Agronomy **2013**, *3*, 757-793; doi:10.3390/agronomy3040757

OPEN ACCESS

agronomy

ISSN 2073-4395

www.mdpi.com/journal/agronomy

Review

A Review of the Applications of Chitin and Its Derivatives in Agriculture to Modify Plant-Microbial Interactions and Improve Crop Yields

Russell G. Sharp

Moulton College, Moulton, Northamptonshire, NN3 7RR, UK; E-Mail: russell.g.sharp@gmail.com; Tel.: +44-1604-491-131

Received: 9 August 2013; in revised form: 8 September 2013 / Accepted: 9 September 2013 / Published: 21 November 2013

Abstract: In recent decades, a greater knowledge of chitin chemistry, and the increased availability of chitin-containing waste materials from the seafood industry, have led to the testing and development of chitin-containing products for a wide variety of applications in the agriculture industry. A number of modes of action have been proposed for how chitin and its derivatives can improve crop yield. In addition to direct effects on plant nutrition and plant growth stimulation, chitin-derived products have also been shown to be toxic to plant pests and pathogens, induce plant defenses and stimulate the growth and activity of beneficial microbes. A repeating theme of the published studies is that chitin-based treatments augment and amplify the action of beneficial chitinolytic microbes. This article reviews the evidence for claims that chitin-based products can improve crop yields and the current understanding of the modes of action with a focus on plant-microbe interactions.

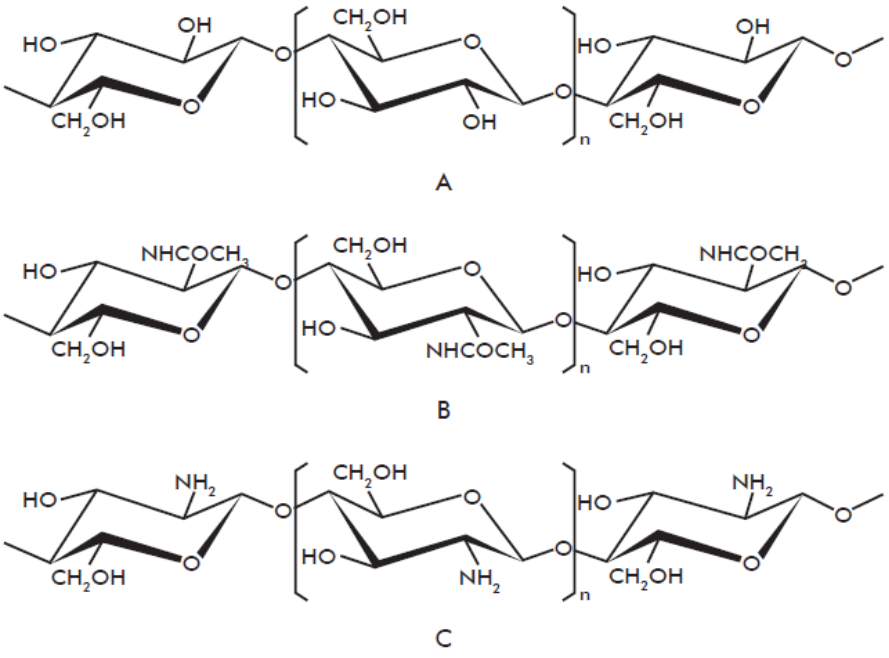
Keywords: chitin; chitosan; plant growth-promoting rhizobacteria; induced defenses

1. Introduction

After cellulose, chitin is the second most abundant polysaccharide on the planet [1]. Chitin is found in, and can be sourced from, a variety of different organisms, with the notable exceptions of higher plants and vertebrate animals. Chitin-rich animal tissues include the exoskeletons of arthropods (including insects, crustaceans and arachnids), the beaks of cephalopods and the eggs and gut linings of nematodes [2]. Various microbes also produce chitin in cell walls, membranes and spores, including fungi [3], and the spines of diatoms [4].

Chitin shares a number of biochemical similarities with the cellulose found in plant cell walls. In common with cellulose, it is a long-chained linear, neutrally charged polymeric polysaccharide. Furthermore, like cellulose, chitin is used to construct mechanical and physical barriers that provide structural stability. However, unlike cellulose, chitin has an innate rigidity. Chitin is composed of repeating saccharide monomers of *N*-acetylglucosamine, which is a modified form of glucose with an amino group substituted at carbon 2 (Figure 1). As is the case with the cellulose in plant cell walls, the chitin polysaccharide is combined with other compounds to produce strengthened tissues. Both polysaccharides form microfibrils which differ in length and construction depending on the species and cellular location [5]. In fungi this involves cross linkages to glucan polymers to create a meshed hyphal wall [6,7]. Due to the involvement of other polymers, such as glucans, the chitin content of fungal cell walls ranges from 22%–40% [8]. In invertebrate tissues the chitin is supplemented with substantial amounts of proteins and calcium minerals [9].

Figure 1. The structural representation of the repeating polymer chains of (**A**) cellulose, (**B**) fully acetylated chitin and (**C**) fully deacetylated chitosan, evidencing their structural similarity. In addition to being deacetylated, chitosan applied in agriculture is also commonly shorter chained. Taken from Ramírez *et al.* [10].



1.1. Chitin Biochemistry and Production

The majority of the chitin produced for agricultural purposes is sourced from the exoskeletons of crustaceans farmed/harvested for human consumption, chiefly shrimp, crab, and lobster. In addition to possessing a high chitin content, the use of crustacean exoskeletons provides a way of utilizing a major source of waste in the shrimp farming industry. Accurate data on global crustacean farming do not exist, but the Food and Agriculture Organization of the United Nations estimate that in 2011 global crustacean production was 5.9 Mt [11], with 35%–45% of this amount being discarded waste (head and thorax). This means that the global chitinous waste production from this source is 2.1–2.7 Mt,

most of which could be productively utilised in agriculture. While attempts have been made to extract chitin from the waste produced from edible fungi cultivation [12,13] or by using fungi to ferment plant material [14], these enterprises are currently conducted on a much smaller scale to crustacean-derived chitin production.

The chitin polysaccharide can be partially depolymerized to produce oligosaccharide derivatives [10]. These oligosaccharides can be produced with varying polymer length or completely depolymerized to *N*-acetylglucosamine. If the chitin oligomers are deacetylated, the resultant compound produced is called chitosan (Figure 1). The protonation of the amino group resulting from its deacetylation makes chitosan one of the few cationic polymers found in nature [10]. Chitosan is produced commercially by exposing crustacean exoskeletons to high temperatures and alkali conditions which deacetylates the polymer and aids the removal of proteins and calcium minerals. Further purification steps are required if pigments and fats need to be removed, but these contaminations may be acceptable depending upon the final use. Purer forms of chitin and its derivatives are white, odorless and tasteless crystalline solids [10]. Chitosan is soluble in weak acid and so, once the alkali is neutralized, it can be safely applied to plants/soil as a solution or as a dry powder.

As is the case with the production of other natural polysaccharide products, such as fibres [15], considerable research effort is now focused on optimizing methods for enzymatic digestion to replace the use of strong acids/alkali, which are themselves a problematic waste product of chitosan production. Chitinases, chitosanases, chitin deacetylases and proteases [16] from natural sources have been isolated and trialed to develop environmentally friendly chitin and chitosan production [17], as has lactic acid fermentation methods [18].

The cationic properties of the chitosan oligosaccharide imbue it with unique properties that can be exploited by biotechnologists; including applications in the fields of medicine [19–21], material science [7], and crop science. Chitin, chitosan (of various chain lengths), and glucosamine have all been experimentally trialed on crop plants with a range of beneficial agronomical responses recorded. These can be broadly divided into four main areas, each dealt with in a separate section in this review: 1. *Direct antibiosis against pests and pathogens of crops; 2. Enhancement of beneficial microbes, both in plant defense and growth; 3. Stimulation of plant defense responses against biotic stress; 4. Up-regulation of plant growth, development, nutrition, and tolerance to abiotic stresses.* Positive responses to chitin and its derivatives have been reported in numerous economically important crop species that themselves represent a broad coverage of the plant kingdom, including monocotyledons, eudicotyledons, magnoliids and gymnosperms [22,23].

2. Direct Antibiosis of Chitin

Chitosan has been repeatedly found to exhibit potent antimicrobial activity (Reviewed in Ramírez *et al.*, 2010 [10] and El Hadrami *et al.*, 2010 [24]), which has been attributed to its cationic properties and the disruption of potassium signaling in pathogens [25,26]. However, chitosan could also be acting by creating barrier films, chelating mineral nutrients making them inaccessible to pathogens, and/or preventing the release of mycotoxins from the pathogen [27–29]. The polymeric form of chitin does not show substantial antimicrobial activity and this lack of antimicrobial activity

has been attributed to chitin's insolubility and uncharged nature [10]. Thishypothesis is supported by the finding that uncharged chitin oligomers lack antifungal activity [30]. While it is possible to show direct toxicity of pathogens in *in vitro* cultures, when chitosan is applied to field-grown crops it is less clear if the effects observed are due to direct toxicity of chitosan to the pathogen, the induction of plant defenses, and/or the stimulation of beneficial microbes.

2.1. Effectiveness of Chitin-Based Treatments against Fungal Pathogens

Soil amendment with chitosan has repeatedly been shown to control fungal diseases in numerous crops, especially *Fusarium* wilts [31–33] and grey mould [34,35]. It is also of note that these studies show chitosan to be fungistatic against both biotrophic and necrotrophic pathogens.

The control of oomycete pathogens has also been achieved with chitosan treatment, with *Phytophthora capsici* controlled on peppers [26] and *Phytophthora infestans* in potato [36]. This is despite oomycetes lacking chitinous cell walls, like true-fungi (eumycota). In the study by Xu *et al.* [26] on *Phytophthora capsici* in peppers, it was reported that the main effect observed in the pathogen was the disruption of the endomembrane system, especially the integrity of the vacuoles.

2.2. Effectiveness of Chitin-Based Treatments against Bacterial Pathogens

Despite chitin not being a component of bacterial cells [2], chitosan has been shown to possess antibacterial activity [37,38]. The majority of studies have been concerned with the control of human pathogens such as *Escherichia coli, Staphylococcus aureus* and certain *Bacillus* species. While *in vitro* studies show clear antibiotic activity, there is limited evidence for the antibiotic action of chitosan against major bacterial pathogens *in planta*. Chitosan toxicity has been shown in the major bacterial plant pathogen *Pseudomonas syringae* [39], but again, this study was conducted *in vitro*. Chitin in the form of ground shrimp waste was found to control the pathogen *Streptomyces scabies,* which causes scab disease on potato tubers [40], minimising the infection of the scab susceptible potato cultivar "Bentje" to 4%, compared to 22% in the control group. However, rather than direct antibiosis, it was concluded by the author that chitin was active by promoting the growth of microbial species with antagonistic action against the pathogen.

2.3. Antiviral Action of Chitin

Chitosan has been shown to control viral diseases in plants [41]. However, it is yet to be shown that viruses are directly inactivated by chitosan, which in itself would appear to be unlikely as viruses are not composed of chitin or related polysaccharides. Therefore, rather than direct toxicity, it has been proposed that chitosan is effective against plant viruses by modifying the plant's response to infection. It is hypothesised that viral particle transfer is disrupted by chitosan application and its induction of the hypersensitivity response [42–44].

2.4. Effectiveness of Chitin-Based Treatments against Insect Pests

Chitosan has been found to show strong insecticidal activity in some plant pests [45]. Rabea *et al.* [45] found that a chitin derivative (*N*-(2-chloro-6-fluorobenzyl-chitosan) caused 100%

mortality of larvae of the cotton leafworm (*Spodoptera littoralis*) that consumed it when incorporated into an artificial diet at 5 g kg^{-1}. Despite these positive results and the ubiquitous nature of insect pests, there are still only a limited number of studies on the effects of chitin derivatives on insect pests of plants. Of the reports published in peer-reviewed journals effective control with chitosan has been demonstrated for insect pests in the orders Hemiptera (including aphids) [46] and Lepidoptera (chiefly moth pests) [45,46]. However, there is a notable absence of information on the effects on pests in the orders Coleoptera (beetles), Diptera (true flies), and Hymenoptera (wasps, termites, ants and sawflies), which together represent thousands of economically important plant pests.

Mites are another group of economically important arthropod pests for which there is no information on the effects of chitin-based treatments. Mites, being arachnids, possess a chitinous exoskeleton [47]. There are reports that the chitin synthesis inhibitor nikkomycin disrupts many aspects of the development in the glasshouse mite (*Tetranychus urticae*); especially cuticular development [48], but there are no published reports of the effects of chitin/chitosan treatments on phytophagous mites on searchable databases.

While chitosan treatments have been found to effective at controlling herbivorous insect pests, it has actually been used successfully as an ingredient in the artificial diet fed to carnivorous insects being reared for use in the biological control of chitinous pests [49]. This finding suggests that chitin-based products could potentially be less harmful to non-target insects than conventional insecticides. However, there is not enough published data on other beneficial insects, such as pollinators, to come to firm conclusions on this matter.

2.5. Effectiveness of Chitin-Based Treatments against Nematodes

From the 1980s onwards a number of studies found that chitin was effective at controlling plant pathogenic nematode populations [50–52]. Chitinous amendments resulted in impressive reductions in the levels of the phytopathogenic nematode species *Meloidogyne arenaria* [50,51] and *Heterodera glycines* [52]. The level of control of nematodes by chitin-based products was sufficient for them to be registered and marketed as commercial nematocides (e.g., ClandoSan®618) [53]. However, Westerdahl *et al.* [54] found in an independent study that, although the level of control of nematodes on potatoes and walnuts was good, it was not at the level achieved with the synthetic nematicide 1, 3-dichloropropene. Furthermore, in a study on tomato Belair and Tremblay [55] found that, while plant growth was improved by chitin addition, no nematode control was observed.

It has been proposed that chitin controls pathogenic nematodes by acting as a prebiotic promoting the growth of the beneficial chitinolytic microbes that parasitized the eggs of the nematodes [52,56]. However the exact mode of action remains unclear. Both Duncan [57], and Stirling [58] concluded that there was insufficient evidence to back up this mode of action with no detectible parasitism of eggs by chitinolytic fungi when chitin was applied to soil. Therefore, an alternative mode of action, whereby chitin breaks down in the soil to release nematicidal levels of ammonia has been proposed. This would therefore represent a more direct nematicidal action for chitin treatments. This hypothesis is supported by the finding that chitin decomposition in the soil releases significant amounts of ammonia [51]. However, the control of nematode populations by chitin addition has also been found over longer

periods than would be expected from the short-term release of ammonia gas, which would quickly dissipate [56], thus indicating another control mechanism may be operating in chitin-amended soils.

