

Commercial extract from the brown seaweed *Ascophyllum nodosum* reduces fungal diseases in greenhouse cucumber

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Abstract This study examined the effects of Stimplex™, a marine plant extract formulation from *Ascophyllum nodosum*, on some common cucumber fungal pathogens. Greenhouse cucumber plants were sprayed and/or root drenched using Stimplex™ at 0.5% or 1% concentration twice at 10-day intervals. Treatments also included application of fungicide (chlorothalonil, 2 g L⁻¹) alternating with Stimplex™ application. Treated plants were inoculated with four cucumber fungal pathogens including *Alternaria cucumerinum*, *Didymella applanata*, *Fusarium oxysporum*, and *Botrytis cinerea*. Stimplex™ application resulted in a significant reduction in disease incidence of all the pathogens tested. The disease control effect was greater for *Alternaria* and *Fusarium* infection, followed by *Didymella* and *Botrytis*. Combined spray and root drenching with Stimplex™ was more effective than either spray or root drenching alone. The alternation of one fungicide application, alternated with Stimplex™ application, was highly effective and found to be the best treatment in reducing the disease ratings. Plants treated with Stimplex™ showed enhanced activities of various defense-

related enzymes including chitinase, β -1,3-glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, and lipoxygenase. Altered transcript levels of various defense genes, including chitinase, lipoxygenase, glucanase, peroxidase, and phenylalanine ammonia lyase were observed in treated plants. Cucumber plants treated with Stimplex™ also accumulated higher level of phenolics compared to water controls. These results suggest that seaweed extracts enhance disease resistance in cucumber probably through induction of defense genes or enzymes.

Keywords *Ascophyllum* · Cucumber · Fungal diseases · Resistance · Mechanism

Introduction

Plants are capable of defending themselves against pathogens with a variety of preformed structures and inducible reactions. Inducible reactions essentially require the perception of signal molecules, which may result from pathogen attack or an external chemical treatment. This kind of recognition leads to triggering of a plethora of reactions, which result in augmentation of resistance to the invading pathogens. This enhanced state of resistance is effective against a broad range of pathogens and parasites, including fungi, bacteria, viruses, nematodes, parasitic plants, and even insect herbivores (Hammerschmidt 1999; Ton et al. 2006). The reactions include stimulation of the phenylpropanoid pathway, production of defense-specific signal molecules such as salicylic acid (SA), jasmonates, and accumulation of antimicrobial compounds/proteins such as phytoalexins and pathogenesis-related (PR) proteins (Kombrink and

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Somssich 1995). The chemical stimuli or elicitors, which bring about these induced reactions, are diverse and include oligosaccharides, polysaccharides, lipids, glycoproteins, peptides, and proteins (Vander et al. 1998; Klarzynski et al. 2000). Many elicitors generally enhance non-host plant resistance. It has become a fascinating crop protection strategy by mimicking pathogen attack using non-specific elicitors (Klarzynski et al. 2000; Anderson et al. 2006), and many such elicitors from synthetic and natural sources are being examined for efficacy in crop disease control.

Cucumber is a semi-tropical vegetable crop and grows best under conditions of high light, humidity, moisture, temperature, and fertilizer. These conditions are provided in a greenhouse environment. The production of greenhouse cucumbers has become a profitable industry in many parts of the world and parallels that of greenhouse tomatoes. Greenhouse cucumbers are sold for the fresh market (Rose et al. 2003) and are grown hydroponically with computer systems that continually monitor and regulate temperature, light, humidity, irrigation, and nutrient levels. Damage caused by diseases is one of the chief constraints in the greenhouse cucumber industry. The unique growing substrate (rockwool) used in most greenhouses in Canada presents both challenges and opportunities for management of plant diseases. Since rockwool is an inert substrate initially free of any competing microorganisms, introduced pathogens have universal access to the substrate and plant roots (Postma et al. 2000). Diseases such as *Fusarium* root rot, Gummy stem blight, *Alternaria* blight, *Botrytis* rot, and powdery mildew are prevalent in greenhouse cucumbers. Disease control methods usually involve multiple fungicide applications. Although fungicidal control is an option, owing to practical difficulties, including development of resistant strains, non-target effects, phytotoxicity, cost, accumulation of residue, and environmental and health hazards, minimal usage is always preferred (Rose et al. 2003). The growing popularity of organic production necessitates development and adoption of non-fungicidal approaches for disease control. There are earlier reports of induction of resistance in cucumbers through application of elicitors. Treatment of cucumber plants with acibenzolar-*S*-methyl offered protection against anthracnose and powdery mildew (Lin et al. 2008), and β -aminobutyric acid protected against a wide range of diseases including downy mildew and anthracnose (Andreas and Oliver 2009). A pectinase extract from the fermentation product of *Penicillium oxalicum* induced resistance against *Cladosporium cucumerinum* (Peng et al. 2004). However, no reports are available with regard to greenhouse cucumbers, and this system demands development of new crop protection strategies, in particular, non-fungicidal approaches for both conventional and organic

cultivation. Further, no single compound was effective against multiple pathogens. This particular limitation led us to undertake the current study.

