are mixtures of product types, they include the product types not covered in the review, or the biostimulant component of the product is not disclosed. Where there are multiple product types mixed with additional nutrients, it can be difficult to state the specific biostimulant effect.

There are data available to demonstrate the effectiveness of mixed applications against untreated controls. Table 17 summarises studies on wheat and maize that have received mixed inoculants. In all six experiments, there were significant increases in above-ground biomass reported, but in 2 experiments there were also decreases in above-ground growth reported. Thus, it is important to understand which combinations of species produce positive and negative responses. The specific response will depend upon the specific species mix, crop and environmental conditions. There was limited information available on yield as most studies were pot based. In one study mixed cultures of PGPB, fungi and AMF (*Pseudomonas* spp., *Aspergillus awamori*, and/or *Penicillium chrysogenum*, *Glomus intraradices*) produced the highest yield and grain P concentration in field-grown wheat (Babana & Antoun, 2006). However, this study was carried out in Mali, and yields never exceeded 3 t/ha, thus it is difficult to compare this to conventional UK cereal and OSR crops.

Products are also often produced as complexes. For example, De Sangosse produce a biostimulant product mixed with micronutrients (Radiate), for which field trial data shows positive effects on yield (Table 18).

Table 17. Effect of product mixes on above-ground growth, below-ground growth or yield of cereal, maize and/or oilseed rape crops.

Year	Crop	Organism name	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
2009[a]	Maize	Range of *Glomus, Gigaspora* and some non-pathogenic fungal species, humic substances, sea kelp, micronutrients	AgBio-Endos, AM 120, BEI, BioGrow Endo, DieHard Endo Starter, MycorMax, Mycor Nursery/Media Mix, MycorTree Root Dip, Root Dip Universal	Controlled-environment glasshouse, South Carolina, USA	3/3*	-	-	33-227†	-	-	Wiseman *et al*. 2009
2011-2012	Wheat	Mix of *Bacillus* spp. plus mix of AMF spp. Including *Scutellospora calospora, Acaulospora laevis, Gigaspora margarita, Glomus aggregatum, Rhizophagus irregulare* (syn *G. intraradices), Funneliformis mosseae* (syn *G. mosseae), G. fasciculatum, G. etunicatum, G. deserticola*.	-	Field, Sicily, Italy	1/1	-	-	107-125	-	-	Saia *et al*., 2015
2000[a]	Maize	*Glomus mosseae, G. deserticola*, natural AMF, *Azospirillum, Pseudomonas, Trichoderma* spp.	-	Sterilised and re-inoculated soil, glasshouse, Spain	1/1	1/1	-	NA	NA	-	Vázquez *et al*., 2000

Table 17 continued.

Year	Crop	Organism name	Product Name	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
					Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
2001-2002	Wheat	Combinations of: *Glomus intraradices, Aspergillus awamori, Penicillium chrysogenum, Pseudomonas* spp.	-	Field, Mali	1/1	-	1/1	102-160	-	102-142	Babana & Antoun, 2006

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.

NA = data only presented in graph format

[a] Publication year

[†] High doses of one product were toxic, plants died

[*] 3/3 studies had significant increases in biomass, 2/3 studies also had significant decreases in biomass

Significance level $P < 0.05$

Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.

If a trait was not measured this is indicated by '-'.

The entire range of responses is included, regardless of significance for the treatments containing the product listed.

Table 18. Effect of product complexes on above-ground growth, below-ground growth or yield of cereal and oilseed rape crops.

Year	Crop	Product Name & contents	Location	Proportion of experiments showing a significant effect			Range of plant responses as a percentage of the untreated mean (%)			Reference
				Above-ground	Below-ground	Yield	Above-ground	Below-ground	Yield	
2004	Winter barley	Radiate (seed treatment, 7% N, 8.5% Zn, biostimulant)	Field, Norfolk, UK	-	-	1/1	-	-	109	NIAB TAG, 2004* (De Sangosse)
2011	Winter wheat	Radiate (seed treatment, 7% N, 8.5% Zn, biostimulant)	Field, Yorkshire, UK	-	-	1/1	-	-	103-129	Metcalfe, 2011* (De Sangosse)
2012	Winter oilseed rape	Radiate (seed treatment, 7% N, 8.5% Zn, biostimulant)	Field, Lincolnshire, UK	-	-	1/1	-	-	119	Curtis, 2012* (De Sangosse)

Significant responses include both positive and negative responses, any negative responses are indicated in the footnotes.
*Raw data analysed by ADAS.
Significance level $P < 0.05$
Above- and below- ground growth includes fresh or dry weights of shoots and roots respectively at any growth stage.
If a trait was not measured this is indicated by ‘-‘.
The entire range of responses is included, regardless of significance for the treatments containing the product listed.

4.4. Product types not covered in this review

This review has covered the most commonly used biostimulant groups and types which have been agreed in consultation with the industry as being priority areas. It should be recognised that there are other biostimulants available, which are either used less frequently in commercial agriculture or have received little research attention. Biostimulant types which were not covered include plant extracts, sterols, nitrophenols and complexes. Categories that were not considered to fall under the biostimulant topic area included nitrification inhibitors, since they are not thought to have any direct effects on plant growth besides modifying the available nitrogen; and biochar, which is most commonly used as a soil stabiliser. Biochar also contains no nutritional components and the main benefits are thought to be via improved soil properties rather than interactions with plants.