3. Enhancement of Beneficial Microbes, both in Plant Defense and Growth

There is now a substantial body of evidence that the addition of chitin alters the environmental conditions in the rhizosphere and phyllosphere to shift the microbial balance in favour of beneficial organisms and to the detriment of plant pathogens. Chitinolytic microbes produce extracellular chitinase enzymes to degrade chitin-rich tissues of other organisms. While many chitinolytic organisms are pathogenic or parasites, many are also saprotrophic/necrotrophic feeding off dead material or are in a mutualistic relationship with plants. As a result, chitinolytic microbes are essential to plant and ecosystem health and nutrition. It is also important to note that chitinases are also used by organisms for reasons other than to utilize chitin as a food source. Firstly, chitin-containing organisms (both beneficial and pathogenic) use chitinases to regulate their growth and development by controlling the synthesis and lysis of cell walls and skeletons. Secondly, chitinases are also produced in organisms that do not produce chitin themselves, such as higher plants, bacteria and vertebrates, as well as viruses where they are used for detecting, consuming, and interacting with chitin-containing organisms [59,60]. Therefore, adding chitin to a growing environment can have a range of effects on the organisms present.

3.1. Stimulation of Antagonistic Biological Control Agents

One of the best-studied responses to chitin addition is the effect on the microbial species that act as antagonists of crop pathogens. Antagonistic microbes employ a number of methods to attack plant pests and pathogens. This includes, but is not limited to, the production of chitinases [61], the production of toxins (e.g., antibiotics and toxins), direct parasitism, competition for nutriment, and the induction of defense responses in the plant. Therefore, adding chitin-based products to the growing environment may aid beneficial antagonists by stimulating the production and activation of chitinases that can then be used to attack pests and pathogens, or be used as a stable nitrogen-rich polysaccharide food source that boosts the population to the level where other mechanisms control the plant pathogens.

While the addition of chitin to the soil around cultivated crops may promote the growth of antagonistic microbes, owing to the nature of such a complex system, this is extremely difficult to monitor precisely. As a result, the majority of trials have monitored the effect of chitin addition on isolated and cultured antagonists applied to the same plants.

The bacterium *Bacillus subtilis* is a pathogen of fungi and is one of the most widely used biopesticide in agriculture (product name = Serenade ASO) [62]. *B. subtilis* is known to secrete chitinases into the medium in which it is growing [63]. Manjula and Podile [64] showed that the addition of chitin to the carrier material improved the multiplication of *B. subtilis*, and improved the bacteria's fungicidal action and improved the control of *Fusarium* wilt in pigeon pea and crown rot in peanut caused by *Aspergillus niger*. Chitosan addition also improved the action of *B. subtilis* against powdery mildew in strawberry [65].

The beneficial effect of chitin-based treatments to antagonistic bacteria is not restricted to *B. subtilis,* with both chitin and chitosan improving the control of *Fusarium* wilt in both tomato [66] and cucumber [67] when applied to the soil with a range of different species of chitinolytic microbes. Kishore *et al.* [68] found that chitin addition improved the control of *Phaeoisariopsis personata,* the causal agent of late leaf blight in peanut, by the bacterium *Serratia marcescens*. In addition to direct antibiosis, the study by Kishore *et al.* [68] found that these applications also increased the activity of key plant defense enzymes.

A number of soil-borne fungi have been reported to exhibit a chitinolytic activity that surpasses that of bacteria. Strongly chitinolytic species in the *Aspergillus* and *Trichoderma* genera are the most commonly studied, but many more are present in the soil [69]. As with chitinolytic bacteria, chitinase levels and activity are raised upon sensing chitin-containing material [70]. *Trichoderma* species are useful antagonists that utilise chitinases and other hydrolase enzymes against plant pests and pathogens and have now been developed into a number of biopesticide products [62]. The chitinases produced by *Trichoderma* are now known to be extremely antifungal and work on a wide range of fungal plant pathogens [71].

As a substantial body of evidence has built up to support the premise that incorporating chitin and its derivatives enhances the efficacy of natural biological control agents (both bacterial and fungal) [10,72], a number of commercial products have been developed that supply antagonistic microbe strains supplemented with chitin or encapsulated within a chitinous matrix [72,73]. The use of chitin/chitosan to encapsulate microbes also assists with the practicalities of storing and applying microbes on farms and nurseries, which has been one of the major restriction to the use of biopesticides in recent times [74].

In addition to the control of fungal pathogens, chitinolytic bacteria and fungi have considerable potential for the biological control of animal pests, especially insects, mites and nematodes. Of these, the effects of chitinolytic microbes on insects are the best-studied and have been developed as biopesticides. Entomopathogenic fungi, overcome the physical barrier presented by the insect's exoskeleton and gut lining by producing multiple extracellular enzymes, including chitinases, which aid cuticular penetration and subsequent infection [75,76]. As a result, a number of chitinase producing entomopathogenic fungi, such as *Beauveria bassiana*, have been developed into biopesticides that successfully control a range of invertebrate pests [2].

The bacterium *Bacillus thurigensis* is the most widely used biopesticide worldwide (product name = Dipel DF) [62]. *B. thuringiensis* produces the insecticidal Cry-protein toxin. When plant tissue treated with the bacterium is consumed by an insect pest, the Cry-protein is activated by the alkaline conditions in its gut [77]. While the primary mode of action of *B. thuringiensis* is not via chitinase activity, the bacterium can utilize chitin as a source of carbon [78]. In addition, Ortiz-Rodríguez *et al.* [79] showed that *B. thuringiensis* does produce an endochitinase, ChiA74, which when expressed in *Escherichia coli* growing on a chitin-rich media was able to generate chitin-derived oligosaccharides with antibacterial activity against food-borne human pathogenic bacteria. This indicates that chitin can both be used to stimulate the growth of beneficial bacteria with functions other than just degrading chitinous organisms, and that if chitin can be used to upregulate chitinases in a variety of bacteria used for the control of insects (via Cry-protein toxins), they could also potentially be used to control pathogenic microbes (via chitinase activity). In addition, specific

strains of *B. thuringiensis* have been found to produce a chitin-binding protein that both potentiates the insecticidal activity of the Cry-proteins and is directly fungistatic [78].

The caterpillars of the spruce budworm moth (*Choristoneura fumiferana*) died more rapidly when exposed to a mixture of chitinase and *B. thurigensis* than when exposed to either the enzyme or bacterium alone [80]. These findings substantiate the previously stated hypothesis that chitinases can assist the penetration of entomopathogenic bacteria which then use other methods of killing their host. As enzymes are relatively expensive to produce and apply as agrochemicals on farms, the organism that they were isolated from in the above study, the entomopathogenic fungus *Beauveria bassiana* could be used in concert with *B. thurigensis* to increase the effectiveness of bioinsecticide preparations. This synergistic approach of applying both microbial species has already proved successful experimentally for the control of two major beetle pests; the spotted asparagus beetle (*Crioceris quatuordecimpunctata*) [81] and the Colarado potato beetle (*Leptinotarsa decemlineata*) [82].

In addition to promoting bacterial growth, and stimulating the activation of chitinase enzymes, chitin addition has also been shown to have other beneficial effects on rhizobacteria. It was shown by Lo Scrudato and Blokesch [83] that the presence of chitin in the growing media of bacterium *Vibro cloerae* induced horizontal gene transfer (natural competence) where DNA was absorbed and recombined into the chitinolytic bacterium. Horizontal gene transfer allows for quick adaptation to changes in growing conditions with the bacteria being *naturally genetically transformed*. Another potential mechanism by which chitin aids the action of beneficial bacteria is by disrupting the formation of biofilms produced by pathogenic microbes [84]. Such biofilms are increasingly being found to be important regulators of pathogenicity and involve quorum sensing of a diverse range of different species [85]. Therefore, if chitin biopolymers disrupt pathogenic film formation and favour the generation of beneficial microbial ones, it could aid plant health.

Entomopathogenic baculoviruses have also been found to utilize chitinases [60] to aid their penetration of their host [86]. It has also been shown that if viruses are transformed with foreign chitinase genes it can increase their virulence [87]. This work holds promise for increasing the effectiveness of baculoviruses when used as biopesticides. The use of baculoviruses is a relatively minor area of pest control in agriculture at present, but is forecast to increase dramatically as insecticides are withdrawn or replaced with viral products which possess greater specificity to pest species [88]. However, unlike the culturing, activation and delivery of chitinolytic microbe biopesticides, viruses cannot be grown on purified chitin and need a living organism for their multiplication.

3.2. Chitin as a Signalling Molecule for Growth-Promoting Microbes

It is well known that a mutualistic symbiotic relationship exists between legume plants and *Rhizobium* bacteria present in specialised root nodules. However, root nodule formation only occurs after the symbiotic partners exchange specific signalling molecules; flavonoids from the legume stimulating the production of chitin-based "Nod" factors from the bacterium. After successful recognition, there is a series of events that results in nodule formation by the plant and the supply of assimilates to the bacterium, which in turn fix atmospheric nitrogen into a form utilisable by plants.

The Nod factors produced by *Rhizobium* bacteria are classified as lipochitooligosaccharides (LCOs), which are composed of an acylated chitin oligomer backbone with various functional group substituted onto the terminal or non-terminal residues. The number of *N*-acetylglucosamine monomers in a nod factor varies between species; however, generally it is 3 to 5 monomers in length [89]. The exact chemical structure of the Nod factors is thought to vary between bacterial species and strains in order that there is host-symbiont specificity. Staehelin *et al.* [89] demonstrated that the addition of short-chain acetylated chitin derivatives with structural similarity to Nod factors can induce nodulation in *Medicago sativa*. However, this study also showed that there needs to be a fair degree of biochemical similarity between the chitin-derivative applied and the Nod factors excreted by *Rhizobium* sp. This would need to be taken into account when trying to improve nitrogen fixation in legume crops by applying chitin derivatives.

Actinorhizal plants, such as alders (*Alnus*), also possess a symbiotic relationship with a bacterium that fixes nitrogen in their roots. Unlike legume plants, the nitrogen-fixing bacterium associated with actinorhizal plants is not a *Rhizobium* species, but actinobacteria in the genus *Frankia* [90]. No Nod factor genes have yet been found in the *Frankia* genome [91]. However, it is thought that the signaling compounds produced by bacteria are biochemically similar to *Rhizobium* Nod factors, but they have not yet been confirmed as lipochitooligosaccharides [92]; therefore, the involvement of chitinous compounds in these symbiotic relationships remains uncertain.

3.3. Chitin's Interaction with Mycorrhizal Fungi

Considering that an estimated 90% of plant species form mycorrhizal connections with fungi [93], there is currently a dearth of published data on the effect of chitin on mycorrhizal fungi. Lowe *et al.* [65] found that chitosan addition amplified the benefits of mycorrhizal inoculation in strawberry with *Glomus* sp.; specifically increased growth, fruit yield and a delay of the onset of powdery mildew. Gryndler *et al.* [94] found that chitin addition to a soil-based growing media promoted the growth of *Glomus claroideum* mycelium and its colonization of the roots of a number of plant species. However, this is countered by two reports that chitin addition inhibited the growth of mycorrhizae-infected sorghum and broad bean plants [95,96].

Recently it was found that mycorrhizal fungi in the *Glomus* genus secrete lipochitooligosaccharides which stimulate the formation of root connections in plant species belonging to diverse families [97]. At least three genes in *Medicago truncatula* that are stimulated by Nod factors are also involved in the formation of an arbuscular mycorrhizal symbiosis [98]. The fact that the genes that are essential to the response to chitinous Nod factors by legumes are also present and functional in non-legumes [99] also suggests that lipochitooligosaccharides could play a wider role in plant-microbe interactions and plant development, and not just nodule formation. This indicates that both rhizobial and mycorrhizal symbioses may share some common mechanisms and hints at the existence of a chitin-based "Myc factor".

It is also thought that, in addition to Nod/Myc factors and flavonoids, plant chitinases also play a key role in the recognition and formation of connection with mycorrhizal fungi and nitrogen-fixing bacteria [100]. In *Medicago truncatula* different sets of chitinase genes are expressed depending upon

whether it is a mycorrhizal fungus, nodulating bacterium, or a pathogen being interacted with [100]. It could therefore be that chitin-based products make the plant super-sensitized to the presence of fungi.

4. Chitin-Induced Plant Defense Responses against Biotic Stress

It has been repeatedly found that certain chemicals and compounds applied to plants in low concentrations can activate biochemical, genetic and physical defense mechanisms [101]. These compounds, known as effectors/elicitors, include compounds released by pathogens as they attempt to colonize plants [101,102]. Chitin and especially chitosan have been shown in a number of studies to be potent elicitors of plant defenses, which in turn have allowed plants to resist or tolerate a range of diseases. There is little information on how chitosan treatments compare to other elicitors (such as methyl jasmonates, methyl salicylate and harpin proteins), however the findings that diseases can be controlled using chitosan suggests that its effects are sufficiently strong to match up to other elicitors. These findings, combined with the relative low cost of chitosan compared to other types of elicitors, means chitin-based products hold promise commercially to protect crops in large scale agriculture.

4.1. Detection of Chitin in Plants

It is hypothesised that chitin-based treatments activate plant defenses because they mimic the compounds that a plant would normally respond to when being attacked by chitin-containing organism. Plant cell membranes contain chitin-specific receptors, which are known to activate induced defense mechanisms [103]. A range of "chitin elicitor binding proteins" (CEBiP) have been isolated from a number of crops [104] and all these glycoproteins possess a highly conserved extracellular lysin motif (LysM) that binds chitin directly when in contact with the plasma membrane in which it is embedded. In *Arabidopsis* a kinase, CERK1, is also required for chitin sensing and essential for triggering the numerous downstream responses to chitin [105,106]. Plants possess CEBiPs and respond to chitin oligomers because chitin is a structural component of many pathogens, and crucially, is not produced by the plant themselves. Therefore, chitin is classed as a microbe-associated molecular pattern (MAMP). While many elicitors of plant defenses are MAMPs, such as the bacterial flaggelin protein [102], others act by mimicking the hormonal signals that act downstream of MAMP detection, such as jasmonates [107]. As a result, "elicitor" and "MAMP" are not necessarily interchangeable terms.

It is thought that CEBiP receptors respond to chitin oligomers released from pests and pathogens when they are degraded by chitinases that the plant produces in both the apoplast and symplast [108,109]. While plants do produce chitinases to generate chitin fragments from pests and pathogens, and possess CEBiP receptors to detect the chitin and then activate defenses, it is also the case that pests and pathogens can overcome this mechanism by producing chitin-binding proteins to prevent the detection of chitin fragments by CEBiPs by the host plant and thus attack without defenses being induced [110]. This mechanism is well-studied in rice where CEBiP detects chitosan oligosaccharides released by fungal pathogen cell walls, but some pathogens, such as rice blast fungus (*Magnaporthe oryzae*) can mask the chitin released by producing and secreting a chitin-binding protein (LysM Protein1 (Slp1)) that provides a barrier in the apoplastic space to CEBiP activity [109]. Therefore, chitin treatments applied before or during the early stages of attack could swamp the

chitin-binding proteins produced by the pest or pathogens and thus the plant can detect the presence of chitin fragments and therefore activate its defenses.