Ascophyllum nodosum is a temperate seaweed found in the Atlantic and Arctic seas and has been widely studied for its properties, which include plant growth promotion and use in animal feed (Colapietra and Alexander 2006). There are a few reports available which indicated enhanced plant yield and health in different crops following application, although the mechanisms of action have not been determined (Norrie et al. 2002; Colapietra and Alexander 2006). In a previous study, we investigated the potential elicitor and disease-suppressive activities of *A. nodosum* extract. Spray application of *Ascophyllum* extract on greenhouse-grown carrots significantly reduced disease incidence levels of *Alternaria* and *Botrytis* foliar blights (Jayaraj et al. 2008). In order to evaluate efficacy of seaweed extract on greenhouse cucumber, we utilized a commercial formulation of seaweed extract, “Stimplex™”, which is water-soluble. We examined different methods and dosages of application, integration of fungicide, and their effects on four commonly occurring fungal pathogens including *Alternaria cucumerinum*, *Didymella applanata*, *Fusarium oxysporum*, and *Botrytis cinerea*. The possible biochemical and molecular basis of induced resistance was also investigated.

Material and methods

Plant material and treatments

Cucumber (*Cucumis sativus* var. *sativus*) seeds (cv. Salad Bush) (Westcoast Seeds, Delta, BC, Canada) were surface-sterilized in 10% Clorox solution for 10 min and rinsed in sterile water, planted into rockwool blocks, and irrigated with an aqueous solution containing water-soluble fertilizer (7:11:27-N:P:K, Plant Products Company Ltd., Brompton, ON, Canada) 1.15 g L⁻¹ and calcium nitrate 0.78 g L⁻¹. Rockwool blocks containing seedlings (2 weeks old) were transplanted into pots (15 cm diameter) containing sawdust. Plants were regularly fertilized using the above nutrient solution (30 mL, every third day). The potted plants were grown in a greenhouse at 22–28°C, 70–85% relative humidity, and 600–1,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ light intensity and a 12-h photoperiod. After 30–40 days in the greenhouse, plants were sprayed or drenched (30 mL plant⁻¹) or spray+drench with Stimplex™ (containing protein/amino acids 3–6%, lipid 1%, alginic acid 12–18%, fucose-containing polymers 12–15%, mannitol 5–6%, other carbohydrates 10–20%; Acadian Seaplants Limited, Dartmouth, NS, Canada), at 0.5% or 1% concentration. The treatments were repeated twice at 10-day

intervals. For fungicide control, chlorothalonil 50% (Syngenta Canada, Guelph, ON, Canada) was sprayed or drenched at 2 g L^{-1} concentration. For treatments involving Stimplex™+fungicide, the plants were sprayed or drenched with chlorothalonil on the tenth day after inoculation. Two independent trials were conducted in each case.

The following are the treatments tested: T1, 0.5% foliar spray; T2, 0.5% drench; T3, 0.5% spray+drench; T4, 1% foliar spray; T5, 1% drench; T6, 1% spray+drench with Stimplex™; T7, 0.5% Stimplex™ spray alternating with fungicide (chlorothalonil, 2 g L^{-1}); T8, 1% Stimplex™ spray alternating with fungicide (chlorothalonil, 2 g L^{-1}); T9, fungicide (chlorothalonil, 2 g L^{-1}) spray control; and T10, water control.

After 6 h following treatment with Stimplex™/water, plants were inoculated with conidial suspensions of pathogens (1×10^6 spores mL^{-1}). Each trial was conducted separately for each pathogen. The fungal pathogens *A. cucumerinum* (*Alternaria* blight), *D. appplanata* (Gummy stem blight), *F. oxysporum* (*Fusarium* root and stem rot), and *B. cinerea* (*Botrytis* blight) were cultured from infected tissues of plants collected from the grower's greenhouses. Koch's postulates were conducted by inoculating onto test cucumber plants. The inoculum was multiplied on potato dextrose agar or V-8 agar or broken maize medium. The conidial suspensions from 15- to 20-day-old cultures were used for plant inoculations. The inoculated plants were incubated in a humid chamber for 72 h and then transferred back to the greenhouse and grown under conditions described earlier. Twelve replicate plants were maintained per treatment.

Plants were scored for disease severity 15 and 25 d after inoculation using a six-point disease rating scale. For *Alternaria* and *Botrytis* blights, the following six-point disease rating scale based on percentage of leaf area infected was used (1=0%, 2=1–10%, 3=11–25%, 4=26–40%, 5=41–55%, and 6=>56%). For scoring *Didymella* gummy blight infection, the following scale was used: 1=no damage; 2=single lesion (1–10 mm long) or coalesced lesions (1–20 mm); 3=lesion length 21–80 mm, girdling of the stem or both; 4=withered stem; and 5=dead plant (Zuniga et al. 1999). Percent disease index was calculated as (sum of disease ratings of individual leaves/total number of leaves) \times (100/maximum rating). Plants inoculated with the *Fusarium* root and stem rot pathogen were scored for percent disease incidence by counting the number of plants showing disease symptoms. *Fusarium*-inoculated plants were carefully uprooted after disease assessment and dried at 60°C for 4 days, and dry biomass yields were recorded. The greenhouse experiments were conducted twice with 12 replicates for each treatment. All data were analyzed by analysis of variance, and mean disease severity values were separated using Fisher's protected least significant difference test ($P=0.05$).