5. Considerations for use in commercial agriculture

5.1. Common themes to consider when using a biostimulant product

Specific information on the management of biostimulant product types has been included in the individual product type sections above. Across all groupings some common themes are apparent. Most product types exhibit a wide range of potential effects on plants and rhizosphere organisms, which should be considered before application. Furthermore, different biostimulant products within the same product type can still produce different effects. This is not only due to the mixtures of biostimulant types that make up a given product, but also because the efficacy of a biostimulant can be affected by extraction procedure, source material, nutrient composition of the substrate, presence of substances that may interfere, and environmental conditions at application. It is therefore important to follow manufacturer's guidelines and not assume that guidelines for one product will be applicable to another, even if it is the same product type.

Other management considerations are similar to those for more standard farm inputs, for example crop growth stage, cultivations, application rate and timing. Given the limited field-based evidence for many products on UK cereal and OSR crops, there will need to be more research to optimise management guidance in order to maximise efficacy of many products on these crops.

In addition, many biostimulant products were first developed for use in horticultural crops, therefore the methods of application in arable fields will need development. For example, since chitosan is a viscous product, it can cause issues when applied as a seed coating by ‘gumming up’ the mechanical seed-treatment operations (Hadwiger, 2013). Hadwiger (2013) also emphasised that biological products do not work in the same way as standard chemical products such as fungicides, in that the response is usually not a direct kill; instead, it takes time for the induced responses and biological interactions to take effect. This is one reason that these products may form part of an integrated crop management scheme, rather than replacing standard farm inputs. The interactions with standard inputs must therefore be better understood, since there is potential for these to be negative, neutral or synergistic. Furthermore, the benefits of some biostimulant products depending heavily on crop conditions: assessing yield responses under ideal conditions may not identify the potential benefits of improved stress tolerance (e.g. under drought conditions).

5.2. Management of microbial inocula

A common theme for microbial biostimulant production and use is that these types of inocula require more considerate management than non-microbial applications. This is because microbial activity varies significantly under different environmental conditions (Killham, 1994), in the presence of different native microorganisms (Raaijmakers *et al.*, 2009), and between different soil types (Owen *et al.*, 2015). Because these are living organisms, they require careful management both before, during and after application to soils. The specific management will vary depending upon the organism in question, but there are some general guidelines that should be followed (Killham, 2006), and a range of biological, chemical and physical factors that need to work together for successful inoculation (Table 19).

Table 19. Factors affecting inoculum success as outlined by Killham (2006) with practical guidelines for managing these risks.

Factor affecting inoculum success	Options to manage the risk
Method of inoculum production, storage and introduction	Manufacturers should provide guidelines, request this information. Consider the range of available forms of the product, for example - seed coatings may provide better access to plant roots at early developmental stages
Soil physical factors – water potential, temperature, soil texture	Consider where it’s being applied, is it compatible with the conditions provided in the management guidelines? Are there any guidelines relating to soil management (e.g. cultivations)?
Soil chemical factors – pH, available nutrients, pesticides, redox	If unknown, consider getting these factors assessed, incorrect chemical environments may mean the environment is inhospitable for the bioinoculant, and therefore it’s unlikely to be effective.
Climatic factors – seasonal, freezing, thawing	Take note of the optimum working temperature of the inoculant, e.g. is it affected by freezing? Microbial activity generally declines with temperature.

Factor affecting inoculum success	Options to manage the risk
Effect of viruses, particularly bacteriophage	Refer to product guidelines
Interactions with soil animals – protozoan predation, dispersal	One way to reduce this risk is by ensuring that the product is applied under ideal conditions – follow all product guidelines very closely. If there are known pests that cause problems for the inoculant, consider not applying to that area.
Competition from indigenous soil microbes	It is important that the applied microorganism will not be easily out-competed by other soil organisms. Again, following guidelines will be very important to minimise this risk. This is always a risk as the local microbial community will be well adapted
Vegetation factors host specificities, diffusates, rhizosphere effects	Understand the needs of the organism that you are applying – for example, brassicas cannot form the mycorrhizal association, so adding a mycorrhizal inoculum to an OSR crop will be ineffective. Also, be mindful of the product type that you are applying, if it is a fungal inoculant, it is unlikely to respond well to being tank mixed with certain fungicides.

6. Summary table – evidence for effects across product types

There is a significant amount of information in the sections above, which is not easy or quick to digest when faced with a range of potential biostimulant products. It is clear that the level of evidence available to demonstrate effects and modes of action for each product type varies considerably between product types. This review has therefore summarised the information above to indicate the level of evidence available for effects on the plant for each of the product types covered in this review (Table 20). This does not prove the effect of a product under different scenarios, but indicates that evidence is available to support these effects under certain conditions, which may be limited to controlled environment studies. It is therefore important to recognise that interactions of product types with plants identified under controlled conditions may not follow through under field conditions (Owen *et al.*, 2015). This is because field systems are inevitably far more complex. Nonetheless, given the limited field based evidence available in the UK, glasshouse studies have been included to demonstrate that these effects are occurring, and identify the need for field based research to understand whether these effects are continued under field conditions.