Signalling events downstream of chitin detection in plants include the expression of a number of early response defense-related genes [111–113] as well as the activation of jasmonate hormones [114]. Numerous genetic and physiological studies show a clear link between jasmonate signalling and the induction of local and systemic defenses in plants. In addition to elevated jasmonate levels, exogenous methyl-jasmonate application also activates many of the same systemic responses [107,115–117] and genes [115,118,119] as chitin-based treatments in plants, which also indicates a link between chitin treatment and jasmonate activation of defenses. Elevations in the level of the stress hormone ABA has also been found following chitin-based treatments [120] and both are known to reduce stomatal aperture [121]. However the link between chitin addition and ABA signalling is not currently well-studied.

4.2. Chitin-Induced Defense Mechanisms

Once chitin oligomers have been detected by CEBiP receptors and the signal transported around the plant by jasmonates, a number of downstream responses of the plants are activated. Various studies have analysed the defenses activated by chitin and include the production, release, and/or activation of phytoalexins [122,123], phenolics [124], terpenes [23], and reactive oxygen species [125]. Cellular changes detected following chitin derivative application include membrane depolarization resulting in alterations in ion fluxes and cytoplasmic acidification [125,126].

In addition to biochemical defenses, chitin addition has also been found to induce the formation of physical barriers to attack including; the deposition of callose [120] and lignin [127,128], and the formation of tyloses [129]. These physical barriers allow for quick wound formation and sealing in order to compartmentalise an infection. This compartmentalisation of wounds and infection sites is an especially important defense response in woody perennials to prevent pathogens travelling systemically around trees [130]. The induction of programmed cell death (PCD) in the hypersensitive responses in the epidermis [131] has also been observed following chitin treatment, and this provides both a physical and biochemical barrier to further infection of pathogens in herbaceous tissues.

4.3. The Role of Chitinases in Plant Defense

A range of pathogen-related (PR) proteins have also been found to be activated after chitin-based treatments have been applied to plants. Most notably this includes chitinases, but also includes glucanases [68] peroxidases, polyphenoloxidases [124] and MAP-kinases [132]. Protease inhibitors are also produced [118]; presumably to limit the activity of the pathogen's own enzymes involved in attack. In addition to their role in the early stages of pest and pathogen detection, plant chitinases have also been shown to be effective at controlling fungal growth [133] and are thus thought to also be an induced defense in their own right and in this role are classified as pathogen-related (PR) proteins. However, although effective against fungal pathogen, it appears insect pests are not controlled by plant chitinases [134]. For example, even when a rice chitinase was overexpressed in rice, the transformed plants still had no resistance to attack by the caterpillars of the fall armyworm moth (*Spodoptera frugiperda*) [135].

From the early 1990s [136] onwards, a number of horticultural and agricultural crops have been genetically engineered to express foreign chitinases (summarized in Herrera-Estrella and Chet, 1999 [134]) in order to confer resistance to pathogens, pests and abiotic stress. As plant chitinases have been found to have a lower activity against insect pests, the chitinases used in transgenic crops are insect, bacterial or fungal in origin [137]. In addition to the genetic modification of plants with chitinases, several rhizobacteria species that aid plant growth, but which lack the ability to produce chitinases have also been transformed with chitinase genes (summarised in Someya and Akutsu, 2006 [138]). When these GM rhizobacteria were applied to plants it improved the protection against a range of pathogens. This approach has been found to be successful when foreign chitinases are inserted into symbiotic endophytic bacteria living inside the plant. This approach was successfully used by Sitrit *et al.* [139] who introduced the ChiA gene that codes for an extracellular chitinase from *Serratia marcescens* into the nitrogen-fixing bacterium *Sinorhizobium meliloti* (syn. *Rhizobium melliloti*). When alfalfa plants were inoculated with the transformed bacterium it provided control of fungal diseases and retained its ability to fix nitrogen. In another study [140] the same ChiA chitinase gene from *Serratia marcescens* was inserted into the genome of the endophytic *Pseudomonas fluorescens* which controlled the *Rhizoctonia solani* pathogen in beans (*Phaseolus vulgaris*). The effects of chitin-based products on these transgenic plants and microbes is yet to be studied.

4.4. Chitin Synthase Disruption by Pesticides

A number of pesticides have been developed that disrupt chitin synthesis in target organisms [141]. The acylurea insecticides are thought to disrupt the function of transmembrane chitin synthases [142] which in turn weakens the insect's cuticle and disrupts the moulting process. The effectiveness and apparent safety [143,144] of acylurea led to the development of other urea-based insecticides, such as hexaflumuron, which proved effective against termite larvae [145]. Unlike the juvenile hormone analogue insecticides that are most effective against adult insects, chitin synthesis inhibitors cause mortality mostly in larvae and nymphs [146].

In addition to synthetic compounds, a number of natural compounds inhibit chitin synthesis when applied to chitin-containing organisms, and have thus been identified as potential biopesticides [147]. This includes trehazolin and allosamidin, two pseudo-saccharides, which are both inhibitors of key enzymes involved in chitin synthesis [148–150]. While both trehazolin and allosamidin are effective *in vitro*, the large number of hydroxy groups in the molecules prevent them penetrating the insect cuticle and reaching their specific targets [151] and this has limited their utilisation for *in vivo* pest control. Therefore, a number of synthetic structural analogues have been developed [147,152]. Trehazolin was initially isolated from a strain of *Micromonospora* bacteria [153], in which it is an active antibiotic. As a result, it is now thought that an increased understanding of plant-*Micromonospora* interactions could produce further biological control agents against pests and pathogens [154]. Another chitinase-inhibiting compound nikkomycin is one of the most potent chitin synthase inhibitors isolated [155] and was first obtained from a culture of *Streptomyces tendae*. Unlike trehazolin and allosamidin, nikkomycin is a nucleoside peptide and so the utilisation of both

Streptomyces and *Micromonospora* could improve the control of insect pests via the production of both pseudo-saccharide and peptide-based biopesticides.

In addition to insect pests, chitin synthase inhibitor could potentially be used to control other chitin-containing pathogens. For example, nikkomycin produced by *Streptomyces tendae* shows both antifungal and insecticidal activity [156]. In addition, the chitin synthase inhibitor polyoxin-d is already registered for use as a fungicide in turf grass [157], and while not currently registered as an insecticide, it has been found experimentally to assist the action of a bioinsecticide [158]. In a study by Bixby-Brosi and Potter [158] the infection of black cutworms (larvae of the dark sword-grass moth *Agrotis ipsilon*) by the baculovirus *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (AgipMNPV) was improved by the addition of polyoxin-d.

5. Regulation of Plant Growth, Development, Nutrition, and Tolerance to Abiotic Stresses

5.1. Plant Growth Promotion

Improvements in plant growth have been reported after the application of chitin-based treatment to a range of crops, which are thought to be independent of the effects on pest and disease control. Significant improvements in growth have been reported in daikon radishes (*Raphanus sativus*) [159], cabbage (*Brassica oleracea*) [22], soybean sprouts [160], sweet basil [161], grapevine [162], as well as ornamental crops, such as *Gerbera* [163] and *Dendrobium* orchids [164].

In three studies on orchids it was found that chitosan was effective at a very low concentration of 10 mg L^{-1} [164–166]. This indicates that the chitosan was acting due to mechanisms other than simply improving nitrogen nutrition or as a carbohydrate energy source. The reports of chitosan treatments stimulating growth should be tempered by the findings of other trials showing no significant effect on growth, biomass production, or yield in rice and soybean [167], and maize and soybean [168].

Both Pornpeanpakdee *et al.* [165] and Nahar *et al.* [166] found that the growth of orchids (*Dendrobium* and *Cymbidium* respectively) was enhanced when chitosan was supplied to micropropagated plants growing under aseptic conditions. These findings show that chitin can promote the growth of plants independently of its actions on plant growth promoting rhizobacteria (PGPR). This is backed up by other studies showing enhanced growth in sterile conditions such as tissue cultured grapes [162] and the growth of the medicinal herb *Phyla dulcis* being cultivated in liquid bioreactors [169].

5.2. Physiological Responses to Chitin Treatment

Bittelli *et al.* [121] found that the water use of pepper plants treated with chitosan reduced by 26%–43%, with no significant change in biomass production or yield. These findings indicate that chitosan has potential to be developed as an antitranspirant in agricultural situations where excessive water loss is undesirable. While both ABA and jasmonic acid have both been found to raise in concentration in response to chitosan treatment [114,120], and it is well-documented that these hormones are involved in the control of stomatal aperture [170], it was recently shown that the stomatal closure recorded after chitosan treatment does not involve JA and ABA signaling. This conclusion was reached because JA/ABA mutants still respond to chitosan with stomatal closure [171].

The results that chitosan induced stomatal closure should be tempered by a report by Khan *et al.* [168] who found that foliar application induced stomatal opening and increased transpiration in soybean and maize.

5.3. Chitin's Effects of Plant Development

In addition to increases in photosynthesis and vegetative growth, chitin-based treatments have also been shown to modify developmental processes. Limpanavech *et al.* [172] studied the application of chitosan on *Dendrobium* orchids using a range of concentrations (1–100 mg L^{-1}) and a range of deacetylation levels (70%–90%) with all treatments inducing no changes in vegetative growth, but chitosan did induce precocious flowering. Utsunomiya and Kinai [173] also recorded precocious flowering and increased flower numbers when chitosan was applied to passionfruit (*Passiflora edulis*) as a soil drench. Induction of flowering by chitosan was again observed in *Eustoma grandiflorum* [174] and in a separate study on *Eustoma grandiflorum* chitosan produced more intense petal pigmentation when dissolved into the holding solution of excised inflorescences [175]. Unfortunately, there is no information on the mechanism by which chitin-based treatments induced flowering in these ornamental crops.

In addition to their role in defense mechanisms, plant chitinase are also thought to be involved in regulating the embryogenesis process [176] in seed formation, but their exact function and the effect of exogenous chitin addition on this process remains unclear. The germination of seeds has been shown to be improved in a range of crops following chitin-based treatments including maize [177] and wheat [178]. In these studies chitosan accelerated germination and/or increased the percentage of seeds germinating. Unfortunately, many of the other reports of positive germination responses cited as original articles are in obscure publications or have not undergone rigorous statistical analysis.

As with other responses to chitin-based treatments, the addition of chitin alongside a beneficial chitinolytic microbial agent augment and may amplify the positive effects on germination. In addition to its role in protecting plants against pathogens, the chitinolytic bacterium *B. subtilis* AF 1 was found to promote seed germination and subsequent plant growth in pigeon pea even under pathogen pressure [64] with this response amplified by the addition of chitin to the carrier medium [179]. As *B. subtilis* is known utilize chitin [179] and strains of this bacteria are known to produce plant hormones (auxins) [180] the promotion in germination could potentially be due to changes in plant hormone levels in the rhizosphere.

5.4. Plant Nutrition

Chitin, and all its derivatives, have a high nitrogen content of 6.1%–8.3% [181]. This is a comparable level to other organic fertilizers such as dried blood, bone meal, and hoof and horn meal [182]. While chitin has a high thermal and chemical stability [181], making it possible to store dry product for a good length of time, it can quickly be utilized as both a nitrogen source and energy source by plants and microbes when added to crops. Plants can access the nitrogen in chitin via microbial breakdown and the release of inorganic nitrogen, or directly taking up monomers as organic nitrogen [183,184]. Spiegel *et al.* [183] demonstrated that Chinese cabbages treated with chitin-based products grew faster than plants treated with a standard mineral fertilizer. The utilization of chitin by

microbes will be slowest in cold [1] and dry conditions [185]. This could be of benefit if chitin was used in controlled-release fertilizers (CRF), as inorganic forms of nitrogen will not be released when plants do not need them in winter, and thus could minimize the leaching of nutrients from soils and their damaging impact on waterways. Partially purified chitin also has promise in plant nutrition as it can be used to add organic matter to soils without raising the C:N ratio. This problem occurs when organic matter of plant origin is added in excess, and leads to nitrogen deficiency via a range of different processes [186]. Chitin-rich edible fungi waste has a long history of use in agriculture and landscape horticulture, with 'spent mushroom compost' used primarily to add organic matter and raise soil pH (due to the chalk used in fungi cultivation). In areas of major seafood production chitinous "crab compost" also has a history of use as a soil amendment in agriculture [187].

In addition to supplying nitrogen, the exoskeletons of crustaceans from which chitosan is commercially extracted are also high in calcium minerals where they aid structural rigidity [9]. Therefore, chitin-based products that have only been partially purified will also contain substantial levels of calcium, an important macronutrient.

The cationic properties of chitosan also make it suitable as a medium for supplying additional essential nutrients. The functional hydroxyl and amino groups on deacetylated chitosan allow the formation of coordination compounds (complexes) with ions of copper, zinc, iron and others, but not with those of alkaline metals (e.g., potassium) or alkaline earth metals (e.g., calcium or magnesium) [10]. This makes chitosan a sustainable alternative to synthetic chelation agents, such as EDTA that are routinely used to deliver iron and other nutrients to overcome their poor solubility in calcareous/neutral soils [188]. It may be possible to utilise the cationic nature of chitosan to boost a soil/growing medium's anionic exchange capacity (CAE), which are generally low, and far lower than their cationic exchange capacity (CEC) [188]. Thus soils treated with chitosan could suffer less from leaching of anionic nutrient fertilizers, such as nitrates and phosphates, but this hypothesis remains untested. In addition to the controlled release of nutrients, chitosan polymers have also been used successfully to improve thedelivery of certain pesticides to crops to improve their effictiveness and reduce the environmental impact [189,190].

Chitosan can also form gels that absorb substantial volumes of water due to its high molecular weight, and porous structure [191]. These "hydrogels" can improve the water retention levels of soils [192]. As a result, chitosan hydrogels are potential natural alternatives to polyacrylamide products that are used to improve the water retention in sandy soils, and containerized growing media in ornamental horticulture. In combination the nutrient chelation and water holding properties of chitosan have been utilized to produce new prototype control release fertilizers (CRF) [193].

5.5. Chitin's Ability to Alter a Plant'S Resistance to Abiotic Stress

Chitosan has repeatedly been shown to possess antioxidant activity [194–197]. The hydroxylated amino groups present on chitosan oligomers make them extremely effective scavengers of hydroxyl radicals, hydrogen peroxide and anion superoxide [194,196]. Plant chitinases are also considered to be a component of the resistance mechanisms to a range of abiotic stresses [176]. Unfortunately there is a dearth of published information on how chitin-based treatments can improve plant survival and performance in stressful growing environments. Boonlertnirun *et al.* [198] found that chitosan

treatments had a significant effect on the growth or yield of drought-stressed rice plants compared to control plants, and that the effect was greatest when chitosan was applied before the onset of stressful conditions. These findings could be linked to the previous reports of chitosan inducing stomatal closure and reducing transpiration rates in plants [120].

