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# The influence of extracts of Ascophvllum nodosum on plant and soil-borne pathogen interactions

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# The influence of extracts of *Ascophyllum nodosum* on plant and soil-borne pathogen interactions

by

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# ABSTRACT

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This thesis presents an investigation into the responses to extracts of *Ascophyllum nodosum* (Maxicrop seaweed extracts - MSE) of two different plants species – wheat and strawberry, and their interactions with two soil-borne pathogens, *Gaeumannomyces graminis* and *Phytophthora fragariae* respectively, under various environmental conditions.

The responses to MSE using hydroponic, glasshouse and field experiments showed that levels of Take-all infection in wheat were reduced by some of the treatments applied. Repeat experiments showed that consistency of results was poor but a positive trend for disease suppression followed MSE treatments.

Studies of strawberry infection by *Phytophthora fragariae* revealed a significantly reduced level of disease severity in plants grown both in hydroponics and in the growth chamber in response to MSE. *In vitro* studies of the fungus demonstrated that the seaweed extract treatments severely altered mycelial growth, which drastically reduced formation of sporangia and release of zoospores. Experiments using β-glucan, β-glucanase and laminarin showed that these could not reproduce the effects observed for MSE treatments suggesting that these components were not responsible for the MSE effect. Applications of potassium salts however, did reproduce the responses observed when applied at concentrations similar to the ones found in the seaweed extract. In these investigations, no significant benefits to non-inoculated strawberry plants could be identified as a response to MSE. Measurement of growth of disease infected plants, however clearly demonstrated that they benefited in terms of growth from the MSE amendments probably as a consequence of the disease suppression obtained.

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## DECLARATION

I declare that the research presented in this thesis was conducted by myself under supervision. At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

Relevant scientific seminars and conferences were regularly attended at which work was often presented.

Conferences attended:

1. SEB Annual Meeting (York, March 1998).
2. "Detection, Isolation and Manipulation of Soil Rhizosphere Microorganisms", organized by the Society for Applied Microbiology, (Warwick, December 1999).
3. SEB Annual Meeting (Exeter, March 2000). "*In-vitro* suppression of sporangia production in *Phytophthora fragariae* by Liquid Seaweed Extracts" – Poster Presentation.
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Signed:.. Felicidade Maria de Jesus Cunha..

Date:.....8/10/02.....

"...O tempo é um tesouro que se vai, que se escapa, que escorre entre as nossas mãos como a àgua pelos seixos. Ontem já passou e o hoje está passando. Amanhã será em breve outro ontem. A vida é muito curta. No entanto, quanto pode realizar-se neste pequeno espaço por amor..."

Escrivá de Balaguer (1977).

("...Time is a treasure that runs out, that escapes, that runs through our hands like water through the pebbles. Yesterday is gone and today is passing. Tomorrow will soon be yesterday. Life is short. However, how much can be done during this brief spell for love...")

Escrivá de Balaguer (1977).

# CHAPTER I - LITERATURE REVIEW

## 1. PLANTS AND DISEASE

The principal objectives of Pathology are to establish the causes for dysfunction and consequently find ways to control or eliminate the factors that allow establishment and spread of a pathogen in the individual or group of individuals (Parry, 1990; Roberts & Boothroyd, 1984; Bateman, 1978). Plant pathological studies can thus be considered essential as diseases still cause large reductions in the amount of food and fibre that could potentially be produced around the world (OECD Agricultural Outlook, 2000; Herwitt, 1998).

The word, disease, is normally used to denote the loss of the normal state of the living plant body, or any of its components, that interrupts or modifies the performance of its vital functions, being a response to environmental factors, to specific infective agents, to inherent defects of the organism, or to a combination of these factors (Beringer & Johnston, 1984; Bateman, 1978). Agrios (1978) specified that disease is "any disturbance brought about by a pathogen (organism which causes disease) or an environmental factor which interferes with manufacture, translocation, or utilisation of food, mineral nutrients, and water in such a way that the affected plant changes in appearance and/or yields less than a normal, healthy plant of the same variety". Infection by microorganisms (fungi, bacteria, viruses, mycoplasmas), nutrient deficiencies or excesses, toxic materials in the soil or atmospheric environment, infestation by pests and colonisation by plants or algal parasites are all causal agents of disease (Parry, 1990). The changes induced by the causal agents in the plant are called symptoms and vary in significance in terms of the overall effect that they have on plant physiology and, consequently, on the final yield obtained from crops. Plants have developed a range of mechanisms that allow them to withstand

disease and their efficiency determines whether they survive attack or whether they succumb (Dean & Kuc, 1987; Kozlowski, 1978).