Table 20. A summary of the evidence for positive biostimulant effects on plant nutrition, growth and stress tolerance, based on published and unpublished information analysed by this review.

***Low level of evidence: principally laboratory experiments, including little or no data on cereals or oilseed rape;**

****Moderate evidence: greater number of experiments including some that were field-based and/or on cereals or oilseed rape;**

*****Good evidence: wide evidence base including multiple field-based experiments on cereals or oilseed rape.**

Effect Category	Nutrient uptake or access			Plant function & Growth						Abiotic stress tolerance				Biotic stress tolerance	
Product type	N	P	Other	Hormonal	Growth[†]	Yield	Reduced Transpiration	Delay senescence	Improved photosynthesis	Salt	Alkaline	Drought	Cold	Pathogen[††]	Pest[††]
Seaweed extracts	*	*	*	**	**	**			*	*		*	*	*	*
Humic substances	**	*	*	*	**	**			*	*		*			
Phosphite & inorganic salts				*	**	**								**	
Chitin & chitosan derivatives					**	**	*			*		*	*	***	*
Anti-transpirants				***		**[a]	***		*			**			
Protein hydrolysates & amino acids	*		*		*	*				*		*	*		
Non-essential chemical elements	*	*			*	*		*	*	*	*	*		**	**
Plant growth promoting bacteria	**	**	*	*	***	***				*		*		**	*
Non-pathogenic fungi	*	*	*	*	**	**				*		*	*	**	
Arbuscular mycorrhizal fungi	*	**	*		**	**						*		*	*
Protozoa & nematodes	*			*	*	*									

[†] Above and/or below-ground growth

[††] Resistance or tolerance of pathogen/pest, induced or physical

[a] Yield and other benefits depend on severity of drought conditions; yield penalties may occur when water is plentiful.

7. Conclusions, knowledge gaps, and recommendations for future work

This review has focused on UK cereal and OSR crops, which is an emerging market for biostimulant use. A more substantial research base is available for horticultural crops in the UK and Europe. Nonetheless, numerous biostimulant products are available for use on UK cereal and OSR crops, and the range is likely to expand now that some large agrochemical companies have shown interest in this area, for example by becoming members of the EBIC.

The research here has demonstrated clearly that this is a very complex area, with wide ranging product types and product mixes, making them difficult to compare. The lack of data on biostimulant use on cereals and OSR, relative to on horticultural crops, in combination with the complex range of product types, makes it a confusing area for growers and agronomists to enter into. The current review aimed to distil and summarise the information that is currently available on biostimulant products that are marketed for use with UK cereals and OSR, using academic literature and commercial data. It became apparent in the early stages of the review that a certain level of summarising and grouping of products was required in order to make general conclusions. This lead to the adoption of product type groupings (e.g. seaweed extracts, humic substances) which have been discussed with industry. There was also limited evidence available for cereal and OSR crops, therefore the literature search was expanded to look for effects on all plant species to aid understanding of the products. For all product type groups, there was at least some evidence that biostimulants can positively affect plant growth. The majority of product type groups had at least some evidence available for these effects on cereal crops, but consistently less information available for OSR crops. Many product types also had some level of a plant protectant role. Much of the evidence is laboratory or glasshouse based, or from field sites outside the UK.

This review has shown that each biostimulant category is at a different stage of understanding. As a result there are different research requirements for each biostimulant category. Elucidating the mode of action will require very detailed experimentation such as growth cabinet experiments where the growing environment can be fully controlled, microscopy analysis to understand effects at the plant tissue and cellular levels and biochemical assays to understand effects on for example plant growth hormones.

Many biostimulant products are mixtures of active ingredients, nutrients and/or organisms and it will be important to disentangle these effects by first focussing on individual actives and subsequently the interactions between actives. Some biostimulant categories, such as protozoa and nematodes, have received little research outside of controlled conditions, or on cereals and OSR. Therefore controlled environment experiments (e.g. pot experiments) to determine the interaction of these organisms with plants would be most appropriate initially since this would allow a much wider range of treatments (e.g. rate, timings, environmental conditions) to be tested. If fruitful, this will provide the basis for more focussed field experiments.

Biostimulant product types including seaweed extracts, chitin and chitosan and phosphite have received some research on cereals and in these cases, field experimentation to improve understanding in a commercial environment is an appropriate level of research. This will include replicated small plot experiments and larger scale tramline and split field experiments. However, since varying extraction procedures can mean that products within the same category can differ, new products will still benefit from detailed controlled experiments to determine the mode of action.

For most microbial biostimulants, applied research would be most appropriately focussed on the best researched species (E.g. *Bacillus, Trichoderma* spp.), with a focus on understanding the interactions between native organisms, environmental conditions and management practices. Understanding how biostimulants should be targeted according to environmental conditions, crop growth stage and crop characteristics is lacking for most biostimulant categories. Understanding about how to target the products is vital to help practitioners gain confidence about how to use biostimulants. For example, conventional PGRs only started to be used regularly on OSR once it was understood that yield increases only occurred when PGRs were used on crops above a specific canopy size at the start of stem extension. Therefore experiments with different environmental treatments (e.g. drought stress, nutrient levels, pest pressure) and treatments designed to produce different crop characteristics (e.g. canopy size, nutritional status, rooting) will be required develop guidelines for targeting biostimulants. Product efficacy evidence will be required to comply with new legislation. The section below summarises the key areas of research for each biostimulant category.