In addition to their ability to form complexes with plant nutrients, chitin and its derivatives can also form complex with non-nutrient elemental ions, including a number of heavy metals [199]. This has led to interest in chitin treatment to remediate polluted soils and water sources [200–202]. Chitosan has been shown to assist in the remediation of other man-made pollutants that can pollute soil and water sources including dyes [203], and hydrocarbons [204], as well as being able to absorb certain fertilizers and pesticides should they be accidentally applied in excess.

As is the case with growth and defensive responses to chitin, the application of chitin-based products alongside a beneficial microbial agent augments and amplifies the effectiveness of each. Wang *et al.* [204] found that the chitosan treatment alongside mycorrhizal inoculation aided the bioremediation of soil polluted with a range of heavy metals by *Elsholtzia splendens*. Angelim *et al.* [205] recently showed that encapsulating a consortium of different PGPR within chitosan helped with delivery, and stimulated the growth and activity of the bacteria for bioaugmentation and biostimulation of hydrocarbon-polluted soils. An additional study on crude oil contaminated seawater found that chitin/chitosan encapsulation improved the effectiveness and survival of bioremediating chitinolytic bacteria [206].

6. Agronomic Considerations

6.1. Efficacy and Phytotoxicity

Variability in the chitin extraction and modification processes are thought to change the effectiveness of the chitin-based products produced. This in turn has resulted in considerable variability in the literature in the responses to treatments. Properties that are thought to be important for the action of chitin and its derivatives include polymer length, degree of acetylation, pH, and the presence of contaminants. For example, a common finding from studies on phytopathogenic fungi is that as the degree of acetylation increases in a chitin derivative, the antifungal activity increases [207].

In addition to variations in the chitin products themselves, differences in the application method, solvent used, quantity applied and local environmental conditions are all likely to alter a plant's response to a treatment. These many variables differ in the trials highlighted in this review which has limited the potential for comparisons of the effects seen in each trial and precludes the use of meta-analysis techniques to analyze across multiple datasets. In addition, while direct antibiosis of fungal pathogens may occur after chitosan application, it may also be combined with positive responses by the plant and beneficial microbes. For example, both direct antifungal action and host defense responses (increased phenolic content) were observed in response to chitosan addition to control *Fusarium* wilt on date palm [207].

Despite the many reports of positive responses of plants to chitin-based treatments there are also reports of phytotoxicity when super-optimal concentrations were supplied. Pornpeanpakdee *et al.* [165] found that in micropropagated *Dendrobium* orchids growth was enhanced when chitosan was supplied

at 10 and 20 mg L^{-1}, but that growth was inhibited at 80 mg L^{-1} and the orchids killed in the 160 mg L^{-1} treatment. In addition to the volume and type of chitin supplied, Westerdahl *et al* [54] also proposed that soil water content has a major impact on chitin phytotoxicity. This could mean that chitin treatments need to be 'wetted in' after application as is standard practice for certain other pesticides and fertilizers.

6.2. Human/Animal Toxicity of Chitin and Chitosan

The Environmental Protection Agency (EPA) concluded in 2008 that there are no major identifiable risks to human health of chitin and chitosan products. As chitin is not a biopolymer in vertebrates and is consumed regularly in the human diet, it is considered safe for the control of chitinous pests [149]. Unfortunately there is dearth of information regarding the toxicity of chitin/chitosan to beneficial microbes, nematodes or insects other than those purposely applied as biological control agents alongside chitin products, including saprotrophic/nectrophic organisms. One study [208] did show that chitosan was more toxic to phytopathogenic fungi than to beneficial nematophageous and entomopathogenic fungi. Such information is useful for improving the integration of chitin treatments into Integrated Pest Management (IPM) systems on farms and nurseries.

6.3. Chitin Delivery Systems

Application methods used to apply chitin largely depends upon the desired effects. Even though systemic induced defenses are activated, for the control of soil-borne diseases and nematodes, clearly a soil drench is optimal. Soil drenches are essential for long-chain chitin addition as it is poorly soluble in water. Foliar sprays of chitosan can be used to control foliar fungal diseases of plants or to coat stored produce. Other application methods include dissolving chitosan into the fertigation stream in hydroponic systems, the growing media of micropropagated plants, or the vase water of cut flowers. Seed coating has been found to be effective at inducing plant defenses and protecting against *Fusarium* ear rot [178], However, another study on tomato found that while coating seeds in chitosan induced defenses in tomato, these were not sufficient or prolonged enough to fully protect against *Fusarium* root rot [209]. In addition, for fungi and nematodes used as biological control agents to control fungal pathogens and insects, it is not currently known how they are able to express chitinases to digest these organisms without compromising their chitinous tissues.

Despite plants responding to chitin and chitosan by activating induced defenses, no successful attempts have been made or published regarding inserting chitin/chitosan synthase genes into plants. This could potentially elicit permanent systemic defensive responses by the continual production of chitin oligomer elicitors in the plant's symplast. The permanent elicitation of plant defenses by inserting genes from pathogens that code for specific MAMP elicitors has already been achieved. One of the best examples of a MAMP-expressing GM crop is the plum variety "Honey Sweet", which has been transformed with a gene coding for a protein coat from Plum Pox Virus (PPV) [210]. "Honey Sweet" is fully resistant to PPV, with all non-GM plum varieties being susceptible to this devastating disease [211].

As chitin-based treatments allow plants to overcome an inability to identify the presence of certain pests or pathogens, there are situations when this will not be sufficient for control. If a plant's induced

defenses are not sufficient to control the pest or pathogen then chitin elicitation will be unlikely to be effective (unless chitosan is directly toxic to that organism). In addition, as chitin derivatives allow the "pre-emptive" or early response to pathogen attack, applications made on plants visibly harmed by disease are unlikely to be effective. Other scenarios where the activity of chitin may be compromised include plant species which rely strongly on constitutive defenses that remain activated regardless of the presence/absence of pathogens.

Further work is still required to optimize the application methods for chitin-based treatments. Notable areas where there is currently a lack of information include the effects on mite, beetle and fly pests, as well a requirement for an improved understanding of the phytotoxicity resulting from super-optimal application. There is also a need to streamline the legislative procedures for registering biopesticides to make chitin-based products realistic alternatives to conventional synthetic pesticides [62].

6.4. Use of Chitin-Based Treatments in Non-Field Agricultural Systems

In addition to the positive responses to chitin-based treatments in field-grown crops, numerous studies have identified positive effects in other sectors of agriculture and horticulture. The growth of micropropagated crops has been shown to increase following the inclusion of chitosan to the growth medium [162,165,166,169]. As many of the defensive secondary metabolites activated by chitin elicitation are useful as medicines, or their precursors, the use of soluble chitosan in tissue culture and hairy root culture laboratories to produce medicinal plants with elevated levels of active compounds has been widely studied. Examples include chitosan increasing the concentration of the anticholinergic alkaloids scopolamine and hyoscyamine in *Brugmansia candida*, from which they are commercially extracted for antispasmodics in the treatment of motion sickness [212]. The same response to chitosan was discovered in *Hyoscyamus muticus* with it produced a 5-fold increase in hyoscyamine content compared to controls [213]. Chitosan supplementation induced a three-fold increase in the steroid drug precursor diosgenin in *Trigonella foenum-graecum* hairy root cultures [214]. Also, chitosan boosted the production of the potential drug and pigment indirubin from Chinese indigio (*Persicaria tinctoria*) growing in tissue culture [215]. Another aspect of micropropagation where chitin-based products have proved effective is in the breeding of hybrid varieties. Chitosan has successfully been used as a component of artificial seed coats that are required for the embryo rescue procedures when breeding certain hybrid plant varieties [216].

Hydroponics is now widely used for the production of many edible and ornamental plants. The addition of soluble chitosan amendments to hydroponically cultivated tomatoes was found to successfully supress root rot (*Fusarium oxysporum* f. sp. *radicis-lycopersici*) [129] a pathogen that can thrive if the growth of microbes in the fertigation stream is not controlled. The inclusion of soluble chitosan to hydroponic fertigation streams also promoted the growth and final yield of hydroponically cultivated potato microtubers [217].

Chitin-based treatments show promise as alternatives to the use of synthetic pesticides on fresh produce in post-harvest storage [207,218]. This is an issue of particular concern for human health because of the temporal proximity of use to the point of consumption of food. Fungal rots have been successfully prevented by applying chitosan treatments in a range of fruits, including; apples, cherries,

citrus, grapes, kiwifruit, litchi, papaya, peaches, pears, and strawberries [207,218–225]. El Ghaouth *et al.* [218] found that chitosan was antifungal when applied on its own, but also improved the action of the chitinolytic biological control agent *Candida saitoana,* and as such is line with the findings of improved efficacy of biopesticides on field-grown crops by chitin-based treatments.

Sivakumar *et al.* [225] found that as well as controlling anthracnose (*Colletotrichum gloeosporioides*) rots on stored papaya, chitosan treatment improved fruit quality by retarding the ripening process, retarding color development, and the fruits being firmer and losing less weight that control treatments. Chitin-based products have also been successfully used to extend the storage life of non-food crops, including cut flowers [226]. Further advances in post-harvest storage may also come from developments in chitin-based films and threads, which are being developed as biodegradable polymers and packaging [7,227]. The antimicrobial properties of the chitin in these products could further improve the shelf life of crops once they leave storage facilities.

There is also potential for incorporating chitin derivatives into the sealant products that are used to cover pruning wound sites on trees and shrubs. Hirano *et al.* [22] found that the application of chitin films to tree bark wounds resulted in faster wound healing. This is backed up by the findings of increased callose [120] and lignin [127,128] deposition following chitin treatment. There is a particular need for the development of improved wound sealant products in orchards, landscape horticulture and arboriculture, as the wax/resin-based products currently used are known to perform poorly, with minimal independent evidence of effectiveness [228].

6. Conclusions

Chitin and its derivatives have been repeatedly shown to protect crops from pests, pathogens and physiological disorders. A number of modes of action have been identified for the beneficial effects of chitin-based treatment on crops, including direct antibiosis and the induction of plant defences. However, their action in stimulating beneficial microbes has proved particularly impressive, with chitin/chitosan amplifying the effect of beneficial microbes in controlling pathogens, promoting plant growth and remediating soil pollutants. Combined, these effects of chitin addition and the subsequent responses of plants and microbes have led to improvements in disease control, plant growth, and ultimately improved crop yield and quality. The effectiveness of chitin-based treatments has been found to be comparable to those achieved with current synthetic pesticides and fertilizers. This effectiveness combined with the low cost, low concentration required, ample supply (recycled waste) and health/environmental safety lead to a forecast that a range of chitin-based/augmented products (Table 1) will become a more common feature in agriculture in the near future.

Table 1. Summary of the potential commercial products that chitin and its derivatives could be used for in agriculture and horticulture and their proposed mode of action.

Product class	Product type	Primary crop	Current product used	Hypothesised mode of action	Reference to evidence to chitin activity
Biocide	Fungicide	Most	Various synthetic and organic pesticides	Direct antibiosis, promotion of antagonists, induction of plant defenses.	[45,229]
Biocide	Oomyceticide	Most	Few of the fungicides available are effective	Disruption of endomembranes	[26]
Biocide	Bactericide	Most	Various synthetic and organic pesticides	Direct antibiosis, promotion of antagonists, induction of plant defenses.	[40]
Biocide	Viricide	Most	Few viricides currently commercially available	Induction of hypersensitivity and restriction in viral movement	[41–43]
Biocide	Nematocide	Bulbous/tuberous crops	Various synthetic and organic pesticides	Direct antibiosis, release of ammonia, promotion of antagonists	[50]
Biocide	Insecticide	Most	Various synthetic and organic pesticides	Direct antibiosis, promotion of antagonists, induction of plant defenses.	[45,229]
Biocide	Miticide	Glasshouse and orchard crops	Various synthetic and organic pesticides	Direct antibiosis, promotion of antagonists, induction of plant defenses.	No published studies identified.

Table 1. *Cont.*

Product class	Product type	Primary crop	Current product used	Hypothesised mode of action	Reference to evidence to chitin activity
Fertilizer	Nitrogen fertilizer	Most	Various synthetic and organic fertilizers	Utilisation of nitrogen from the chitin's amino groups	[181,183]
Fertilizer	Controlled release fertilizer	Most	Resin-based fertilizer granules	Direct uptake of monomers and decomposition to ammonium by microbes	[192]
Growth regulator	Direct growth regulator / biostimulant/ Stress eliviator	Most	Various synthetic growth regulators and hormone inhibitors. Plus humic and fulvic acid, and seaweed extracts.	Action on specific signalling pathways; tomatal closure; direct antioxidant activity	[120,162,165,166,169,198]
Growth regulator	Elicitor for the production of secondary metabolites	Medicinal herbs	Jasmonates, salicylates, benzoic acid	Induction of plant biochemical defenses	[212,213,215]
Growth regulator	Antitranspirant	Stored fresh produce, evergreen trees	Synthetic polymers: e.g., di-1-p-Menthene	Formation of a film minimising evaporation from tissues, plus action on stomatal aperture.	[120,121]
Growth regulator	Wound sealant	Trees and shrubs	Wax and resin-based paints	Barrier formation, liginin and callose formation, and direct antibiosis.	[22,127,128]

Table 1. *Cont.*

Product class	Product type	Primary crop	Current product used	Hypothesised mode of action	Reference to evidence to chitin activity
Growth regulator	PGPR stimulator	Most	none	Supply of suitable food source and signalling compounds	[89]. No studies on hormone producing bacteria.
Growth regulator	Mycorrhizal stimulator	Perennial crops	none	Supply of suitable food source and signalling compounds	[65]
Growth regulator	Ripening retardant	Stored fresh produce	Controlled-environment storage, 1-MCP	Unknown	[225]
Soil conditioner	Pollution absorber	Crops growing in polluted soils	Activated charcoal, synthetic chelating agents	Cationic binding of metal ions	[199,201–203]
Soil conditioner	Water retainer	Crops in sandy/dry soils	Polyacrylamide and cellulose-based products	Gel formation	[193]
Biopolymer	Packaging	Stored fresh produce	Predominantly Polyethylene films	Formation of antiseptic film	[7,227,230]

Acknowledgments

Thanks go to Julia Lock and Gordon Sharp for assistance with manuscript submission, and to Miguel Á Ramírez for the use of the image in Figure 1.