In agricultural systems, crop protection measures intend not only to avoid plant death but also the onset of the causal agents of disease and/or the reduction of their aggressiveness in order to ensure that the final yield is not reduced below a certain economic level. In this context, the identification of disease symptoms and the assessment of their severity play vital roles, allowing to discriminate whether control measures are required and/or whether those measures were effective (Parry, 1990).

Although some may consider that research into plant protection strategies is not as relevant at present as it was in the past due to the production surplus available in developed countries, a substantial number of scientists and politicians defend the opposite view (Longemann, 1994; Sequeira, 1993). These argue that the number of developing countries where food production does not reach the level required out-weighs the number of developed countries where food production is excessive. They also consider that the world population is predicted to continue increasing, therefore, the total food production must rise accordingly in order to cover the future needs. In addition, environmental concerns should spur the search for better and sustainable ways to produce food and fibre (Thomson, 1994; Kareiva *et al.*, 1993). Apart from these arguments, one should also consider that unpredictable natural cataclysms might occur without warning at any given moment, therefore, surplus can always be regarded as potentially useful (Chet, 1994; Longemann, 1994).

## 1.1. Impact of Disease on Plant Growth and Nutrition

The disease impact on the plant is variable depending on the many factors that associate the disease causal agent, the plant and the environment (Benson, 1994; English &

Mitchell, 1994; Manners, 1993; Parry, 1990). Environmental factors such as temperature, water, light, concentration of certain ions, balance between oxygen and carbon dioxide and nutrients affect both the capacity of the pathogen to cause infection and the ability of the plant to resist disease (Manners, 1993; Roberts & Boothroyd, 1984).

A successful pathogen attack generally follows several steps from searching and recognizing a compatible host, attaching and penetrating the surface of the host, through outplaying host defences and growing using nutrients provided by the host to reproduction (Deacon, 1996; Ward *et al.*, 1994; Hoch & Staples, 1991; Nicholson & Epstein, 1991). Various mechanisms are thought to be involved in the stimulation and orientation of pathogens towards their hosts. In soil-borne plant pathogens, some exudates liberated by roots of host plants have been identified that will stimulate the germination, orientation and penetration of infective microbes (Deacon, 1996; Sacks, 1993; Hitoshi, 1991; Hoch & Staples, 1991). Following this first orientation step, pathogen penetration is required for disease to occur, this may happen through various pre-existent routes, like wounds and natural openings (*eg.* stomata, hydathodes). Many microorganisms, however, have the ability to invade the plant through the unbroken surface by using means such as mechanical pressure and the production of various degradative enzymes (Manners, 1994; Roberts& Boothroyd, 1984; Bracker & Littlefield, 1973).

Ensuing penetration an array of communication processes occur between the plant cell and the invading organism. These will determine whether they are compatible and, if so, to what extent will the pathogen disrupt the host functions (Hammerschmidt, 2000; Deacon, 1996; Ouchi, 1991; Ward, 1986). Compatibility is thought to be genetically controlled and a great variability of responses exists within the same plant species. Compatibility between the pathogen and the plant ultimately determines the establishment of resistant and susceptible cultivars (Deacon, 1996; Hitoshi, 1991; De Wit, 1986).

Disease symptoms are variable depending on the infectivity of the pathogen and the magnitude of the physiologic malfunctioning. Frequent visible symptoms are wilting, changes in coloration of leaves and stems, abnormal growth, inhibition of flowering and fruit formation, lesions and rots. At the physiological level all of the processes can be disrupted, thus, photosynthesis for example is often disturbed. Some pathogens growing over or killing cells on green leaves and stems ultimately lead to senescence and even death of the organ infected. Some pathogens produce toxins that directly cause death of photosynthetic tissue while others stimulate the formation of the structures called green islands, which some authors think could be used as a survival measure by the invader (Manners, 1993; Parry, 1990, Dickinson & Lucas, 1982).

Pathogenic attacks and establishment act as sinks for fixed carbon, therefore, even though photosynthetic areas might be affected or reduced, in many instances photosynthetic rates have been found to increase following infection. In parallel, as observed for other stress stimulus, diseased plants commonly have increased respiration rates after infection (Ayres, 1991; Geiger & Servaites, 1991; Pell & Dann, 1991). Increased respiration accompanies the acceleration of host metabolism and oxidative phosphorylation required by defence mechanisms (Ayres, 1991; Vaadia, 1985; Whitney, 1976).