The sections to follow summarise the main conclusions, knowledge gaps and recommendations for future work for each of the product type categories covered.

7.1. Non-microbial biostimulants

7.1.1. Seaweed extracts

Commonly studied species of seaweed include *Ascophyllum nodosum, Ecklonia maxima* and *Kappaphycus alvarezii*, with *A. nodosum* the most commonly cited. These are also the most commonly used species from which extracts are produced. Despite the consistency in species used, the extracts produced are inherently different as they are derived from different environments, using different extraction procedures and will have different extract stability properties. There are a wide range of demonstrated biostimulant effects on plants, including increases in yield and crop biomass (root and shoot), increased nutrient uptake (N, P, K and often Mg) and increases in chloroplast number, as well as prevention of chlorophyll degradation. The modes of action for these effects are not well understood, although it is reported that seaweed components include phytohormones (cytokinins, auxins and abscisic acid), betaine and proline to buffer against osmotic changes, alginate and diverse polysaccharides, and minerals and trace elements, all of which can affect plant growth. There is also evidence for increased resilience to biotic and abiotic stresses, including osmotic and salinity stress. The majority of research on seaweed extracts has been on plants other than cereals and OSR. For cereals, significant increases in above-ground biomass, below-ground biomass and yield were found in 7 out of 11, 6 out of 6 and 3 out of 7 experiments respectively with significant yield responses of cereal crops ranging from 73-134% of the untreated control, with no significant negative effects. Fewer data were available for effects on OSR crops (significant responses were found in 2 out of 2, 3 out of 5 and 0 out of 0 for above-ground biomass, below-ground biomass and yield, respectively), with biomass (above- and below-ground) responses ranging from 89-173% of the untreated control, of which no effects were significantly negative. Apart from three OSR experiments, these data were from either controlled conditions (e.g. glasshouse) or field studies outside of the UK. A key knowledge gap is therefore information about effects of seaweed extracts on UK cereal and OSR crops grown in field conditions. Future research priorities should therefore include:

- For each product, understand the main effect and mode of action. If phytohormone effects are key, this includes understanding which growth hormones are involved and how they affect the plant; this is likely to require controlled environment work and/or bioassays.
- Field experiments on cereals and OSR to identify the environmental conditions, growth stage and type of crop which will give the greatest benefits. Cereals should be an initial target, looking at a range of rates and timings with appropriate controls

where applicable (e.g. for nutrient content) to determine whether effects can be demonstrated under field conditions.

7.1.2. Humic substances

Humic substances are the products of decomposition of plant and microbial remains, the properties can vary between sources but overall are relatively consistent. They are often applied with other fertiliser products. Demonstrated effects of humic substances on plants include: acting as plant hormone-like substances (e.g. acting like cytokinins), increasing above- and below-ground growth, increasing root hair length density and cell proliferation; improved nutrient use efficiency, influencing primary metabolism and photosynthesis, and alleviation of salinity stress in beans and maize. However, humic substances can also have negative effects on growth and nutrient uptake at high doses. Other less well understood effects may include chelating nutrients and buffering pH which may increase nutrient availability to crops and reduce the impact of pH changes. Three out of four cereal and maize experiments showed a significant yield increase to humic substances with reported ranges from 78 to 139% of the untreated control. One of these experiments detected a significant decrease in yield under deficit irrigation conditions, but significant increases in yield under adequate water conditions. Available data more strongly supports increases in shoot and root dry weight in wheat, maize and barley from laboratory and glasshouse studies, with 9/11 and 6/7 reported experiments showing significant increases and one experiment with a significant negative effect on shoot weight. Again, most of these studies were non-UK based and only 3 of the 12 reported were field based. A key knowledge gap is therefore whether humic substances can produce responses in UK cereal and OSR crops grown in field conditions. Future research should therefore consider:

- Experiments to elucidate the mode of action, particularly which growth hormones are involved and how they affect the plant, in order to reduce variation in crop responses.
- Field experiments on cereal species to identify the environmental conditions, growth stage and type of crop which will give the greatest benefits.
- Controlled environment pot experiments on OSR to test a wide range of treatments (product rate and timing, environmental stress such as drought). This will help to focus future field experiments.

7.1.3. Phosphite and other inorganic salts

Phosphite is a reduced form of phosphate with the chemical formula $H_2PO_2^-$ (Phi). It is often applied in the form of phosphorus acid (H_3PO_3) to soils. There is no evidence for fertiliser benefits of phosphite, but there is evidence for both biostimulant and pesticide effects.

Biostimulant effects on plants include increased growth, P content, grain quality, mycorrhizal colonisation, and chlorophyll content. Other inorganic salts include biocarbonates, phosphates, silicates and chlorides. These are often more frequently used as fertilisers and/or pest control, e.g. 14 studies have reported inorganic salt application to significantly reduce various diseases in cereal crops. Four out of seventeen UK industry studies demonstrated a significant yield increase and no significant decreases in yield of phosphite on cereals. No OSR experiments were available for yield responses, but one did show a significant increase in above- and below-ground biomass. Yield responses ranged from 95-112% of the untreated control. There have also been significant above- and below-ground biomass increases found in cereals (3/5 and 4/4 respectively), with one study reporting a decrease in above- and below-ground biomass under low P conditions. It is difficult to distinguish between pesticide and biostimulant effects on growth response, but there is some evidence to support biostimulant action of phosphite. Therefore, an important knowledge gap is understanding the mode of action under a range of UK field conditions. Future research priorities should therefore include:

- Focus on understanding the relative roles of biostimulant and disease control effects in cereal and OSR growth and yield responses. Treatments should include high and low disease pressure.
- Field based UK studies on cereals and OSR to determine the optimum application rates and timings.