References

1. Gooday, G.W. The ecology of chitin degradation. *Adv. Microb. Ecol.* **1990**, *11*, 387–419.
2. Gohel, V.; Singh, A.; Vimal, M.; Ashwini, P.; Chhatpar, H.S. Bioprospecting and antifungal potential of chitinolytic microorganisms. *Afr. J. Biotechnol.* **2006**, *5*, 54–72.
3. Castro, S.P.M.; Paulín, E.G.L. Is chitosan a new panacea? Areas of application. In *the Complex World of Polysaccharides*; Karunaratne, D.N., Ed.; InTech: Rijeka, Croatia, 2012; doi:10.5772/51200. Available online: http://www.intechopen.com/books/the-complex-world-of-polysaccharides/is-chitosan-a-new-panacea-areas-of-application (accessed on 4 July 2013).

4. Bartnicki-Garcia, S.; Lippman, E. Fungal wall composition. In *CRC Hand book of Microbiology*, 2nd ed.; Laskin, A.J., Lechevalier, H.A., Eds.; CRC Press: Boca Raton, FL, USA, 1982; pp. 229–252.
5. Gow, N.A.R.; Gooday, G.W. Ultrastructure of chitin in hyphae of *Candida albicans* and other dimorphic and mycelial fungi. *Protoplasma* **1983**, *115*, 52–58.
6. Bueter, C.L.; Specht, C.A.; Levitz, S.M. Innate sensing of chitin and chitosan. *PLoS Pathog.* **2013**, *9*, e1003080, doi:10.1371/journal.ppat.1003080.
7. Pillai, C.K.S.; Paul, W.; Sharma, C.P. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Prog. Polym. Sci.* **2009**, *34*, 641–678.
8. Muzzarelli, R.A.A. *Chitin*; Pergamon Press: Oxford, UK, 1977.
9. Boßelmann, F.; Romano, P.; Fabritius, H.; Raabe, D.; Epple, M. The composition of the exoskeleton of two crustacea: The American lobster *Homarus americanus* and the edible crab *Cancer pagurus*. *Thermochim. Acta* **2007**, *463*, 65–68.
10. Ramírez, M.Á.; Rodriguez, A.T.; Alfonso, L.; Peniche, C. Chitin and its derivatives as biopolymers with potential agricultural applications. *Biotecnol. Apl.* **2010**, *27*, 270–276.
11. FAO, Fishery Statistical Collections; Global Aquaculture Production. Available online: http://www.fao.org/fishery/statistics/global-aquaculture-production/en (accessed on 15 May 2013).
12. Andrade, V.; Neto, B.; Fukushima, K.; Campos-Takaki, G. Effect of medium components and time of cultivation on chitin production by *Mucor circinelloides* (*Mucor javanicus* IFO 4570)—A factorial study. *Revista Iberoamericana de Micología* **2003**, *20*, 149–153.
13. Law, W.M.; Lau, W.N.; Lo, K.L.; Wai, L.M.; Chiu, S.W. Removal of biocide pentachlorophenol in water system by the spent mushroom compost of *Pleurotus pulmonarius*. *Chemosphere* **2003**, *52*, 1531–1537.
14. Crestini, C.; Kovac, B.; Giovannozzi-Sermanni, G. Production and isolation of chitosan by submerged and solid-state fermentation from *Lentinus edodes*. *Biotechnol. Bioeng.* **1996**, *50*, 207–210.
15. Akin, D.E.; Condon, B.; Sohn, M.; Foulk, J.A.; Dodd, R.B.; Rigsby, L.L. Optimization for enzyme-retting of flax with pectate lyase. *Ind. Crops Prod.* **2007**, *25*, 136–146.
16. Yang, J.K.; Shih, I.L.; Tzeng, Y.M.; Wang, S.L. Production and purification of protease from a *Bacillus subtilis* that can deproteinize crustacean wastes. *Enzyme Microb. Technol.* **2000**, *26*, 406–413.
17. Hoell, I.A.; Vaaje-Kolstad, G.; Eijsink, V.G.H. Structure and function of enzymes acting on chitin and chitosan. *Biotechnol. Genet. Eng. Rev.* **2010**, *27*, 331–366.
18. Cira, L.A.; Huerta, S.; Hall, G.M.; Shirai, K. Pilot scale lactic acid fermentation of shrimp wastes for chitin recovery. *Process Biochem.* **2002**, *37*, 1359–1366.
19. Bowman, K.; Leong, K.W. Chitosan nanoparticles for oral drug and gene delivery. *Int. J. Nanomed.* **2006**, *1*, 117–128.
20. Jayakumar, R.; Prabaharan, M.; Nair, S.V.; Tamura, H. Novel chitin and chitosan nanofibers in biomedical applications. *Biotechnol. Adv.* **2010**, *28*, 142–150.
21. Towheed, T.E.; Anastassiades, T.P.; Shea, B.; Houpt, J.; Welch, V.; Hochberg, M.C. Glucosamine therapy for treating osteoarthritis. *Cochrane Database Syst. Reviews* **2001**, doi:10.1002/14651858.CD002946.pub.

22. Hirano, S.; Kitaura, S.; Sasaki, N.; Sakaguchi, H.; Sugiyama, M.; Hashimoto, K.; Tanatani, A. Chitin biodegradation and wound healing in tree bark tissues. *J. Environ. Polym. Degrad.* **1996**, *4*, 261–265.
23. Croteau, R.; Gurkewitz, S.; Johnson, M.A.; Fisk, H.J. Monoterpene and diterpene biosynthesis in lodgepole pine saplings infected with *Ceratocystis clavigera* or treated with carbohydrate elicitors. *Plant Physiol.* **1987**, *85*, 1123–1128.
24. El Hadrami, A.; Adam, L.R.; El Hadrami, I.; Daayf, F. Chitosan in Plant Protection. *Mar. Drugs* **2010**, *8*, 968–987.
25. Harish Prashanth, K.V.; Tharanathan, R.N. Chitin/chitosan: Modifications and their unlimited application potential—An overview. *Trends Food Sci. Technol.* **2007**, *18*, 117–131.
26. Xu, J.; Zhao, X.; Han, X.; Du, Y. Antifungal activity of oligochitosan against *Phytophthora capsici* and other plant pathogenic fungi *in vitro*. *Pestic. Biochem. Physiol.* **2007**, *87*, 220–228.
27. Sudarshan, N.R.; Hoover, D.G.; Knorr, D. Antibacterial action of chitosan. *Food Biotechnol.* **1992**, *6*, 257–272.
28. Kendra, D.F.; Hadwiger L.A. Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum*. *Exp. Mycol.* **1984**, *8*, 276–281.
29. Sekiguchi, S.; Miura, Y.; Kaneko, H.; Nishimura, S.I.; Nishi, N.; Iwase, M.; Tokura, S. Molecular weight dependency of antimicrobial activity by chitosan oligomers. In *Food Hydrocolloids: Structures, Properties and Functions*; Nishinari, K., Doi, E., Eds.; Plenum: New York, NY, USA, 1994; pp. 71–76.
30. Parra, Y.; Ramírez, M.A. Efecto de diferentes derivados de quitina sobre el crecimiento *in vitro* del hongo *Rhizoctonia solani* Kuhn. *Cultivos Tropicales* **2002**, *23*, 73–75.
31. Rabea, E.I.; El Badawy, M.T.; Stevens, C.V.; Smagghe, G.; Steurbaut, W. Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules* **2003**, *4*, 1457–1465.
32. Laflamme, P.; Benhamou, N.; Bussiéres, G.; Dessureault, M. Differential effect of chitosan on root rot fungal pathogens in forest nurseries. *Can. J. Bot.* **1999**, *77*, 1460–1468.
33. Bell, A.A.; Hubbard, J.C.; Liu, L.; Davis, R.M.; Subbarao, K.V. Effects of chitin and chitosan on the incidence and severity of *Fusarium* yellows in celery. *Plant Dis.* **1998**, *82*, 322–328.
34. Aziz, A.; Trotel-Aziz, P.; Dhuicq, L.; Jeandet, P.; Couderchet, M. Vernet, G. Chitosan oligomers and copper sulphate induce grapevine defense reaction and resistance to grey mould and down mildew. *Phytopathology* **2006**, *96*, 1188–1194.
35. Ben-shalom, N.; Ardi, R.; Pinto, R.; Aki, C.; Fallik, E. Controlling gray mould caused by *Botyrtis cinerea* in cucumber plants by means of chitosan. *Crop Prot.* **2003**, *22*, 285–290.
36. O'Herlihy, E.A.; Duffy, E.M.; Cassells, A.C. The effects of arbuscular mycorrhizal fungi band chitosan sprays on yield and late blight resistance in potato crops from microplants. *Folio Geobotanica* **2003**, *38*, 201–208.
37. Muzzarelli, R.A.A.; Tarsi, R.; Filippini, O.; Giovanetti, E.; Biagini, G.; Varaldo, P.E. Antimicrobial properties of *N*-carboxybutyl chitosan. *Antimicrob. Agents Chemother.* **1990**, *34*, 2019–2023.
38. Jia, Z.; Shen, D.; Xu, W. Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. *Carbohydr. Res.* **2001**, *333*, 1–6.

39. Ferrante, P.; Scortichini, M. Molecular and phenotypic features of *Pseudomonas syringae* pv. actinidiae isolated during recent epidemics of bacterial canker on yellow kiwifruit (*Actinidia chinensis*) in central Italy. *Plant Pathol.* **2010**, *59*, 954–962.
40. Vruggink, H. The effect of chitin amendment on actinomycetes in soil and on the infection of potato tubers by *Streptomyces scabies*. *Neth. J. Plant Pathol.* **1970**, *76*, 293–295.
41. Kulikov, S.N.; Chirkov, S.N.; Il'ina, A.V.; Lopatin, S.A.; Varlamov, V.P. Effect of the molecular weight of chitosan on its antiviral activity in plants. *Prikl Biokhim Mikrobiol.* **2006**, *42*, 224–228.
42. Pospieszny, H.; Chirkov, S.; Atabekov, J. Induction of antiviral resistance in plants by chitosan. *Plant Sci.* **1991**, *79*, 63–68.
43. Faoro, F.; Sant, S.; Iriti, M.; Appiano, A. Chitosan-elicited resistance to plant viruses: A histochemical and cytochemical study. In *Chitin Enzymology*; Muzzarelli, R.A.A., Ed.; Atec: Grottammare, Italy, 2001; pp. 57–62.
44. Chirkov, S.N. The antiviral activity of chitosan (review). *Appl. Biochem. Microbiol.* **2002**, *38*, 1–8.
45. Rabea, E.I.; El Badawy, M.T.; Rogge, T.M.; Stevens, C.V.; Höfte, M.; Steurbaut, W.; Smagghe, G. Insecticidal and fungicidal activity of new synthesized chitosan derivatives. *Pest Manage. Sci.* **2005**, *61*, 951–960.
46. Badawy, M.E.I.; El-Aswad, A.F. Insecticidal activity of chitosans of different molecular weights and chitosan-metal complexes against cotton leafworm *Spodoptera littoralis* and oleander aphid *Aphis nerii*. *Plant Prot. Sci.* **2012**, *48*, 131–141.
47. Gibbs, K.E.; Morrison, F.O. The cuticle of the two-spotted spider mite, *Tetranychus telarius* (Linnaeus) (Acarina: Tetranychidae). *Can. J. Zool.* **1959**, *37*, 633–637.
48. Mothes-Wagner, U. Comparative histopathology of the chitin synthesis inhibitors nikkomycin X/Z, nikkomycin Z and polyoxin D. I: Effects on moulting, reproduction and digestion in the spider mite *Tetranychus urticae*. *Pest Manage. Sci.* **1986**, *17*, 607–620.
49. Tan, X.; Wang, S.; Li, X.; Zhang, F. Optimizing and application of microencapsulated artificial diet for *Orius sauteri* (Hemiptera: Anthocoridae). *Acta Entomol. Sin.* **2010**, *53*, 891–900.
50. Godoy, G.; Rodriguez-Kabana, R.; Shelby, R.A.; Morgan-Jones, G. Chitin amendments for control of *Meloidogyne arenaria* in infested soil. II. Effects on microbial population. *Nematropica* **1983**, *13*, 63–74.
51. Mian, I.H.; Godoy, G.; Shelby, R.A.; Rodriguez-Kabana, R.; Morgan-Jones, G. Chitin amendments for control of *Meloidogyne arenaria* in infested soil. *Nematropica* **1982**, *12*, 71–84.
52. Rodriguez-Kabana, R.; Morgan-Jones, G.; Ownley-Gintis, B. Effects of chitin amendments to soil on *Heterodera glycines*, microbial populations, and colonization of cysts by fungi. *Nematropica* **1984**, *14*, 10–25.
53. EPA. Final Registration Review Work Plan for Chitin and Chitosan 2008. Available online: http://www.epa.gov/oppsrrd1/registration_review/chitin/ (accessed on 4 July 2013).
54. Westerdahl, B.B.; Carlson, H.L.; Grant, J.; Radewald, J.D.; Welch, N.; Anderson, C.A.; Darso, J.; Kirby, D.; Shibuya, F. Management of plant-parasitic nematodes with a chitin-urea soil amendment and other materials. *J. Nematol.* **1992**, *24*, 669–680.