Enzyme and biochemical assays

For enzyme and biochemical assays, plants were grown in rockwool blocks in a growth chamber at 23°C , 70% relative humidity, and $700 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and a 12-h photoperiod. Plants were sprayed/drenched with Stimplex™ (0.5%), and leaves were sampled (triplicate) at 0, 12, 24, 48, 72, and 96 h after treatment. For extraction of total proteins, leaf samples from treated plants were frozen in liquid nitrogen and homogenized and suspended in 0.1 M phosphate buffer, pH 6.5, containing 0.5 mM phenylmethyl sulfonyl fluoride and centrifuged at $10,000 \times g$ for 15 min at 4°C . Protein content of extracts was determined by the bicinchoninic acid microtiter plate assay kit (Pierce, Rockford, USA).

The activity of peroxidases (PO) was assayed spectrophotometrically using pyrogallol as a substrate; PO activity was expressed as changes in absorbance $\text{s}^{-1} \text{ g}^{-1}$ fresh weight of tissue. Polyphenol oxidase (PPO) activity was assayed using catechol as substrate, and the enzyme activity was expressed as catechol equivalents. Phenylalanine ammonia lyase (PAL) activity was assessed spectrophotometrically by assaying the rate of conversion of L-phenylalanine to *trans*-cinnamic acid at 290 nm, and the amount of *trans*-cinnamic acid synthesized was calculated using its absorption coefficient of $9,630 \mu\text{mol sec}^{-1} \text{ g}^{-1}$ (Rahman and Punja 2005). Chitinase activity was determined using *N*-acetyl glucosamine (NAG) as a substrate, and activity was expressed as NAG units (Singh et al. 1999).

Glucanase activity was assessed using laminarin as a substrate, and β -1,3-glucanase activity was expressed as micromole glucose equivalents (Wood and Bhat 1988). Lipoygenase activity was estimated employing linoleic acid as a substrate, and activity was expressed as linoleic acid equivalents (Alexrod et al. 1981).

For the estimation of total phenolic content, leaf tissues (1 g) were homogenized in 10 mL of 80% methanol and agitated for 15 min at 70°C . One milliliter of the methanolic extract was added to 5 mL distilled water and 250 μL Folin–Ciocalteu reagent (1 N), and the solution was kept at 25°C . After 3 min incubation, 1 mL saturated Na_2CO_3 solution and 1 mL distilled water were added, and the reaction mixture was further incubated for 1 h at 25°C . The absorption of the developed blue color was measured spectrophotometrically at 725 nm. The total phenolic content was calculated based on standards prepared with phenol and expressed as phenol equivalents g^{-1} fresh weight (Rahman and Punja 2005).

Effect of Stimplex™ treatment on defense gene expression

RNA was extracted from 300 mg of leaf tissue using Trizol reagent (Invitrogen) as per the manufacturer's

protocol. For total RNA extraction, cucumber leaf samples were collected in triplicate at 0, 12, 24, 48, 72, and 96 h after elicitor treatment and immediately frozen in liquid nitrogen and stored at -80°C . Total RNA (10 μg) was run

on a 1.4% (w/v) agarose formaldehyde gel and transferred overnight onto membrane (Hybond N+, Amersham). The blots were probed with [α - ^{32}P] dCTP-labelled probes prepared from the cloned cDNAs of *C. sativus*, chitinase