7.1.4. Chitin and chitosan derivatives

Chitin and chitosan derivatives are natural polysaccharides, mainly sourced from waste products of marine shellfisheries. They often contain impurities and varying polysaccharide chain lengths which can vary between products, but they are reported to be relatively comparable. Demonstrated effects on plants include direct reduction of bacteria, fungi and nematode pests, induced plant defences, improved tolerance to abiotic stress (inc. drought stress), a stimulatory effect on beneficial microorganisms, and regulation of plant growth. Other possible effects include improved tolerance to salt and temperature stress, improved seedling germination rates under low temperature and, since it has a relatively high N content (6-8%), it may also act as a slow release fertiliser. Studies are available for wheat crops, with significant yield increases reported in 9 out of 12 experiments, ranging from 94-134% of the control, and no significant negative effects. Above-ground growth increases have also been reported in 4 out of 7 studies, none of which reported significant negative effects. It seems likely that there are pest/pathogen control effects of chitin and chitosan derivatives, but these can be difficult to distinguish from any biostimulant benefits. Key knowledge gaps include information on effects on OSR and how these biostimulants interact with native microorganisms including AMF. Future research priorities should therefore include:

- Focus on understanding the relative roles of biostimulant and pest-control effects in cereal and OSR growth and yield responses to enable better product targeting. Controlled conditions (e.g. pot experiments) may be more appropriate for this work. It will be important to test different stress conditions, both biotic (e.g. disease) and abiotic (e.g. drought).
- Research into the interactions between chitin/chitosan derivatives and native soil microorganisms, to better understand whether these are synergistic and/or biocidal under UK conditions (e.g. AMF & nematodes).
- UK field research to determine effects on cereal and OSR crops at a commercial scale.

7.1.5. Anti-transpirants

Anti-transpirants are chemicals applied to plant leaves to reduce transpiration (water loss). There are two types, which have different modes of action: film anti-transpirants form a colourless film over the leaf surface, and metabolic inhibitors (e.g. abscisic acid) which reduce stomatal opening. Recent research has led to the hypothesis that, under drought conditions and targeted at key growth stages where drought sensitivity is highest (e.g. just before booting in wheat), the benefits of anti-transpirants may outweigh the costs. There is evidence for significant yield increases of wheat under drought stress in response to film anti-transpirants (13 out of 14 experiments), but also negative responses have been reported in 5 out of 5 of studies under well-watered conditions. There was no evidence for effects on OSR, and the one field experiment showing a wheat yield response to abscisic acid under drought conditions was not conducted in the UK. Future research priorities should therefore include:

- Research on OSR under controlled conditions (e.g. pots) to determine product effects on OSR transpiration rates and growth. A range of treatments should be used, including product rates and timings under adequate water and drought conditions.
- Research under controlled conditions (e.g. pots) investigating the effects of metabolic inhibitors on cereal and OSR transpiration rates and growth.
- Field based research on cereal crops to further investigate the optimum timing of film anti-transpirants under various environmental conditions (e.g. soil type, water availability).

7.1.6. Protein hydrolysates and free amino acids

Protein-based products can be split into two main categories: protein hydrolysates, which consist of a mixture of peptides and amino acids of animal or plant origin, and individual amino acids such as glutamate and proline. Products often include a mix of amino acids and/or protein hydrolysates. They may be animal, plant or microbial in origin. Demonstrated effects on plants include stimulation of root and leaf biomass, abiotic stress tolerance (inc. salinity, drought, extreme temperature and oxidative conditions), increased nutrient uptake, water use and nutrient use efficiencies for macro and microelements (esp. N). Modes of action for increased nutrient uptake have been hypothesised to include increases in soil microbial activity and soil enzymatic activities, improved micronutrient mobility and solubility, modifications in plant root architecture, and increases in activity of plant nutrient acquisition enzymes (e.g. nitrate reductase, glutamine synthase and Fe(III)-chelate reductase). However, there is very limited evidence for these effects on cereal and OSR crops. There is some evidence for increased above-ground biomass in three experiments on maize. In 2/3 of these experiments there were also increases in below-ground biomass, but a significant decrease in below-ground biomass was observed in one study. Future research priorities should therefore consider:

- The impact of the source of the protein hydrolysates/amino acids (animal or plant) and implications for consumers.
- Controlled experiments (e.g. pot and growth cabinet) on cereal and OSR crops would be the most appropriate at this stage to determine the potential effects on growth and identify the main mode of action. Using various stress treatments (e.g. drought, cold, and nutrient availability) will help to elucidate the key effects.