55. Belair, G.; Tremblay, N. The influence of chitin-urea amendments applied to an organic soil on a *Meloidogyne hapla* population and on the growth of greenhouse tomato. *Phytoprotection* **1995**, *76*, 75–80.
56. Rodriguez-Kabana, R.; Morgan-Jones, G.; Chet, I. Biological control of nematodes: Soil amendments and microbial antagonists. *Plant Soil* **1987**, *100*, 237–247.
57. Duncan, L.W. Current options for nematode management. *Annu. Rev. Phytopathol.* **1991**, *29*, 469–490.
58. Stirling, G.R. *Biological Control of Plant-Parasitic Nematodes*; CAB International: Wallingford, UK, 1991.
59. Hamid, R.; Khan, M.A.; Ahmad, M.; Ahmad, M.M.; Abdin, M.Z.; Musarrat, J.; Javed, S. Chitinases: An update. *J. Pharm. BioAllied Sci.* **2013**, *5*, 21–29.
60. Ayes, M.D.; Howard, S.C.; Kuzio, J.; Lopez-Ferber, M.; Possee, R.D. The complete DNA sequence of Autographa californica nuclear polyhedrosis virus. *Virology* **1994**, *202*, 586–605.
61. Maksimov, I.V.; Abizgil'dina, R.R.; Pusenkova, L.I. Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens (Review). *Appl. Biochem. Microbiol.* **2011**, *47*, 333–345.
62. Chandler, D.; Bailey, A.S.; Tatchell, G.M.; Davidson, G.; Greaves, J.; Grant, W.P. The development, regulation and use of biopesticides for integrated pest management. *Philos. Trans. R. Soc. B.* **2011**, *366*, 1987–1998.
63. Chen, F.; Wang, M.; Zheng, Y.; Luo, J.; Yang, X.; Wang, X. Quantitative changes of plant defense enzymes and phytohormone in biocontrol of cucumber *Fusarium* wilt by *Bacillus subtilis* B579. *World J. Microbiol. Biotechnol.* **2010**, *26*, 675–684.
64. Manjula, K.; Podile, A.R. Chitin-supplemented formulations improve biocontrol and plant growth promoting efficiency of *Bacillus subtilis* AF1. *Can. J. Microbiol.* **2001**, *47*, 618–625.
65. Lowe, A.; Rafferty-McArdle, S.M.; Cassells, A.C. Effects of AMF- and PGPR-root inoculation and a foliar chitosan spray in single and combined treatments on powdery mildew disease in strawberry. *Agric. Food Sci.* **2012**, *21*, 28–38.
66. Toyoda, H.; Matsuda, Y.; Fukamizo, T.; Nonomura, T.; Kukutani, K.; Ouchi, S. Application of chitin and chitosan degrading microbes to comprehensive biocontrol of fungal wilt pathogen, *Fusarium oxysporum*. In *Chitin Handbook*; Muzzarelli, R.A.A., Peter, M.G., Eds.; European Chitin Society, Atec: Grottammare, Italy, 1996; pp. 359–370.
67. Singh, P.P.; Shin, Y.C.; Park, C.S.; Chung, Y.R. Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology* **1999**, *89*, 92–99.
68. Kishore, G.K.; Pande, S.; Podile, A.R. Chitin-supplemented foliar application of *Serratia marcescens* GPS 5 improves control of late leaf spot disease of groundnut by activating defense-related enzymes. *J. Phytopathol.* **2005**, *153*, 169–173.
69. Gooday, G.W. Physiology of microbial degradation of chitin and chitosan. *Biodegradation* **1990**, *1*, 177–190.
70. Sivan, A.; Chet, I. Degradation of fungal cell walls by lytic enzymes of *Trichoderma haryianam*. *J. Gen. Microbiol.* **1989**, *135*, 675–682.

71. Lorito, M.; Harman, G.E.; Hayes, C.K.; Broadway, R.M.; Tronsmo, A.; Woo, S.L.; Di-Pietro, A. Chitinolytic enzymes produced by *Trichoderma harzianum:* Antifungal activity of purified endochitinase and chitobiosidase. *Phytopathology* **1993**, *83*, 302–307.
72. Sid Ahmed, A.; Ezziyyani, M.; Pérez Sánchez, C.; Candela, M.E. Effect of chitin on biological control activity of *Bacillus* spp. and *Trichoderma harzianum* against root rot disease in pepper (*Capsicum annuum*) plants. *Eur. J. Plant Pathol.* **2003**, *109*, 633–637.
73. López-Cervantes, J.; Rochin, K.R.F. Microbial Process and Composition for Agricultural Use. US 2012/0084886 A1, 5 April 2012.
74. John, R.P.; Tyagi, R.D.; Brar, S.K.; Surampalli, R.Y.; Prévost, D. Bio-encapsulation of microbial cells for targeted agricultural delivery. *Crit. Rev. Biotechnol.* **2011**, *31*, 211–226.
75. El-Sayed, G.N.; Coudron, T.A.; Ignoffo, C.M. Chitinolytic activity and virulence associated with native and mutant isolates of the entomopathogenic fungus, *Nomumea ileyi*. *J. Invertebr. Pathol.* **1989**, *54*, 394–403.
76. St Leger, R.J.; Cooper, R.M.; Charnley, A.K. Characterization of chitinase and chitobiase produced by the entomopathogenic fungus *Metarhizium anisopliae*. *J. Invertebr. Pathol.* **1991**, *58*, 415–426.
77. Gill, S.S.; Cowles, E.A.; Pietrantonio, P.V. The mode of action of *Bacillus thuringiensis* endotoxins. *Annu. Rev. Entomol.* **1992**, *37*, 615–636.
78. Arora, N.; Sachdev, B.; Gupta, R.; Vimala, Y.; Bhatnagar, R.K. Characterization of a chitin-binding protein from *Bacillus thuringiensis* HD-1. *PLoS One* **2013**, *8*, e66603, doi:10.1371/journal.pone.0066603.
79. Ortiz-Rodríguez, T.; De La Fuente-Salcido, N.; Bideshi, D.K.; Salcedo-Hernández, R.; Barboza-Corona, J.E. Generation of chitin-derived oligosaccharides toxic to pathogenic bacteria using ChiA74, an endochitinase native to *Bacillus thuringiensis*. *Lett. Appl. Microbiol.* **2010**, *51*, 184–190.
80. Smirnoff, W.A. Three year of aerial field experiments with *Bacillus thuringiensis* plus chitinase formulation against the spruce bud worm. *J. Invertebr. Pathol.* **1974**, *24*, 344–348.
81. Gao, Y.; Oppert, B.; Lord, J.C.; Liu, C.; Lei, Z. *Bacillus thuringiensis* Cry3Aa toxin increases the susceptibility of *Crioceris quatuordecimpunctata* to *Beauveria bassiana* infection. *J. Invertebr. Pathol.* **2012**, *109*, 260–263.
82. Wraight, S.P.; Ramos, M.E. Synergistic interaction between *Beauveria bassiana*- and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae. *J. Invertebr. Pathol.* **2005**, *90*, 139–150.
83. Lo Scrudato, M.; Blokesch, M. A transcriptional regulator linking quorum sensing and chitin induction to render *Vibrio cholerae* naturally transformable. *Nucleic Acids Res.* **2013**, *41*, 3644–3658.
84. Bhattacharyya, P.N.; Jha, D.K. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1327–1350.
85. Rudrappa, T.; Biedrzycki, M.L.; Bais, H.P. Causes and consequences of plant-associated biofilms. *FEMS Microbiol. Ecol.* **2008**, *64*, 153–166.

86. Thomas, C.J.; Brown, H.L.; Hawes, C.R.; Lee, B.Y.; Min, M.K.; King, L.A.; Possee, R.D. Localization of a baculovirus-induced chitinase in the insect cell endoplasmic reticulum. *J. Virol.* **1998**, *72*, 10207–10212.
87. Gopalakrishnan, B.; Muthukrishnan, S.; Kramer, K.J. Baculovirus-mediated expression of a *Manduca sexta* chitinase gene: Properties of the recombinant protein. *Insect Biochem. Mol. Biol.* **1995**, *25*, 255–265.
88. Moscardi, F.; de Souza, M.L.; de Castro, M.E.B.; Moscardi, M.L.; Szewczyk, B. Baculovirus pesticides: Present state and future perspectives. In *Microbes and Microbial Technology*; Springer: New York, NY, USA, 2011; pp. 415–445.
89. Staehelin, C.; Schultze, M.; Tokuyasu, K.; Poinsot, V.; Promé, J.C.; Kondorosi, E.; Kondorosi, A. *N*-deacetylation of *Sinorhizobium meliloti* Nod factors increases their stability in the *Medicago sativa* rhizosphere and decreases their biological activity. *Mol. Plant-Microbe Interact.* **2000**, *13*, 72–79.
90. Berry, A.M.; McIntyre, L.; McCully, M.E. Fine structure of root hair infection leading to nodulation in the *Frankia-Alnus* symbiosis. *Can. J. Bot.* **1986**, *64*, 292–305.
91. Normand, P.; Lapierre, P.; Tisa, L.S.; Gogarten, J.P.; Alloisio, N.; Bagnarol, E.; Bassi, C.A.; Berry, A.M.; Bickhart, D.M.; Choisne, N. Genome characteristics of facultatively symbiotic *Frankia* sp. strains reflect host range and host plant biogeography. *Genome Res.* **2007**, *17*, 7–15.
92. Cérémonie, H.; Debelle, F.; Fernandez, M.P. Structural and functional comparison of *Frankia* root hair deforming factor and rhizobia Nod factor. *Can. J. Bot.* **1999**, *77*, 1293–1301.
93. Fitter, A.H.; Moyersoen, B. Evolutionary trends in root-microbe symbioses. *Philosophical Philos. Trans. R. Soc. B.* **1996**, *351*, 1367–1375.
94. Gryndler, M.; Jansa, J.; Hršelová, H.; Chvátalová, I.; Vosátka, M. Chitin stimulates development and sporulation of arbuscular mycorrhizal fungi. *Appl. Soil Ecol.* **2003**, *22*, 283–287.
95. Abdel-Fattah, G.M.; Mohamedin, A.H. Interactions between a vesicular-arbuscular mycorrhizal fungus (*Glomus intraradices*) and *Streptomyces coelicolor* and their effects on sorghum plants grown in soil amended with chitin of brawn scales. *Biol. Fertil. Soils* **2000**, *32*, 401–409.
96. El-Sayed, E.S.A.; El-Didamony, G.; El-Sayed, E.F. Effects of mycorrhizae and chitin-hydrolysing microbes on *Vicia faba*. *World J. Microbiol. Biotechnol.* **2002**, *18*, 505–515.
97. Maillet, F.; Poinsot, V.; André, O.; Puech-Pagès, V.; Haouy, A.; Gueunier, M.; Cromer, L.; Giraudet, D.; Formey, D.; Niebel, A. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhizae. *Nature* **2011**, *469*, 58–63.
98. Oláh, B.; Brière, C.; Bécard, G.; Dénarié, J.; Gough, C. Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. *Plant J.* **2005**, *44*, 195–207.
99. Schultze, M.; Kondorosi, A. The role of lipochitooligosaccharides in root nodule organogenesis and plant cell growth. *Curr. Opin. Genet. Dev.* **1996**, *6*, 631–638.
100. Salzer, P.; Bonanomi, A.; Beyer, K.; Vögeli-Lange, R.; Aeschbacher, R.A.; Lange, J.; Wiemken, A.; Kim, D.; Cook, D.R.; Boller, T. Differential expression of eight chitinase genes in *Medicago truncatula* roots during mycorrhiza formation, nodulation, and pathogen infection. *Mol. Plant-Microbe Interact.* **2000**, *13*, 763–777.

101. Garcia-Brugger, A.; Lamotte, O.; Vandelle, E.; Bourque, S.; Lecourieux, D.; Poinssot, B.; Wendehenne, D.; Pugin, A. Early signaling events induced by elicitors of plant defenses. *Mol. Plant-Microbe Interact.* **2006**, *19*, 711–724.
102. Kaku, H.; Nishizawa, Y.; Minami, N.I.; Tomiyama, C.A.; Dohmae, N.; Takio, K.; Manami, E.; Shibuya, N. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11086–11091.
103. Day, R.B.; Okada, M.; Ito, Y.; Tsukada, K.; Zaghouani, H.; Shibuya, N.; Stacey, G. Binding site for chitin oligosaccharides in the soybean plasma membrane. *Plant Physiol.* **2001**, *26*, 1162–1173.
104. Miya, A.; Albert, P.; Shinya, T.; Desaki, Y.; Ichimura, K.; Shirasu, K.; Narusaka, Y.; Kawakami, N.; Kaku, H.; Shibuya, N. CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 19613–19618.
105. Shinya, T.; Motoyama, N.; Ikeda, A.; Wada, M.; Kamiya, K.; Hayafune, M.; Kaku, H.; Shibuya, N. Functional characterization of CEBiP and CERK1 homologs in arabidopsis and rice reveals the presence of different chitin receptor systems in plants. *Plant Cell Physiol.* **2012**, *53*, 1696–1706.
106. Nojiri, H.; Sugimori, M.; Yamane, H.; Nishimura, Y.; Yamada, A.; Shibuya, N.; Kodama, O.; Murofushi, N.; Ohmori, T. Involvement of jasmonic acid in elicitor-induced phytoalexin production in suspension-cultured rice cells. *Plant Physiol.* **1996**, *110*, 387–392.
107. Ott, P.G.; Varga, G.J.; Szatmári, A.; Bozsó, Z.; Klement, E.; Medzihradszky, K.F.; Besenyei, E.; Czelleng, A.; Klement, Z. Novel extracellular chitinases rapidly and specifically induced by general bacterial elicitors and suppressed by virulent bacteria as a marker of early basal resistance in tobacco. *Mol. Plant-Microbe Interact.* **2006**, *19*, 161–172.
108. Mentlak, T.A.; Kombrink, A.; Shinya, T.; Ryder, L.S.; Otomo, I.; Saitoh, H.; Terauchi, R.; Nishizawa, Y.; Shibuya, N.; Thomma, B.P.H.J.; Talbot, N.J. Effector-mediated suppression of chitin-triggered immunity by *Magnaporthe oryzae* is necessary for rice blast disease. *The Plant Cell* **2012**, *24*, 322–335.
109. Fujikawa, T.; Sakaguchim, A.; Nishizawa, Y.; Kouzai, Y.; Minami, E.; Yano, S.; Koga, H.; Meshi, T.; Nishimura, M. Surface α-1,3-glucan facilitates fungal stealth infection by interfering with innate immunity in plants. *PLoS Pathog.* **2012**, *8*, e1002882; doi:10.1371/journal.-ppat.1002882.
110. Minami, E.; Kuchitsu, K.; He, D.Y.; Kouchi, H.; Midoh, N.; Ohtsuki, Y.; Shibuya, N. Two novel genes rapidly and transiently activated in suspension-cultured rice cells by treatment with *N*-acetylchitoheptaose, a biotic elicitor for phytoalexin production. *Plant Cell Physiol.* **1996**, *37*, 563–567.
111. Nishizawa, Y.; Kawakami, A.; Hibi, T.; He, D.Y.; Shibuya, N.; Minami, E. Regulation of the chitinase gene expression in suspension-cultured rice cells by *N*-acetylchitooligosaccharides: differences in the signal transduction pathways leading to the activation of elicitor-responsive genes. *Plant Mol. Biol.* **1999**, *39*, 907–914.
112. Takai, R.; Hasegawa, K.; Kaku, K.; Shibuya, N.; Minami, E. Isolation and analysis of expression mechanisms of a rice gene, EL5, which shows structural similarity to ATL family from *Arabidopsis*, in response to *N*-acetylchitooligosaccharide elicitor. *Plant Sci.* **2001**, *160*, 577–583.