Soil-borne pathogens may cause major disruptions in root functioning and, consequently, drastically reduce uptake of water and nutrients. For that reason, root rots caused by pathogens like *Phytophthora fragariae*, *Gaeumannomyces graminis*, *Plasmodiophora brassicae* frequently lead to plant death (Manners, 1993; Parry, 1990; Dickinson & Lucas, 1982).

An evaluation of the effects of disease on plant growth is not, therefore a simple task considering the many factors involved. Typically, dry weight, leaf area, growth stage,

number and dry weight of grains can all be used to measure responses to disease and their control measures. These, however, are not always the best tools as fine differences in response can easily be lost and there are differences in plant populations and individuals' responses (Ayres, 1991; Parry, 1990; Roberts & Boothroyd, 1984). Observations and quantifications of disease infection are also important as they can be used to assess whether control measures are required. Disease assessments carried-out with the aid of assessment keys are important tools in integrated crop protection systems for example (Manners, 1993; Parry, 1990).

Despite the damages caused by diseases, plants have evolved to co-exist with microorganisms and have developed several mechanisms to withstand the attack of pathogens and survive infection (Dean & Kuc, 1987). Responses to some pathogen attacks may be rapid and directed to ensure survival even though some normal functions may not be restored. Later or slower reactions occur by altered gene expression that allow physiological and morphological responses that in turn attempt to restore functioning to normal or near-normal levels even while disease persists or progresses (Hammond-Kosack *et* al., 1996; Geiger & Servaites, 1991).

## 1.2. Plant Defence Mechanisms

Investigations on the plant diseases at the molecular level became more feasible through the development of biotechnological techniques and instruments over the last decade and give invaluable tools to help clarify some of aspects of plant-pathogen relations. The processes involved in plant disease have often been investigated independently with the consequence that the understanding of the inter-relations of the mechanisms involved can at times be impaired. In general those sub-divisions are, nonetheless, useful and allow a better understanding of the encompassing biological

processes in question (Sequeira, 1993; Keon *et al.*, 1987; Callow, 1983; Cowling & Horsfall, 1978).

Because pathogens and plants have evolved alongside, pathogens are capable of overcoming preformed histological and chemical barriers such as the cuticle, epidermis, cell walls and release of certain chemical compounds (*eg.* phenolics, flavones, terpenoids and saponins). In addition, plants may use other mechanisms after penetration has occurred to restrict pathogen growth, such as infection-induced barriers (Ward *et al.* 1994; Ayres, 1991; Yoshikawa & Takeuchi, 1991; Köller, 1991; Whitney, 1976). These barriers may occur locally through the synthesis of antimicrobial compounds, deposition of large amounts of compounds to isolate the pathogen (*eg.* gums, resins, callose, etc), the encapsulation of the fungal structures in the invaded cell or modification of neighbouring cell walls (Ayres, 1991; Bowles, 1990; Hahn *et al.*, 1989; Hargreaves & Keon, 1986; Heitefuss, 1980). Lignification and suberization, for example, form part of the physical barriers that some plants use against pathogen attack (Ouchi, 1991; Smart, 1991; De Wit, 1987; Ride, 1983; Whitney, 1976). These and other defensive structures and reactions are relied upon when the plant identifies foreign possibly damaging signals. Many substances of microbiological origin have been identified as phyto-elicitors in this context (Boller, 1995; Scheel, *et al.*, 1991; Anderson, 1989).

Phytoalexins are low-molecular-weight compounds of various classes synthesised by plants that are typically involved in antimicrobial responses (De Wit, 1987, Deverall, 1982; Stoessl, 1982). They are synthesised in response to various stimuli, thus, mycelial walls of fungi and several types of fungal metabolites, such as degrading enzymes used to destroy and penetrate the cell wall, have shown to be capable of eliciting phytoalexin biosynthesis (Yoshikawa, 1995; Sinha, 1995; Keon *et al.*, 1987).

It has been demonstrated that it is possible to obtain phytoalexin responses by submitting susceptible hosts to treatments with non-compatible pathogens and with filtrates containing the cell walls of compatible pathogens prior to infection with the infective strains (Sinha, 1995). Phytoalexin synthesising responses were also obtained using other types of substances, such as phosphonates to suppress *Phytophthora* diseases (Saindrenan & Guest, 1995). Glucans and some glycoprotein compounds from various fungal origins have also been extensively studied in the infection by *Phytophthora* species and have shown strong elicitor activity (Boller, 1995; Ebel, 1991; De Wit, 1987).