7.1.7. Non-essential chemical elements

Non-essential chemical elements are elements that are not required by cereals and OSR crops, but may increase plant growth. Elements which can fall into this category include aluminium (Al), cobalt (Co), sodium (Na), selenium (Se) and silicon (Si). These are often found in biostimulant product mixes and may play a role in more complex biostimulants (e.g. complex organic materials).

Silicon may be the most promising non-essential chemical element in terms of biostimulant benefits. Demonstrated biostimulant effects on plants include alleviation of salt stress, tolerance of adverse climatic conditions, alkaline stress, drought stress and nutrient deficiency, and delay of plant senescence processes. The hypothesised modes of action include anatomical changes in plant tissues, enhancement of the antioxidant defence system in plants, immobilisation of complex metals or modulation of gene expression and signalling via phytohormones. It is also hypothesised that the production of phytoliths (rigid structures made of silica found in some in plant tissues) may reduce lodging. There is evidence to support the link between Si and improved pest and disease tolerance of crops including wheat, with modes of action proposed including; acting as a physical barrier, preventing penetration by pests and stimulation of natural defence. Barley and wheat are accumulators of Si and increased plant growth has been demonstrated, although there are limited studies available to report on, with one study reporting significant increases and decreases in yield, and another reporting a significant increase in above-ground biomass. Both studies were glasshouse based and no studies could be found on OSR. Key knowledge gaps are therefore field studies on UK crops and understanding the modes of action.

The evidence for Se suggests that the main benefit of application would be via increasing the nutritional content of grain rather than any biostimulant benefit. Cobalt can also be toxic to plants at high levels, however, bacterial nitrogenase requires Co and therefore it may be beneficial for N-fixing bacteria. There is some evidence that Co may delay leaf senescence, improve drought resistance or resistance to herbivory. However, there is very little evidence available for beneficial effects on cereal or OSR crops. Since Na and Al can be toxic to plants, and there is very limited evidence available for biostimulant effects on cereal and OSR crops, it is unlikely that they will be beneficial for cereal and OSR crops in the UK. Future research should therefore focus on Si, to investigate the role of Si to protect against abiotic stress, tolerate sub-optimal nutrition and pests. Experimental treatments should include drought stress and different levels of pest pressure.

7.1.8. Complex organic materials

Complex organic materials include products that are derived from any organic material, including, but not limited to, composts, manure, sewage sludge extracts, agro-industrial and urban waste products. Compost teas therefore can be included in this category. Complex organic materials have not been covered in detail in the current review, primarily because many complex organic material products consist of other product type categories that have been covered in detail by the review. It has been shown that complex organic materials can have a broad range of effects which are dependent on the specific constituents of the product. Future research should aim to better understand the specific components of complex organic materials in order to target the best crop and environmental conditions. This may include bioassays, and pot based experiments to separate key components and modes of action as well as field studies to determine effects on cereal and OSR crops under natural conditions.

7.2. Microbial biostimulants

7.2.1. Plant growth promoting bacteria & rhizobacteria

PGPB are found in the rhizosphere, plant roots or aerial parts of the plant. Demonstrated effects on plants include stimulation of plant growth, increased yield, increased nutrient availability, reduced pathogen infection, and increased tolerance to biotic and abiotic stresses. There is a lot of evidence available for effects on cereal crop species, with 13 out of 15 experiments reported showing significant yield increases. However, 7 out of 15 of these were glasshouse or pot based, and no field studies were carried out in the UK. Effects on field crops are further complicated by the interactions with the environment and resident microbial populations which are poorly understood. Thus, more UK based evidence is required for cereal crops and also for OSR for which there was limited evidence available. Future research should therefore include:

- UK-based field studies elucidating the effects of key species (e.g. *Bacillus* and *Rhizobium* spp.) on cereal and OSR growth, yield, pest & disease tolerance.
- Experiments to better understand interactions with the environment and resident microbial population under UK conditions. This complex question is likely to require a mix of controlled conditions and field experiments. Treatments could include N and P availability to help interpret variable effects.
- Controlled experiments (e.g. pots) will be useful to determine key effects of known PGPB strains under different conditions (e.g. disease pressure, N rates, drought, cold conditions) to help determine the most appropriate timing, crop, locations and application rates.
- Identify the mode of action using for example biochemical assays.

7.2.2. Non-pathogenic fungi

Non-pathogenic fungi include species that interact with plants in a positive way. They can be produced more easily on a commercial scale than AMF as they do not require a plant host. The most common species in inoculants or proposed for use include *Trichoderma, Penicillium, Piriformospora* and yeast species. *Trichoderma* and *Penicillium* are commonly used in inoculants around the world. However, whist having a wide range of demonstrated benefits for plants, *Piriformospora* is not available in the UK as it doesn’t grow well in cold conditions. Research at the fundamental level is needed to identify similar species for UK conditions. Demonstrated benefits of non-pathogenic fungi include plant growth stimulation, improved plant nutrition (e.g. by solubilisation of rock phosphate), protection against plant diseases, tolerance to abiotic stress, and bio-remediation via the sequestration of harmful substances. There is limited evidence for effects on cereal crop yield, with 2 out of 7 studies demonstrating significant yield responses, ranging from 106-111% of the control. No evidence for effects on OSR growth could be found. More evidence is required to understand whether these fungi could have commercially beneficial impacts on UK cereal and OSR crops. Future research priorities should therefore include:

- Applied research focusing on common species, e.g. *Trichoderma* spp. UK field-based experiments should be used to determine whether these can be beneficial for cereal and OSR crops under UK conditions.
- Experiments investigating the inoculation methods, interactions with native soil organisms, background nutrient availability and disease pressure.