113. Rakwal, R.; Tamogami, S.; Agrawal, G.K.; Iwahashi, H. Octadecanoid signaling component "burst" in rice (*Oryza sativa* L.) seedling leaves upon wounding by cut and treatment with fungal elicitor chitosan. *Biochem. Biophys. Res. Commun.* **2002**, *295*, 1041–1045.
114. Doares, S.H.; Syrovets, T.; Weiler, E.W.; Ryan, C.A. Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 4095–4098.
115. Bohland, C.; Balkenhohl, T.; Loers, G.; Feussner, I.; Grambow, H.J. Differential induction of lipoxygenase isoforms in wheat upon treatment with rust fungus elicitor, chitin oligosaccharides, chitosan and methyl jasmonate. *Plant Physiol.* **1997**, *114*, 679–685.
116. Linden, J.C.; Phisalaphong, M. Oligosaccharides potentiate methyl jasmonate-induced production of paclitaxel in *Taxus Canadensis*. *Plant Sci.* **2000**, *158*, 41–51.
117. Walker-Simmons, M.; Jin, D.; West, C.A.; Hadwiger, L.; Ryan, C.A. Comparison of proteinase inhibitor-inducing activities and phytoalexin elicitor activities of a pure fungal endopolygalacturonase, pectic fragments and chitosans. *Plant Physiol.* **1984**, *76*, 833–836.
118. Farmer, E.E.; Ryan, C.A. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. *Plant Cell* **1992**, *4*, 129–134.
119. Iriti, M.; Faoro, F. Abscisic acid is involved in chitosan-induced resistance to tobacco necrosis virus (TNV). *Plant Physiol. Biochem.* **2008**, *46*, 1106–1111.
120. Bittelli, M.; Flury, M.; Campbell, G.S.; Nichols, E.J. Reduction of transpiration through foliar application of chitosan. *Agr. Forest. Meteorol.* **2001**, *107*, 167–175.
121. Jones, J.D.G.; Dangl, J.L. The plant immune system. *Nature* **2006**, *444*, 323–329.
122. Vasyukova, N.I.; Zinoveva, L.I.; Iĺinskaya, E.A.; Perekhod, G.I.; Chalenko, N.G.; Iĺina, A.V.; Varlamov, V.P.; Ozeretskovskaya, O.L. Modulation of plant resistance to diseases by water-soluble chitosan. *Appl. Biochem. Microbiol.* **2001**, *37*, 103–109.
123. Kuchitsu, K.; Kikuyama, M.; Shibuya, N. *N*-Acetylchitooligosaccharides, biotic elicitor for phytoalexin production, induce transient membrane depolarization in suspension-cultured rice cells. *Protoplasma* **1993**, *174*, 79–81.
124. El Hassni, M.; El Hadrami, A.; Daayf, F.; Chérif, M.; Ait Barka, E.; El Hadrami, I. Chitosan, antifungal product against *Fusarium oxysporum* f. sp. *albedinis* and elicitor of defense reactions in date palm roots. *Phytopathol. Mediterr.* **2004**, *43*, 195–204.
125. Kuchitsu, K.; Kosaka, H.; Shiga, T.; Shibuya, N. EPR evidence for generation of hydroxyl radical triggered by *N*-acetylchitooligosaccharide elicitor and a protein phosphatase inhibitor in suspension-cultured rice cells. *Protoplasma* **1995**, *188*, 138–142.
126. Amborabé, B.-E.; Bonmort, J.; Fleurat-Lessard, P.; Roblin, G. Early events induced by chitosan on plant cells. *J. Exp. Bot.* **2008**, *59*, 2317–2324.
127. Köhle, H.; Jeblick, W.; Poten, F.; Blaschek, W.; Kauss, H. Chitosan-elicited callose synthesis in soybean cells as a Ca_2^+-dependent process. *Plant Physiol.* **1985**, *77*, 544–551.
128. Pearce, R.B.; Ride. J.P. Chitin and related compounds as elicitors of the lignification response in wounded wheat leaves. *Physiol. Plant Pathol.* **1982**, *20*, 119–123.
129. Lafontaine, J.P.; Benhamou, N. Chitosan treatment: An emerging strategy for enhancing resistance of greenhouse tomato plants to infection by *Fusarium oxysporum* f. sp. *radicislycopersici*. *Biocontrol Sci. Tech.* **1996**, *6*, 111–124.

130. Shigo, A.L. Compartmentalization: a conceptual framework for understanding how trees grow and defend themselves. *Annu. Rev. Phytopathol.* **1984**, *22*, 189–214.
131. Vasil'ev, L.A.; Dzyubinskaya, E.V.; Zinovkin, R.A.; Kiselevsky, D.B.; Lobysheva, N.V.; Samuilov, V.D. Chitosan-induced programmed cell death in plants. *Biochemistry (Moscow Russ. Fed.)* **2009**, *74*, 1035–1043.
132. Lizama-Uc, G.; Estrada-Mota, I.A.; Caamal-Chan, M.G.; Souza-Perera, R.; Oropeza-Salín, C.; Islas-Flores, I.; Zúñiga-Aguilar, J.J. Chitosan activates a MAP-kinase pathway and modifies abundance of defense-related transcripts in calli of *Cocos nucifera* L. *Physiol. Mol. Plant Pathol.* **2007**, *70*, 130–141.
133. Schlumbaum, A.; Mauch, F.; Vogeli, U.; Boller, T. Plant chitinases are potent inhibitors of fungal growth. *Nature* **1986**, *324*, 365–367.
134. Herrera-Estrella, A.; Chet, I. Chitinases in biological control. *Experientia. Suppl.* **1999**, *87*, 171–184.
135. Kramer, K.J.; Muthukrishnan, S.; Lowell, J.; White, F. Chitinases for insect control. In *Advances in Insect Control: The Role of Transgenic Plants*; Carozzi, N., Koziel, M., Eds.; Taylor and Francis: Bristol, UK, 1997; pp. 185–193.
136. Huang, J.K.; Wen, L.; Swegle, M.; Tran, H.C.; Thin, T.H.; Naylor, H.M.; Muthukrishnan, S.; Reeck, G.R. Nucleotide sequence of a rice genomic clone that encodes a class I endochitinase. *Plant Mol. Biol.* **1991**, *16*, 479–480.
137. Ding, X.; Gopalakrishnan, B.; Johnson, L.B.; White, F.F.; Wang, X.; Morgan, T.D.; Kramer, K.J.; Muthukrishnan, S. Insect resistance of transgenic tobacco expressing an insect chitinase gene. *Transgenic Res.* **1998**, *7*, 77–84.
138. Someya, N.; Akutsu, K. Biocontrol of plant diseases by genetically modified microorganisms: Current status and future prospects. In *PGPR: Biocontrol and Biofertilization*; Siddiqui, Z.A., Ed.; Springer: Dordrecht, the Netherlands, 2006; pp. 297–312.
139. Sitrit, Y.; Barak, Z.; Kapulnik, Y.; Oppenheim, A.B.; Chet, I. Expression of *Serratia marcescens* chitinase gene in *Rhizobium meliloti* during symbiosis on alfalfa roots. *Mol. Plant-Microbe Interact.* **1993**, *6*, 293–298.
140. Downing, K.J.; Thomson, J.A. Introduction of the *Serratia marcescens* chiA gene into an endophytic *Pseudomonas fluorescens* for the biocontrol of phytopathogenic fungi. *Can. J. Microbiol*. **2000**, *46*, 363–369.
141. Lopez, O.; Fernandez-Bolanos, J.G.; Gil, M.V. New trends in pest control: the search for greener insecticides. *Green Chem.* **2005**, *7*, 431–442.
142. Abou-Elghar, G.E.; Fujiyoshi, P.; Matsumura, F. Significance of the sulfonylurea receptor (SUR) as the target of diflubenzuron in chitin synthesis inhibition in *Drosophila melanogaster* and *Blattella germanica. Insect Biochem. Mol. Biol.* **2004**, *34*, 743–752.
143. EPA, Green Chemistry, Designing Safer Chemicals Award 2000. Available online: http://www.epa.gov/gcc/dsca00.html (accessed on 4 July 2013).
144. Bayoumi, A.E.; Pérez-Pertejo, Y.; Zidan, H.Z.; Balaña-Fouce, R.; Ordóñez, C.; Ordóñez, D. Cytotoxic effects of two antimolting insecticides in mammalian CHO-K1 cells. *Ecotoxicol. Environ. Saf.* **2003**, *55*, 19–23.

145. Peppuy, A.; Robert, A.; Delbecque, J.P.; Leca, J.L.; Rouland, C.; Bordereau, C. Efficacy of hexaflumuron against the fungus-growing termite *Pseudacanthotermes spiniger* (Sjöstedt) (isopteran, macrotermitinae). *Pest Manage. Sci.* **1998**, *54*, 22–26.
146. Liu, T.X.; Stansly, P.A. Lethal and sublethal effects of two insect growth regulators on adult *Delphastus catalinae* (Coleoptera Coccinellidae) predator whiteflies (Homoptera Aleyrodidae) *Biol. Control* **2004**, *30*, 298.
147. Berecibar, A.; Granjean, C.; Siriwardena, A. Synthesis and biological activity of natural aminocyclopentitol glycosidase inhibitors: Mannostatins, trehazolin, allosamidins, and their analogues. *Chem. Rev.* **1999**, *99*, 779–844.
148. Soderlund, D.M.; Clark, J.M.; Sheets, L.P.; Mullin, L.S.; Piccirillo, V.J.; Sargent, D.; Stevens, J.T.; Weiner, M.L. Mechanisms of pyrethroid neurotoxicity: Implications for cumulative risk assessment. *Toxicology* **2002**, *171*, 3–59.
149. Cohen, E. Chitin synthesis and inhibition: a revisit. *Pest Manage. Sci.* **2001**, *57*, 946–950.
150. Tang, B.; Wei, P.; Chen, J.; Wang, S.G.; Zhang, W.Q. Progress in gene features and functions of insect trehalases. *Acta Entomol. Sin.* **2012**, *55*, 1315–1321.
151. Qian, X.; Liu, Z.; Li, Z.; Song, G. Synthesis and quantitative structure-activity relationships of fluorine-containing 4,4-dihydroxylmethyl-2-aryliminooxazo (thiazo) lidines as trehalase inhibitors. *J. Agric. Food Chem.* **2001**, *49*, 5279–5284.
152. Crimmins, M.T.; Tabet, E.A. Formal total synthesis of (+)-trehazolin. Application of an asymmetric Aldol-Olefin metathesis approach to the synthesis of functionalized cyclopentenes. *J. Org. Chem.* **2001**, *66*, 4012–4018.
153. Ando, O.; Satake, H.; Itoi, K.; Sato, A.; Nakajima, M.; Takahashi, S.; Haruyama, H.; Ohkuma, J.; Kinoshita, I.; Enokita, R. Trehazolin, a new trehalase inhibitor. *J. Antibiot.* **1991**, *4*, 1165–1168.
154. Hirsch, A.M.; Valdés, M. Micromonospora: An important microbe for biomedicine and potentially for biocontrol and biofuels. *Soil Biol. Biochem.* **2010**, *42*, 536–542.
155. Gaughran, J.P.; Lai, M.H.; Kirsch, D.R.; Silverman, S.J. 1994. Nikkomycin Z is a specific inhibitor of Saccharomyces cerevisiae chitin synthase isozyme Chs3 *in vitro* and *in vivo*. *J. Bacteriol.* **1994**, *176*, 5857–5860.
156. Li, R.K.; Rinaldi, M.G. *In vitro* antifungal activity of nikkomycin Z in combination with fluconazole or itraconazole. *Antimicrob. Agents Chemother.* **1999**, *43*, 1401–1405.
157. EPA. Consideration of Eligibility for Registration of the New Pesticide Active Ingredient Polyoxin D Zinc Salt-DECISION MEMORANDUM 2003. Available online: http://www.epa.gov/pesticides/chem_search/reg_actions/registration/related_PC-230000_1-Jul-03.pdf (accessed on 4 July 2013).
158. Bixby-Brosi, A.J.; Potter, D.A. Can a chitin-synthesis-inhibiting turfgrass fungicide enhance black cutworm susceptibility to a baculovirus? *Pest Manage. Sci.* **2012**, *68*, 324–329.
159. Tsugita, T.; Takahashi, K.; Muraoka, T.; Fukui, H. The application of chitin/chitosan for agriculture (in Japanese). In *Proceedings of Special Session of the 7th Symposium on Chitin and Chitosan*; Japanese Society for Chitin and Chitosan: Fukui, Japan, 1993; pp. 21–22.

160. Lee, Y.S.; Kim, Y.H.; Kim, S.B. Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights. *HortScience* **2005**, *40*, 1333–1335.
161. Kim, H.J.; Chen, F.; Wang, X.; Rajapakse, N.C. Effect of chitosan on the biological properties of sweet basil (*Ocimum basilicum* L.). *J. Agric. Food Chem.* **2005**, *53*, 3696–3701.
162. Ait Barka, E.; Eullaffroy, P.; Clément, C.; Vernet, G. Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. *Plant Cell Rep.* **2004**, *22*, 608–614.
163. Wanichpongpan, P; Suriyachan, K; Chandrkrachang, S. Effects of Chitosan on the growth of Gerbera flower plant (Gerbera jamesonii). In *Chitin and Chitosan in Life Science*, Proceedings of the Eighth International Chitin and Chitosan Conference and Fourth Asia Pacific Chitin and Chitosan Symposium, Yamaguchi, Japan, 21–23 September 2000; Uragami, T., Kurita, K., Fukamizo, T., Eds.; pp.198–201.
164. Chandrkrachang, S. The applications of chitin in agriculture in Thailand. *Adv. Chitin Sci.* **2002**, *5*, 458–462.
165. Pornpeanpakdee, P.; Pichyangkura, R.; Chadchawan, S.; Limpanavech, P. Chitosan effects on Dendrobium 'Eiskul' Protocorm-like body production. In Proceedings of the 31st Congress on Science and Technology of Thailand, Nakornrachaseema, Thailand, 18–20 October 2005; pp. 1–3.
166. Nahar, S.J.; Kazuhiko, S.; Haque, S.M. Effect of Polysaccharides Including Elicitors on Organogenesis in Protocorm-like Body (PLB) of *Cymbidium insigne in vitro*. *J. Agric. Sci. Technol.* **2012**, *2*, 1029–1033.
167. Chibu, H.; Shibayama, H.; Arima, S.; Effects of chitosan application on the shoot growth of rice and soybean. *Jpn. J. Crop Sci.* **2002**, *71*, 206–211.
168. Khan, W.; Prithiviraj, B.; Smith, D.L. Effect of foliar application of chitin and chitosan oligosaccharides on photosynthesis of maize and soybean. *Photosynth. Res.* **2002**, *40*, 621–624.
169. Sauerwein, M.; Flores, H.M.; Yamazaki, T.; Shimomura, K. *Lippia dulcis* shoot cultures as a source of the sweet sesquiterpene hernandulcin. *Plant Cell Rep.* **1991**, *9*, 663–666.
170. Herde, O.; Peña-cortés, H.; Willmitzer, L.; Fisahn, J. Stomatal responses to jasmonic acid, linolenic acid and abscisic acid in wild-type and ABA-deficient tomato plants. *Plant Cell Environ.* **1997**, *20*, 136–141.
171. Issak, M.; Okuma, E.; Munemasa, S.; Nakamura, Y.; Mori, I.C.; Murata, Y. Neither endogenous abscisic acid nor endogenous jasmonate is involved in salicylic acid-, yeast elicitor-, or chitosan-induced stomatal closure in *Arabidopsis thaliana*. *Biosci. Biotechnol. Biochem.* **2013**, *77*, 1111–1113.
172. Limpanavech, P.; Pichyangkura, R.; Khunwasi, C.; Chadchawan, S.; Lotrakul, P.; Bunjongrat, P.; Chaidee, A.; Akaraeakpanya, T. The effects of polymer type, concentration and %DD of bicatalyte modigied chitosan on flora production of *Dendrobium* 'Eiskul'. In Proceedings of the National chitin-chitosan conference, Chulalongkorn University, Bangkok, Thailand, 17–18 July 2003; pp. 60–64.
173. Utsunomiya, N.; Kinai, H. Effect of chitosan-oligosaccharides soil conditioner on the growth of passionfruit. *J. Jpn. Soc. Hortic. Sci.* **1994**, *64*, 176–177.