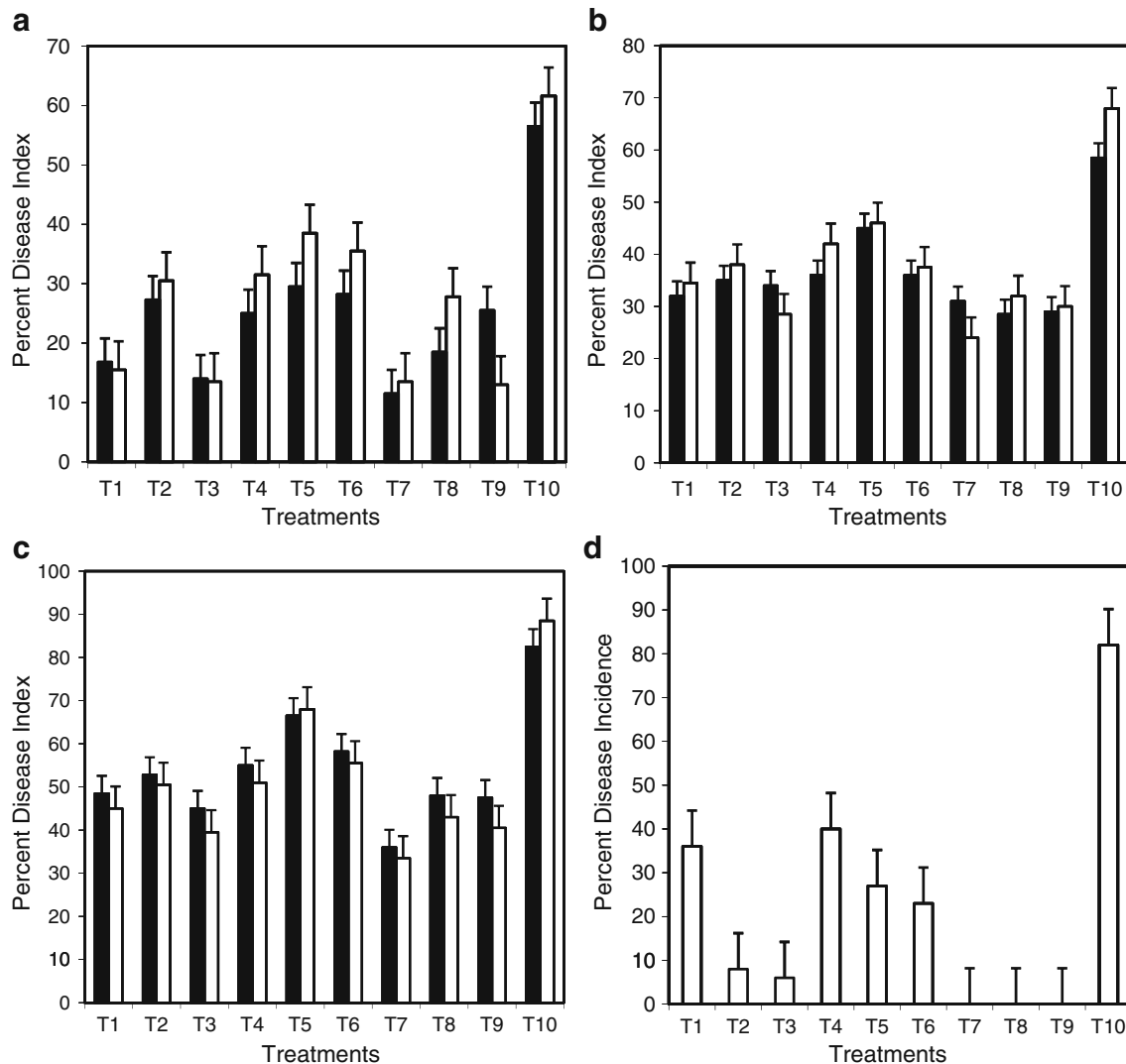


Fig. 1 **a** Effect of Stimplex™ on the incidence of *Alternaria* blight in cucumber. Vertical bars indicate mean \pm SE (from 12 replicates). LSD ($P=0.05$) values: 15 days, 5.2 and 25 days, 2.3. **a–c** Treatments are T1, 0.5% foliar spray; T2, 0.5% drench; T3, 0.5% spray+drench; T4, 1% foliar spray; T5, 1% drench; T6, 1% spray+drench; T7, 0.5% Stimplex™ spray alternating with fungicide (chlorothalonil, 2 g/L); T8, 1% Stimplex™ spray alternating with fungicide (chlorothalonil, 2 g/L); T9, fungicide (chlorothalonil, 2 g/L) spray control; and T10, water control. Two independent trials were conducted. Stimplex™ was applied 6 h before inoculation and 10 and 20 d after inoculation. Disease severity was estimated based on a six (one to six)-point disease rating scale. For fungicide treatment (T9), chlorothalonil was sprayed at 6 d after inoculation. **b** Effect of Stimplex™ on the incidence of *Didymella* blight in cucumber. Treatment and observation details as in **a**. LSD ($P=0.05$) values: 15 days, 2.3 and 25 days, 4.2. **c**

Effect of Stimplex™ on the incidence of *Botrytis* blight in cucumber. Treatment and observation details as in **a**. LSD ($P=0.05$) values: 15 days, 5.8 and 25 days, 1.6. **d** Effect of Stimplex™ on the incidence of *Fusarium* root rot in cucumber. Vertical bars indicate mean \pm SE (from 12 replicates). LSD ($P=0.05$)=2.3. Treatments: T1, 0.5% foliar spray; T2, 0.5% drench; T3, 0.5% spray+drench; T4, 1% foliar spray; T5, 1% drench; T6, 1% spray+drench; T7, 0.5% Stimplex™ spray alternating with fungicide (chlorothalonil, 2 g/L); T8, 1% Stimplex™ spray alternating with fungicide (chlorothalonil, 2 g/L) drench; T9, fungicide (chlorothalonil, 2 g/L) drench control; and T10, water control. Two independent trials were conducted. Stimplex™ was applied 6 h before inoculation and 10 and 20 d after inoculation. Disease incidence was recorded on 25 d after inoculation. For fungicide treatment (T9), chlorothalonil was drenched at 6 d after inoculation

(M84214), β -1,3-glucanase (AF459794), PO (FJ597624), lipoxygenase (X92890), and PAL (AF529240) genes, as described by Jayaraj and Punja (2007).