7.2.3. Arbuscular mycorrhizal fungi

AMF can form a mutualistic symbiosis with over 2/3 of all land plants and are found across all major terrestrial biomes. The demonstrated benefits of AMF include increased nutrient uptake, pest and disease tolerance, nitrogen fixation and improved soil structure. The modes of action are better defined for some effects than others, the best understood effect is plant P acquisition, via extension of the zone of soil from which P uptake can occur. A similar effect can be seen for N too, although this is less well understood. AMF may also release N-fixing bacteria from P limitation, and have been demonstrated to induce systemic resistance against a range of pests and diseases. OSR cannot form the AMF association and therefore AMF should not be applied to OSR crops. Cereal crops including wheat, barley, oats and maize can form the AMF association. There are known limitations and factors to consider before using AMF under field conditions, including availability of N and P, tillage, presence of Brassica species and use of fungicides. Many field experiments are carried out under low N and/or P conditions which must be taken into account when interpreting results for commercial situations. Cereal and maize yield responses ranged from 93-233% of the control in experiments, with 5/7 reporting a significant yield increase in response to AMF application and no negative responses. However, only 2 out of 11 studies were UK based, and 5 out of 11 studies used sterilised soil. There was also a significantly negative response in above-ground biomass in 1 out of 7 reported experiments. A key knowledge gap is understanding the environmental and biological conditions for inoculation with AMF to produce a beneficial response. Future research priorities therefore include:

- Investigating the extent of native AMF interactions with UK cereal crops to determine if native AMF benefit crops. Treatments should be linked to conventional management (e.g. N and P fertilisation, fungicides, tillage).
- Field research to determine if AMF inoculation will produce a beneficial crop response under field conditions, and how this might be achieved. Treatments should include management and inoculation methods (e.g. N, P, tillage, direct drilling etc.).

7.2.4. Protozoa and nematodes

There are interactions between protozoa, nematodes and soil N mineralisation processes, with both being demonstrated to increase the release of the plant growth hormone indole acetic acid (IAA), subsequently enhancing root growth and mineral N availability. Although the modes of action are still under debate, they may be acting via the ‘microbial loop in soil’; a theory describing interactions between protozoa, bacteria and plants that increases N available for plant uptake. There is also evidence for synergistic interactions between AMF and protozoa, with N uptake by AMF increased in the presence of protozoa. However, the only evidence available for biostimulant benefits so far is glasshouse based, there is none in the UK, none on OSR, and no yield data available. Nematodes are not currently promoted for use in cereal and OSR crops, but nematodes are used in hydroponic systems and greenhouses as part of IPM to control certain insect pests. Future research should therefore focus on fundamental research understanding whether inoculating with protozoa and/or nematodes have a beneficial effect on cereal and OSR crops and whether this could be exploited via field inoculation.

7.3. Product mixes and complexes

Whilst the majority of products available on the market today are made up of a mixture of product types, there is very little information available on whether mixing product types together is additive or synergistic. This is a key knowledge gap, applicable across all product types and combinations. Experiments should focus on understanding the role of each component part, both alone and when mixed with other product types to determine whether mixing products is synergistic, additive, or negative. Research should also investigate the optimum crop growth stage for product application when different products are mixed together, given that different actives may have different optimum application timings.

7.4. Research methodologies

Yield improvements from biostimulant products are often less than 0.3 t/ha and yield improvements as little as 0.1 t/ha are required to cover the cost of some biostimulant products. However conventional small plot experiments cannot usually detect statistically significant yield differences of less than 0.5 t/ha. A key requirement is therefore to employ experimental techniques, or develop new techniques, that enable small yield effects to be detected. One approach is to collate and analyse results from multiple experiments in a single analysis as this can give statistically significant effects not found in the individual experiments. For example, a meta-analysis of 44 mainly split-field experiments in the 1980s testing the application of the seaweed extract Seamac 600, found a significant increase in protein content of +0.25% and 56 experiments over 5 years showed that the Hagberg falling number significantly increased from 276 to 320 (Seamac Agriculture Ltd., 1989). In contrast, individual small plot experiments (which were included in the meta-analyses), showed no significant increase in either protein content or Hagberg falling number.

The advent of yield mapping technology now means it is potentially easier to estimate yield effects from tramline or split-field trials. Collating data from multiple tramline trials will provide strong tests of biostimulants in a commercially relevant environment. However, new research is required to develop efficient methods of processing and analysing combine yield data to estimate the yield of tramlines and part-fields. This research is ongoing under IUK Project 101627 ‘Agronomics’. This project is also developing new statistical techniques to enable detection of small differences between adjacent tramline treatments by exploiting the large number of spatially referenced yield measurements generated by GPS linked commercial or research combine harvesters.

Research is also required to improve understanding of the interactions between products, crop types and the environment. In addition, dose response experiments may be useful to determine the optimum product application rate, and whether the product produces a typical dose response curve, or produces an ‘on/off’ response. This will then allow the development and improvement of management guidelines, to enable effective biostimulant deployment.