Proteins have for long been implicated as essential parts in the interactions between plants and their pathogens (Stotz, *et al.* 2000; Boller, 1995; Loon & Van Strein, 1995; Bowles, 1990). Pathogenesis-related (PR) proteins are low molecular-weight polypeptides that accumulate extra-cellularly after infection or stimulation with some chemicals (Loon & Van Strein, 1995; Scholtens-Toma, 1991; Bowles, 1990). It is known that they are involved in the recognition/resistance processes and are primordial to the activation of defence responses (Stotz, 2000; Hammond-Kosack *et al.*, 1996, Scholtens-Toma, 1991). PR proteins are also implicated in catalytic and oxidative reactions that allow the plant to form defence barriers and reinforce the cell structure (Chen *et al*, 2000; Bowles, 1990).

PR proteins have been found to be involved in deterrence and anti-microbial activities, such as internal-enzymatic activity. β-glucanases and chitinases, for example, have been found in great quantity in infected plants and concentrations increased faster in incompatible, i.e. resistant, interactions. It is thought that these enzymes may have degradative functions against β-glucan and chitin rich fungal cell walls although the mechanism of action has not been totally elucidated (Tuzun, 2001; Kini, 2000; Wubben *et al*, 1996; Okinaka *et al*, 1995; Scholtens-Toma, 1991).

The term hypersensitive response (HR) was first used by Ward in 1905 (De Wit, 1987) in reference to host-parasite incompatible relationships resulting in resistance. HR is used to refer to the plant defence strategy where localised and rapid death of host cells invaded by pathogens occurs (Graham & Graham, 1999; Richael & Gilchrist, 1999; Mansfield, 1986; Heitefuss, 1980). HR is induced by elicitors and the synthesis of phytoalexins is generally also part of the complex activation mechanism developed by plants in active defence, thus, these can be seen as inter-related processes (Greenberg, 1997).

Systemic acquired resistance (SAR) and localised acquired resistance (LAR) are also processes of active host defence that have been extensively studied in recent years. SAR in plants has been compared to the immune system in the animal kingdom (Greenberg, 1997; Madamanchi & Kuc, 1991). It is a resistance response to infection induced in plants previously challenged with a pathogenic infection and it has been found to provide broad-spectrum, i.e. non-specific, disease resistance. SAR often occurs at or after a HR event and while in the past some authors have stated that it was dependent on an infection process, more recently evidence has been gathered which shows that it can be generated by other stimuli (Kombrik & Schmelzer, 2001; Reuveni, 2000; Hammerschmidt, 2000; Uknes *et al.*, 1996; Sinha, 1995). Genetic and metabolic studies have indicated that SAR may be mediated by salicylic acid and catalase signalling pathways involving the expression of PR proteins (Metraux, 2001).

Some authors distinguish a second type of induced systemic resistance, (ISR), that is also systemically transmissible and is generated by the root colonization by rhizosphere bacteria (PGPR) that are commonly thought to be responsible for plant growth promotion. ISR, contrary to SAR, does not seem to be dependent on the expression of PR proteins and has been shown to be mediated by jasmonate and ethylene sensitive mechanisms (Tuzun,

2001; Ongena, 2000; Hammerschmidt, 1999). Different bacteria have shown ISR ability, nevertheless, a lot of researchers have focused on fluorescent pseudomonads, trichoderma and other arbuscular mycorrhizal fungi (AMF), possibly due to prior indications that they can act as biocontrol agents (Chen, 2000; Ongena, 2000; Vigo *et al.* 2000; Cordier *et al.*, 1998).

From the practical view of crop protection, the most attractive feature of SAR and ISR is that stimulated plants responded dynamically and vigorously to infection, therefore, energy losses were reduced, pathogens were contained quicker than in non-conditioned plants and the effect remained active for days (Heil, 2001; Baker *et al*, 1997; Sinha, 1995). The use of elicitors of various origins to induce systemic defence reactions as a means to protect plants from specific pathogens may, however, not be totally reliable as a large number of elicitors identified to date are non-specific and their effectiveness varies (Kuc, 2001; Madamanchi & Kuc, 1991; De Witt, 1987).

The knowledge of this type of plant response to certain compounds can, however, play an important role in crop protection. Some researchers envisage that strategies and products may be developed that will allow the stimulation of plant alertness to pathogen invasion, rather like a vaccination procedure in animals. Through these means a stimulation of the synthesis of phytoalexin compounds could potentially guarantee a faster response to actual attacks (Kuc, 2001; Uknes *et al.*, 1996; Madamanchi & Kuc, 1991; De Wit, 1987). Further progress in the knowledge of the subject of plant responses to infection is expected to arise as a result of extensive research being developed and will hopefully help to optimise the protection measures adopted and reduce the level of detrimental chemicals applied in the future (Vigo *et al*, 2000).