Results

Disease incidence and plant growth

Greenhouse cucumber plants were treated with Stimplex™ either as spray or root drench or using both methods, and treated plants showed generally lower infection levels of different pathogens. Two concentrations (0.5% and 1%) of Stimplex™ were tested, of which, 0.5% was found to be more effective than 1%. Further, 1% spray showed some moderate degree of phytotoxicity on leaves. Disease incidence levels were the lowest in plants sprayed and root drenched (T3) with 0.5% Stimplex™. The following were the percentile disease reduction levels observed for various pathogens inoculated under the above treatment: *Alternaria*, 70% (Fig. 1a); *Didymella*, 47% (Fig. 1b); and *Botrytis*, 46% (Fig. 1c). *Fusarium* root rot incidence levels were less in plants either sprayed or sprayed and drenched with 0.5% Stimplex™ (Fig. 1d). The percent disease reduction was between 85% and 88% at the above treatments. However, the best disease control effect was noticed when 0.5% Stimplex™ was integrated with one fungicide application (chlorothalonil, 2 g L⁻¹). The disease reduction was at the level of 75% for *Alternaria*, 60% for *Didymella*, and 55% for *Botrytis*, respectively. Whereas in the case of *Fusarium*-inoculated plants, spraying with 0.5/1% Stimplex™ along with fungi-

cide drench or fungicide drench alone provided complete reduction of disease. In *Fusarium*-inoculated plants, the plant biomass (shoot and root) levels were higher in Stimplex™ 0.5% sprayed/sprayed and drenched plants than control, fungicide, and remainder of the treatments (Fig. 2).

Defense enzyme activities and total phenolic levels

Cucumber plants (spray/drench, spray+drench with Stimplex™ at 0.5%, and control) were extracted for enzyme proteins at 0, 12, 24, 48, 72, and 96 h after treatment. The enzyme extracts were analyzed for the activity of defense enzymes including chitinase (Fig. 3a), glucanase (Fig. 3b), PO (Fig. 3c), PPO (Fig. 3d), PAL (Fig. 3e), and lipoxygenase (Fig. 3f). The enzyme activities were generally high in plants treated with Stimplex™ compared to the control. The highest activities were observed in plant sprayed+drenched/sprayed with Stimplex™. Peak activities were noticed between 24 and 48 h after treatment. The total phenolic contents (Fig. 4) were high in Stimplex™-treated plants when compared to water control. Plants sprayed or spray+drenched with Stimplex™ showed higher phenolic levels compared to control.

Accumulation of defense gene transcripts

Cucumber plants sprayed/sprayed+drenched with Stimplex™ (0.5%) or water were extracted for total RNAs and probed for accumulation of transcripts of defense genes including chitinase, lipoxygenase, glucanase, PO, and PAL. Plants treated with Stimplex™ accumulated the above defense gene transcripts at higher levels compared to water control (Fig. 5). The highest level of transcript accumulation was observed in Stimplex™ spray and spray+drench-treated plants. Increased transcript accumulation occurred rapidly after the application of Stimplex™, and the transcript levels remained high up to 96 h after treatment.

Discussion

Greenhouse cucumber plants treated with Stimplex™ showed reduced infection levels following inoculation with fungal pathogens, *Alternaria*, *Didymella*, *Fusarium*, and *Botrytis*. Similar effects were observed when carrot plants were treated with seaweed extract, wherein infection levels of *Alternaria* and *Botrytis* were strikingly lower compared to controls (Jayaraj et al. 2008). Spray along with drenching of Stimplex™ was more effective compared to one of the above; even more effective was when the above treatment was alternated with one fungicide treatment. Therefore, inclusion of one fungicidal spray in between routine

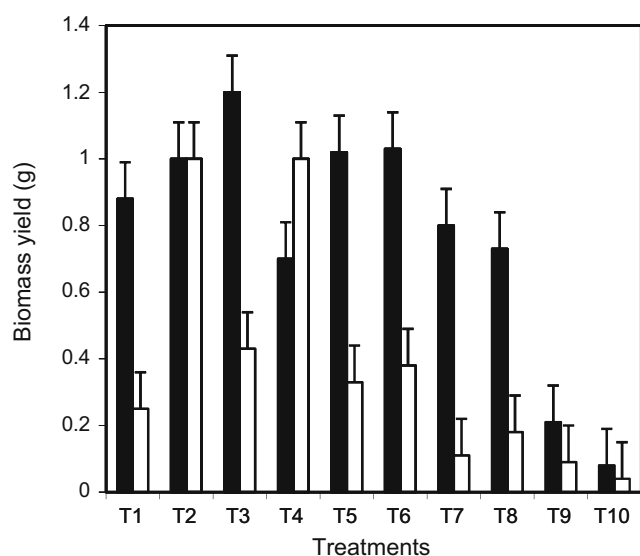


Fig. 2 Effect of Stimplex™ and *Fusarium* inoculation in cucumber on plant biomass. Mean shoot and root dry biomass as grams. Vertical bars indicate mean±SE. LSD ($P=0.05$) values: shoot, 0.23 and root, 0.13. Treatment and observation details as in Fig. 1d