7.5. General conclusions

This is an emerging sector and needs a more substantial UK evidence base to be successfully developed for widespread use in UK arable crops. Product rates and timings are still being optimised for many products, and these often vary between products, therefore following the product guidelines provided by manufacturers will be essential. There also needs to be a clearer understanding and demonstration of the potential economic benefits of these product types, which, given the lack of UK field based data, is unclear at present. Essential for achieving this will be understanding the environmental conditions, crop growth stage and characteristics of the crop which maximise the chance of a biostimulant having a positive effect. Understanding how to integrate biostimulant products into ‘conventional’ crop management systems to produce an integrated management system is also required. There is currently limited understanding from growers and agronomists about how to exploit biostimulants, and evidence is required to build this further. However, with an increase in both independent research and the introduction of legislation due in early 2018, the reliability of products should be demonstrated, along with improved confidence in this area.

The review has indicated three key potential benefits of biostimulant products for use in conventional UK cereal and OSR crops, if their efficacy can be reliably demonstrated. First, they may have a role to play in integrated management schemes, by complimenting and improving the efficiency of use of current crop inputs such as fertilisers (e.g. N and P) and plant protection products. Secondly, there may be opportunities to exploit these new technologies to produce yield gains that cannot be achieved with conventional crop management. Aggregation of these marginal gains could be substantial. Finally, it is also important to recognise that some biostimulant products do not aim to increase yield in a ‘good’ year, they are instead designed to prevent yield loss in a ‘bad’ year, for example under stressed conditions, such as drought. For example, Anjum *et al*., 2011 found that, under drought conditions, application of humic acid brought the yield of maize back up to that of the non-droughted control. The majority of field studies reported are carried out in areas with greater abiotic stress risks such as drought (e.g. Australia and Southern Europe). The risk of abiotic stresses in the UK are comparably much lower than in these locations, however, if products are cost effective enough to warrant regular use in a rotation and can demonstrate their potential benefit as insurance in poor seasons, this may be another area in which biostimulant products become more widespread.

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10. Appendix 1. Glossary

Key terms are described below. Definitions were either taken from within the review text or were sourced from (Lawrence, 2005).

Term	Definition
Anti-transpirant	Product that reduces transpiration by plants
Arbuscular mycorrhizal fungi	Common type of endomycorrhizal fungus that forms a symbiotic association with plant roots.
Ascophyllum nodosum (L.)	A species of seaweed, commonly used in production of seaweed extracts for biostimulant products
Bioamendment	Un-treated organic amendment applied directly to the soil (e.g. green manures)
Biochar	Charcoal produced from biomass (typically plant matter) by pyrolysis
Biofertiliser	Products that contain living organisms, predominantly microorganisms that aid plant uptake of nutrients
Biopesticide	Products derived from naturally occurring substances and/or microorganisms that have a pesticidal action
Bioremediation	Recovery of a contaminated site by the use of living organisms (usually microorganisms) to break down pollutants

Term	Definition
Biostimulant	A material that contains substance(s) and/or microorganisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality, independent of its nutrient content
Chitin	An abundant natural polysaccharide, chitin can be found in a wide range of organisms, most notably exoskeletons of arthropods (e.g. crustaceans and insects) and the cell walls of fungi
Chitosan	Deacetylated form of chitin (poly(D-glucosamine)
Complex organic materials	Broad range of products that contain material derived from the remains of organisms (e.g. plants).
Elicitor	In plant pathology, a compound that induces a defence response to damage or infection in the plant. Can be biological or chemical in origin.
Endophyte	Bacterium, fungus or alga living inside the body or cells of an organism to which they cause no damage
Free amino-acids	Single amino acids, require no digestion
Fulvic acids	Extraction product of humus
Humic substances	Extraction product of humus
Hyperaccumulator	An organism (usually a plant) that can tolerate and accumulate high levels of certain substances (e.g. inorganic salts)
Inorganic salt	Salt that does not contain carbon
Mode of action	Mechanism that explains the effect of a product
Mycorrhizosphere	The volume of soil influenced by plant roots that are colonised by mycorrhizal fungi
Nematodes	Round, unsegmented worms
Nitrification inhibitor	Products that inhibit the rate of conversion of ammonium to nitrate via the process of nitrification
Non-essential chemical elements	Elements that are not necessarily required by all plants but can promote plant growth
Non-pathogenic fungi	A wide range of fungal species that have no direct pathogenic effect on plants
PGPR	Plant growth promoting rhizobacteria
Plant growth promoting rhizobacteria	Bacteria that inhabit the rhizosphere, which have been shown to benefit the plant growth
Protozoa	Single celled organisms found in most soils and in high numbers in the rhizosphere
Rhizobium	A genus of common nitrogen fixing bacteria. Form nodules in leguminous plants (e.g. peas and beans) to establish a symbiotic relationship, providing nitrogen to the plant in exchange for carbon.
Rhizosphere	Volume of soil influenced by plant roots
Seaweed extract	Products that have been extracted from seaweed via either a chemical or natural extraction process

Term	Definition
Systemic acquired resistance	Whole plant resistance response to localised exposure to a pathogen or certain chemicals
Induced systemic resistance	Localised interactions with some plant growth promoting rhizobacteria results in plant becoming resistant to some pathogenic bacteria, fungi and viruses.