174. Ohta, K.; Tanguchi, A.; Konishi, N.; Hosoki, T. Chitosan treatment affects plant growth and flower quality in *Eustoma grandiflorum*. *HortScience* **1999**, *34*, 233–234.
175. Uddin, A.F.M.J.; Hashimoto, F.; Shimiza, K.; Sakata, Y. Monosaccharides and chitosan sensing in bud growth and petal pigmentation in *Eustoma grandiflorum* (Raf.) Shinn. *Sci. Hortic.* **2004**, *100*, 127–138.
176. Grover, A. Plant chitinases: genetic diversity and physiological roles. *Crit. Rev. Plant Sci.* **2012**, *31*, 57–73.
177. Guan, Y.J.; Hu, J.; Wang, X.J.; Shao, C.X. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J. Zhejiang Univ. Sci. B*. **2009**, *10*, 427–433.
178. Bhaskara Reddy, M.V.; Arul, J.; Angers, P.; Couture, L. Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality. *J. Agric. Food Chem.* **1999**, *47*, 1208–1216.
179. Manjula, K.; Podile, A.R. Increase in seedling emergence and dry weight of pigeon pea in the field with chitin-supplemented formulations of *Bacillus subtilis* AF 1. *World J. Microbiol. Biotechnol.* **2005**, *21*, 1057–1062.
180. Malfanova, N.; Kamilova, F.; Validov, S.; Shcherbakov, A.; Chebotar, V.; Tikhonovich, I.; Lugtenberg, B. Characterization of *Bacillus subtilis* HC8, a novel plant-beneficial endophytic strain from giant hogweed. *Microb. Biotechnol.* **2011**, *4*, 523–532.
181. Yen, M.T.; Mau, J.L. Selected physical properties of chitin prepared from shiitake stipes. *Food Sci. Technol.* **2007**, *40*, 558–563.
182. White, R.E. *Principles and Practice of Soil Science: The Soil as a Natural Resource*, 4th ed.; Blackwell: Oxford, UK, 2006.
183. Spiegel, Y.; Kafkafi, U.; Pressman, E. Evaluation of a protein-chitin derivative of crustacean shells as a slow-release nitrogen fertilizer on Chinese cabbage. *J. Hortic. Sci.* **1988**, *63*, 621–628.
184. Roberts, P.; Jones, D.L. Microbial and plant uptake of free amino sugars in grassland soils. *Soil Biol. Biochem.* **2012**, *49*, 139–149.
185. Yaroslavtsev, A.; Manucharova, N.; Stepanov, A.; Zvyagintsev, D.; Sudnitsyn, I. Microbial destruction of chitin in soils under different moisture conditions. *Eurasian Soil Sci.* **2009**, *42*, 797–806.
186. Sinsabaugh, R.L.; Antibus, R.K.; Linkins, A.E.; McClaugherty, C.A.; Rayburn, L.; Repert, D.; Weiland, T. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. *Ecology* **1993**, *74*, 1586–1593.
187. Rich, J.R.; Hodge, C.H. Utilization of blue crab scrap compost to suppress *Meloidogyne javanica* on tomato. *Nematropica* **1993**, *23*, 1–5.
188. Bohn, H.L.; Myer, R.A.; O'Connor, G.A. *Soil Chemistry*; John Wiley & Sons: New Jersey, NJ, USA, 2002.
189. Sukwattanasinitt, M.; Klaikherd, A.; Skulnee, K.; Aiba, S. Chitosan as releasing device for 2,4-D herbicide. In *Chitin and Chitosan in Life Science*, Proceedings of the Eighth International Chitin and Chitosan Conference and Fourth Asia Pacific Chitin and Chitosan Symposium, Yamaguchi, Japan, 21–23 September 2000; Uragami, K., kurita, K., Fukamizo, T., Eds.; pp. 198–201.

190. Hadwiger, L.A.; McBride, P.O. Low-level copper plus chitosan applications provide protection against late blight of potato. In *Plant Health Progress*; 2006. Available online: http://www.plantmanagementnetwork.org/pub/php/research/2006/chitosan (accessed on 23 August 2013).
191. Tamura, H.; Nagahama, H.; Tokura, S. Preparation of chitin hydrogel under mild conditions. *Cellulose* **2006**, *13*, 357–364.
192. Jamnongkan, T.; Kaewpirom, S. Potassium release kinetics and water retention of controlled-release fertilizers based on chitosan hydrogels. *J. Polym. Environ.* **2010**, *18*, 413–421.
193. Wu, L.; Liu, M.; Liang, R. Preparation and properties of a double-coated slow-release NPK compound fertilizer with superabsorbent and water-retention. *Bioresour. Technol.* **2008**, *99*, 547–554.
194. Xie, W.; Xu, P.; Liu, Q. Antioxidant activity of water-soluble chitosan derivatives. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1699–1701.
195. Sun, T.; Zhou, D.; Xie, J.; Mao, F. Preparation of chitosan oligomers and their antioxidant activity. *Eur. Food Res. Technol.* **2006**, *225*, 451–456.
196. Sun, T.; Yao, Q.; Zhou, D.; Mao, F. Antioxidant activity of *N*-carboxymethyl chitosan oligosaccharides. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5774–5776.
197. Chen, W.G.; Liu, X.; Chen, H.X. Preparation of modified chitosan with quaternary ammonium salt. *Textile Bioengineering and Informatics Symposium Proceedings* **2009**, *1*, 226–230.
198. Boonlertnirun, S.; Sarobol, E.D.; Meechoui, S.; Sooksathan, I. Drought recovery and grain yield potential of rice after chitosan application. *Kasetsart J. Nat. Sci.* **2007**, *41*, 1–6.
199. Maruca, R.; Suder, B.J.; Wightman, J.P. Interaction of heavy metals with chitin and chitosan. III. Chromium. *J. Appl. Polym. Sci.* **1982**, *27*, 4827–4837.
200. Correa-Murrieta, M.A.; López-Cervantes, J.; Sánchez-Machado, D.I.; Sánchez-Duarte, R.G.; Rodríguez-Núñez, J.R.; Núñez-Gastélum, J.A. Fe(II) and Fe(III) adsorption by chitosan-tripolyphosphate beads: Kinetic and equilibrium studies. *J. Water Supply Res. Technol. AQUA* **2012**, *61*, 331–341.
201. Babel, S.; Kurniawan, T.A. Low-cost adsorbents for heavy metals uptake from contaminated water: a review. *J. Hazard. Mater.* **2003**, *97*, 219–243.
202. Bailey, S.E.; Olin, T.J.; Bricka, R.M.; Adrian, D.D. A review of potentially low-cost sorbents for heavy metals. *Water Res.* **1999**, *33*, 2469–2479.
203. Sánchez-Duarte, R.G.; Sánchez-Machado, D.I.; López-Cervantes, J.; Correa-Murrieta, M.A. Adsorption of allura red dye by cross-linked chitosan from shrimp waste. *Water Sci. Technol.* **2012**, *65*, 618–623.
204. Wang, F.Y.; Lin, X.G.; Yin, R. Role of microbial inoculation and chitosan in phytoextraction of Cu, Zn, Pb and Cd by *Elsholtzia splendens*—A field case. *Environ. Pollut.* **2007**, *147*, 248–255.
205. Angelim, A.L.; Costa, S.P.; Farias, B.C.; Aquino, L.F.; Melo, V.M. An innovative bioremediation strategy using a bacterial consortium entrapped in chitosan beads. *J. Environ. Manage.* **2013**, *127*, 10–17.
206. Gentili, A.R.; Cubitto, M.A.; Ferrero, M.; Rodriguéz, M.S. Bioremediation of crude oil polluted seawater by a hydrocarbon-degrading bacterial strain immobilized on chitin and chitosan flakes, *Int. Biodeterior. Biodegrad.* **2006**, *57*, 222–228.

207. El Ghaouth, A.; Arul, J.; Asselin, A.; Benhamou, N. Antifungal activity of chitosan on postharvest pathogens: Induction of morphological and cytological alterations in *Rhizopus stolonifer*. *Mycol. Res.* **1992**, *96*, 769–779.
208. Palma-Guerrero, J.; Jansson, H.B.; Salinas, J.; Lopez-Llorca, L.V. Effect of chitosan on hyphal growth and spore germination of plant pathogenic and biocontrol fungi. *J. Appl. Microbiol.* **2008**, *104*, 541–553.
209. Benhamou, N.; Lafontaine, P.J.; Nicole, M. Induction of systemic resistance to *Fusarium* crown and root rot in tomato plants by seed treatment with chitosan. *Phytopathology* **1994**, *84*, 1432–1444.
210. Scorza, R.; Callahan, A.; Levy, L.; Damsteegt, V.; Webb, K.; Ravelonandro, M. Post-transcriptional gene silencing in plum pox virus resistant transgenic European plum containing the plum pox potyvirus coat protein gene. *Transgenic Res.* **2001**, *10*, 201–209.
211. Levy, L.; Damsteegt, V.; Scorza, R.; Kolber, M. Plum pox potyvirus disease of stone fruits. *American Phytopathology Society* 2000. Available online: http://www.apsnet.org/online/feature/plumpox (accessed on 10 July 2013).
212. Pitta-Alavarez, S.; Giulietti, A.M. Influence of chitosan, acetic acid and citric acid on growth and tropane alkaloid production on transformed roots of *Brugmansia candida* Effect of medium pH and growth phase. *In Vitro Cell. Dev. Biol. Plant* **1999**, *59*, 31–38.
213. Sevón, N.; Hiltunen, R.; Oksman-Caldentey, K.M. Chitosan increases hyoscyamine content in hairy root cultures of *Hyoscyamus muticus*. *Pharm. Pharmacol. Lett.* **1992**, *2*, 96–99.
214. Merkli, A.; Christen, P.; Kapetanidis, I. Production of diosgenin by hairy root cultures of *Trigonella foenum-graecum* L. *Plant Cell Rep.* **1997**, *16*, 632–636.
215. Kim, J.H.; Shin, J.H.; Lee, H.J.; Chung, I.S.; Lee, H.J. Effect of chitosan on indirubin production from suspension culture of *Polygonum tinctorium*. *J. Ferment. Bioeng.* **1997**, *83*, 206–208.
216. Tay, L.F.; Khoh, L.K.; Loh, C.S.; Khor, E. Alginate-chitosan coacervation in production of artificial seeds. *Biotechnol. Bioeng.* **1993**, *42*, 449–454.
217. Kowalski, B.; Jimenez Terry, F.; Herrera, L.; Agramonte Peñalver, D. Application of soluble chitosan *in vitro* and in the greenhouse to increase yield and seed quality of potato minitubers. *Potato Res.* **2006**, *49*, 167–176.
218. El Ghaouth, A.; Smilanick, J.L.; Wilson, C.L. Enhancement of the performance of *Candida saitoana* by the addition of glycolchitosan for the control of postharvest decay of apple and citrus fruit. *Postharvest Biol. Technol.* **2000**, *19*, 103–110.
219. Benhamou, N. Potential of the mycoparasite, *Verticillium lecanii*, to protect citrus fruit against *Penicillium digitatum*, the causal agent of green mold: A comparison with the effect of chitosan. *Phytopathology* **2004**, *94*, 693–705.
220. Du, J.; Gemma, H.; Iwahori, S. Effects of chitosan coating on the storage of peach Japanese pear and kiwifruit. *J. Jpn. Soc. Hort. Sci.* **1997**, *66*, 15–22.
221. Fornes, F.; Almela, V.; Abad, M.; Agustí, M. Low concentrations of chitosan coating reduce water spot incidence and delay peel pigmentation of clementine mandarin fruit. *J. Sci. Food Agric.* **2005**, *85*, 1105–1112.
222. El Ghaouth, A.; Arul, J.; Grenier, J.; Asselin, A. Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. *Phytopathology* **1992**, *82*, 398–402.

223. Romanazzi, G.; Nigro, F.; Ippolito, A. Short hypobaric treatments potentiate the effect of chitosan in reducing storage decay of sweet cherries. *Postharvest Biol. Technol.* **2003**, *29*, 73–80.
224. Romanazzi, G.; Mlikota Gabler, F.; Smilanick, J.L. Preharvest chitosan and postharvest UV-C irradiation treatments suppress gray mold of table grapes. *Plant Dis.* **2006**, *90*, 445–450.
225. Sivakumar, D.; Sultanbawa, Y.; Ranasingh, N.; Wijesundera, R.L.C. Effect of the combined application of chitosan and carbonate salts on the incidence of anthracnose and on the quality of papaya during storage. *J. Hortic. Sci. Biotechnol.* **2005**, *80*, 447–452.
226. Azian, E.; Zaki, A.R.M.; Mohamed, M.T.M.; Kamuruzaman, S. The use of chitosan on vase life of cut chrysanthemum (*Dendranthema morifolium* Ramat). In Proceedings of APEC Symposium on Quality Management in Postharvest System, Bangkok, Thailand, 3–5 August 2004; p. 403.
227. Aranaz, I.; Mengibar, M.; Harris, R.; Panos, I.; Miralles, B.; Acosta, N.; Galed, G.; Heras, A. Functional characterization of chitin and chitosan. *Curr. Chem. Biol.* **2009**, *3*, 203–230.
228. Lonsdale, D. Available treatments for tree wounds: an assessment of their value. *Arboricultural J.* **1984**, *8*, 99–107.
229. Badawy, M.E.I.; Rabea, E.I.; Rogge, T.M.; Stevens, C.V.; Steurbaut, W.; Höfte, M.; Smagghe, G. Fungicidal and insecticidal activity of *O*-acyl chitosan derivatives. *Polym. Bull.* **2005**, *54*, 279–289.
230. Rodríguez-Núñez, J.R.; López-Cervantes, J.; Sánchez-Machado, D.I.; Ramírez-Wong, B.; Torres-Chavez, P.; Cortez-Rocha, M.O. Antimicrobial activity of chitosan-based films against *Salmonella typhimurium* and *Staphylococcus aureus*. *Int. J. Food Sci. Technol.* **2012**, *47*, 2127–2133.