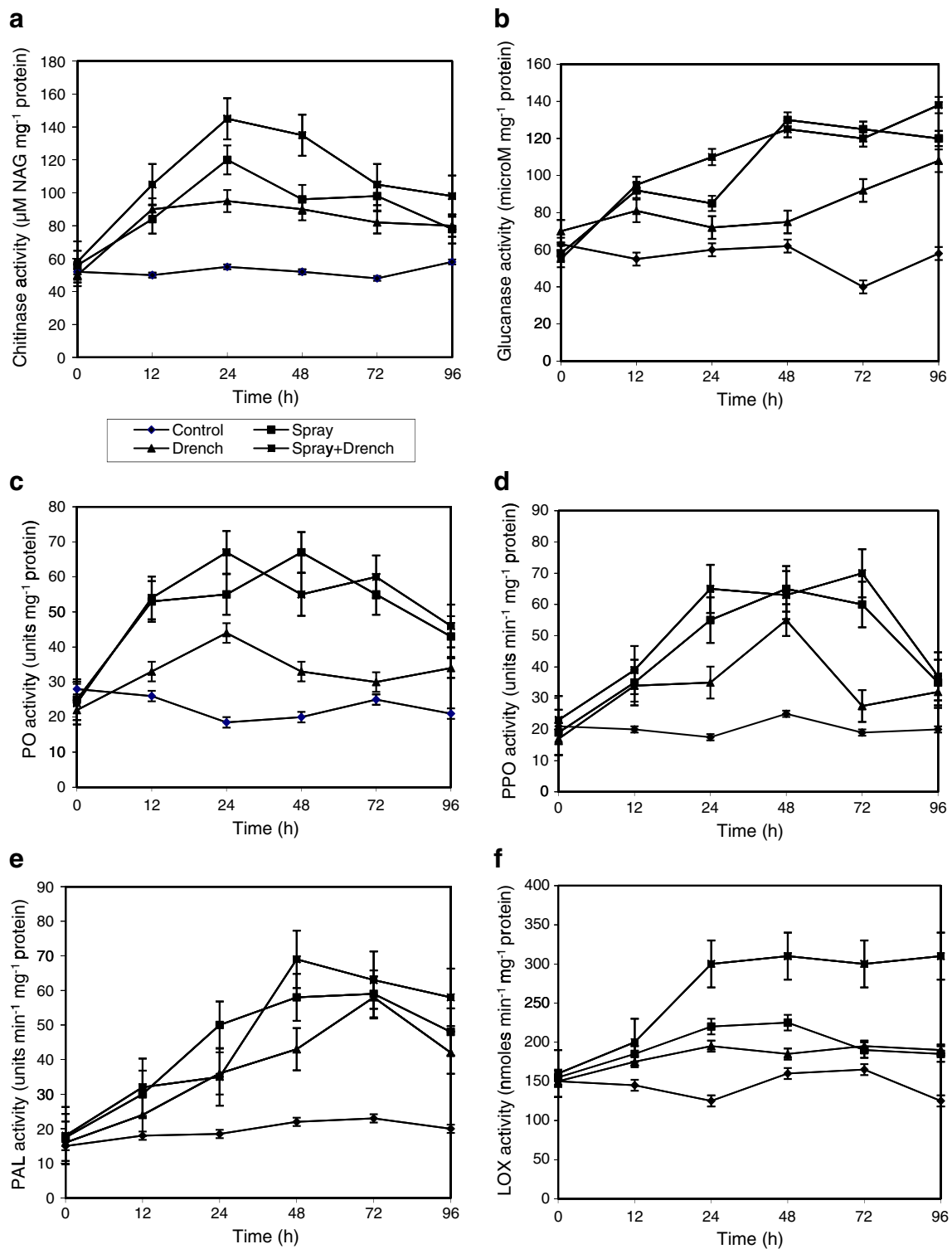


Fig. 3 Defense enzyme activities in cucumber plants treated with StimplexTM (0.5%): **(a)** chitinase, **(b)** β -1,3-glucanase, **(c)** peroxidase, **(d)** polyphenol oxidase, **(e)** phenylalanine ammonia lyase, and **(f)** lipoxygenase. Vertical bars indicate mean \pm SE. Data are means of three replicates

StimplexTM sprays is more effective and practically significant compared to conventional fungicidal sprays for disease control. StimplexTM-treated plants accumulated higher levels of defense-related gene transcripts, showed

higher activities of defense-related enzymes, and accumulated enhanced levels of phenols. Similar effects were observed in carrot plants when treated with seaweed extract (Jayaraj et al. 2008).

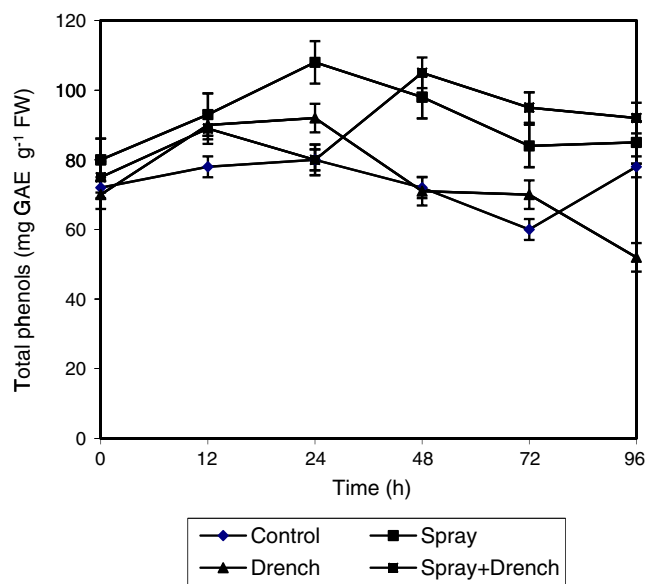


Fig. 4 Total phenolic content in cucumber plants treated with Stimplex™ (0.5%). Vertical bars indicate mean \pm SE

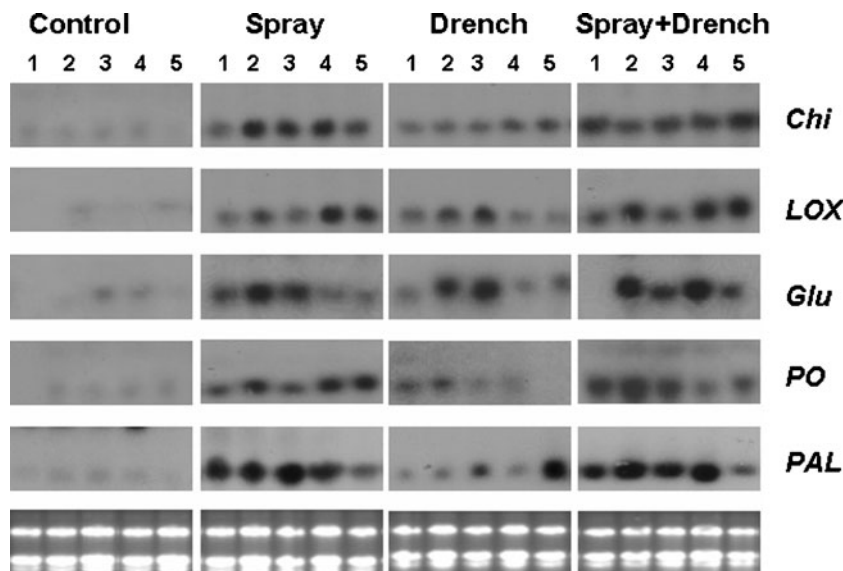
Stimplex™ is a commercial formulation of the seaweed extract derived from *Ascophyllum* which contains a plethora of compounds, including carbohydrate molecules mostly in the form of oligosaccharides, including oligogalacturonides, and some polysaccharides (Dr. Jeffrey Norrie, Acadian SeaPlants Limited, unpublished report). Oligosaccharides are known to act as elicitor and signal transduction molecules in plants (Ryan and Farmer 1991; Vidhyasekaran 1997; Walters et al. 2005). Oligogalacturonides can be mobile systemically, depending on their molecular weight, and can induce expression of various defense-related proteins and proteinase inhibitors in vivo (Cluzet et al. 2004). The algal polysaccharides, laminarin and carra-

geenans, were demonstrated to induce signalling and defense gene expression in tobacco leaves (Mercier et al. 2001). Laminarin is a linear β -(1,3)-glucan, and sulfated fucans abundantly found brown algae can elicit multiple defense responses in alfalfa and tobacco (Kobayashi et al. 1993; Klarzynski et al. 2000). Carrageenans are a family of sulfated linear galactans, and they are effective inducers of defense responses in tobacco plants (Mercier et al. 2001).

In the present study, the observed upregulation of various PR protein genes as well as enhanced activities of different defense enzymes in Stimplex™-treated plants might be the result of induction of resistance pathways, which are JA- and/or SA-mediated. Therefore, the disease control effect observed in Stimplex™-treated carrot plants could be attributed to the elicitor activity of carbohydrate (oligosaccharides) fractions and other unresolved compounds of potential elicitor activity present in the seaweed formulation. In our earlier studies with carrot, induction of PR protein genes and other defense-related genes was observed following treatment with seaweed extract (Jayaraj et al. 2008). Treatment of *Medicago truncatula* with an elicitor from the green algae, *Ulva* sp., induced the expression of defense-related gene *PR-10* without inducing necrosis. Spraying at a minimum concentration of $500 \mu\text{g mL}^{-1}$ was sufficient to cause maximum induction of *PR-10* after 2 d. Further treatment of plants with an extract of *Ulva* sp. reduced subsequent infection by *Colletotrichum trifolii*. Cluzet et al. (2004) observed increased expression through cDNA arrays, a broad range of defense-related transcripts, notably genes involved in phytoalexin and PR proteins.

Stimplex™ treatment also enhanced the activities of chitinase, glucanase, PO, PPO, PAL, and lipoxygenase enzymes in cucumber leaves. The increased activities were stable and prolonged for up to 72 h. A similar increase in

Fig. 5 Northern blots showing accumulation of transcripts of defense genes in cucumber leaves. Chitinase (*Chi*), lipoxygenase (*LOX*), glucanase (*Glu*), peroxidase (*PO*), phenylalanine ammonia lyase (*PAL*). Bottom panel indicates RNA loading controls. Treatments: water control, Stimplex™ spray (0.5%), Stimplex™ drench (0.5%), and Stimplex™ spray+drench (0.5%). Numbers 1, 2, 3, 4, and 5 correspond to 12-, 24-, 48-, 72-, and 96-h intervals, respectively, after treatment



the activity of defense enzymes was observed in carrot plants when sprayed with an extract of *Ascophyllum* (Jayaraj et al. 2008). In pepper plants, spray of *Ascophyllum* extract induced PO activity in pepper plants, and one application of extract at 0.8 or 1.6 l ha⁻¹ stimulated PO activity, and two applications caused an eightfold increase in PO activity (Lizzi et al. 1998). There are numerous reports describing the induction of defense enzymes by various elicitor compounds (Vallard and Goodman 2004). Chitosan sprays on grapevine leaves caused a marked induction of lipooxygenase and PAL activities (Trotel-Aziz et al. 2006). Chitosan foliar spray induced the activities of chitinase, β -1,3-glucanase, and lipooxygenase in potato and tomato (Vasuikova et al. 2001). The above enzymes are involved with defense reactions and further have significant antimicrobial activities (Vidhyasekaran 1997; Jayaraj et al. 2004). In addition, hydrolytic activities of chitinases and glucanases on pathogen cell wall chitin and glucan would result in the release of oligosaccharides, which may in turn act as elicitors and thereby sustain the induction levels of defense reactions (Jayaraj et al. 2004).

In carrot plants, seaweed extract spray showed visible brown spots/patches in leaves following DAB staining (Jayaraj et al. 2008), which was indicative of H₂O₂ accumulation as a consequence of production of reactive oxygen species (ROS; Ganesan and Thomas 2001). Lipooxygenase activity generates ROS and superoxide anion radicals and singlet oxygen (Ohta et al. 1990). In the present study, a significant increase in lipooxygenase activity was observed, and this might be involved in the induction of related defense reactions. The active oxygen may be involved in the oxidation of membrane lipids that results in the production of several antifungal compounds, including phytoalexins, initiation of cell wall lignification, and signal transduction leading to resistance responses (Sutherland 1991).

Elicitor applications may also increase the constitutive phenolic levels in plants. Application of seaweed extract on carrot plants also enhanced the phenolic levels in leaves (Jayaraj et al. 2008). This phenomenon was very commonly observed following treatment with elicitors (Jayaraj et al. 2008). For example, BTH treatment of strawberry plants caused an increased accumulation of soluble and cell wall-bound phenolics in leaves (Hukkanen et al. 2007). Application of chitosan–oligosaccharides to tomato induced the accumulation of phenolics, which led to resistance to fungal infection (Benhamou et al. 1994). The enhanced accumulation of phenolics in elicitor-treated carrot plants might be related to the increased activity of PAL, PO, and other phenol-oxidizing enzymes, which would have in turn caused an increase in the available free phenolic pool. This could be well witnessed in the present studies wherein the activities of the above phenylpropanoid pathway enzymes were always high in Stimplex™-treated plants. Availability

of a surplus free phenolic pool might also otherwise trigger polymerization reaction leading to lignin synthesis, which in turn enhances disease resistance (Vidhyasekaran 1997).

Reports on effective usage of marine plant extracts for plant disease control are few, and these were confined to investigation with individual pathogens. Pepper plants treated with *Ascophyllum* extract enhanced foliar resistance to *Phytophthora capsici* (Lizzi et al. 1998), and the acquired resistance was found to be proportional to the concentration of the extract and the number of applications. Incorporation of *A. nodosum* extract into the planting medium caused delayed and reduced incidence of *Verticillium* wilt of pepper plants, which also contained higher levels and early accumulation of phenolics (Garcia-Mina et al. 2004). In the present study, spray application was more effective than root application, which might be due to increased mobility or availability of elicitor molecules through spray than root treatment.

The enhanced plant growth effects in Stimplex™-treated plants might be correlated with auxins, gibberellins, cytokinins, precursors of ethylene, betaine, and cytokinins, which are present and potentially involved in enhancing plant growth responses (Khan et al. 2009). Apart from hormonal activity, seaweed extract is also reported to enhance the nutrient uptake. Foliar spray of seaweed extracts maximized the grain and straw yield and also enhanced the nutrient (N, P, K, and S) uptake by soybean plants (Rathore et al. 2009). In the present study also, Stimplex™-treated plants despite pathogen (*Fusarium*) inoculation showed superior root and shoot growth and biomass. The enhanced plant growth and biomass could be the result of growth-promoting activity of the seaweed extract and enhanced nutrient uptake by plants as well.

There is more promise for use of non-chemical approaches in crop production in the light of recent shift towards organic farming and growing public concern to minimize the use of chemical fungicides. Since greenhouse cucumber is a commercially important crop, there is more scope for use of alternative strategies for crop protection, and they are ultimately economically viable. The advantage of using seaweed extract is that it conditions the plant and triggers and contributes optimum level of resistance to multiple pathogens apart from promoting plant growth. This particular potential widens the scope of seaweed extract formulation for use in other greenhouse crops also. However, extensive studies need to be conducted under large-scale conditions and locations to evaluate their effectiveness for disease control in other crops.

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