## 1.3. SOIL-BORNE PLANT PATHOGENS

In a simplified way, plant pathogens have been classified as soil-borne when any part of its life cycle is obligatorily spent on the soil (Yarham, 1995; Parry, 1990; Wallace, 1978). According to this classification, they may range from those whose propagules contaminate the soil and function there in some way, to those that exist entirely in the soil. Excluded from the group will be organisms that, for example, are only found in the soil in a casual way and pathogens that spread from host to host by root grafting or through seeds but do not live in the soil itself (Parry, 1990; Bruehl, 1987).

Although in the laboratory a vast number of microorganisms can be isolated from soil, many of which are plant pathogens, fortunately only very rarely do devastating epidemics occur in the field. This demonstrates that although pathogens may be present in the soil, whether or not they are capable of infecting a host depends on a combination of several factors (Ward *et al*, 1994, Campbell, 1989). Apart from the initial inoculum potential, which has to be compatible with the host, germination of propagules, movement to the root and growth in the root are required (Benson, 1994 and Bowen, 1979). The succession of these different stages through to infection is greatly dependent on the rhizosphere environment.

### The Rhizosphere as an environment for the growth of Soil-borne Pathogens

The concept of the rhizosphere has evolved through the years but its inclusion as an essential part of the soil occurred as early as 1904, when it was categorised by Hiltner (Bruehl, 1987) as the portion of soil in the immediate vicinity of roots that is directly influenced by substances which originated in roots and which favoured certain bacteria. Distinguishing properties of this section of the soil are the higher numbers and activity of

microorganisms as compared to other parts of the soil which are root free. The influence of roots in the soil microbial population diminishes with distance and in 1965 Katznelson (Bruehl, 1987) specified that the term should be used to refer to "the thin layer adhering to the root after the loose soil and clumps have been removed by shaking".

The root surface itself, or rhizoplane, is the host of especially intense biological activities which in turn reflect upon the close soil environment thus allowing for the establishment of the rhizosphere (Bruehl, 1987; Foster, 1985). In comparison with bulk soil, populations of bacteria, fungi, nematodes, protozoa and algae are all greater in the rhizosphere. In the rhizosphere microflora and microfauna can find a more stable environment than in the bulk soil as well as a continuous source of food from root exudates. Several metabolites have been identified as exudates: volatile and gaseous molecules, like ethanol and methanol, sugars and amino acids (Trolldenier, 1979). Researchers have found that the rhizosphere influence in the soil environment follows the development of the plant, thus, its activity is greater when the plant is actively growing (Rovira, 1959).

According to Bowen (1979) microbial growth over the rhizoplane concentrates in the grooves between epidermal cells where the highest exudation rates occur. These exudates have also been linked with the germination of spores in the soil, including those of pathogens (Bowen, 1979). Furthermore, it is thought that root exudates not only provide the initial stimulus for germination of propagules, they also seem to orientate the pathogen towards a suitable host and provide energy and enzymes essential for the initiation of the infection process that leads to pathogenesis (Mitchell, 1979). On the other hand, there is evidence indicating that some root exudates are toxic to microorganisms and that they might be released as part of a *voluntary* anti-microbiological action (Bowen, 1979).

Although root exudates released to the rhizosphere may present a window for pathogen attack, they also favour the development of non-pathogenic microorganisms. These, in turn, can be either commensals (with no significance to plants either negative nor positive) or beneficial to the plant in various ways, as in the case of mycorrhizal fungus (Bowen, 1979). Some beneficial rhizoplane microorganisms produce, for example, metabolites which can have an antibiotic nature that affects directly or indirectly potential plant pathogens. Indirectly, the microfauna and microflora which subsist in the rhizosphere can also protect the roots from pathogen attack through competition for the same type of basic needs for survival such as nutrients, water, space, atmosphere. It is evident therefore, that there is a fine balance in the rhizosphere established between the root and its environment. The successful control of plant soil-borne pathogens requires an understanding of the factors that affect them and the inter-actions in which they are involved (Bowen, 1979).

## 2. PLANT PROTECTION STRATEGIES

Plants exploited in agricultural systems are at a greater risk of disease attack than in other ecosystems primarily due to the adoption of mono-culture systems which favour pathogen specialisation and the spread of epidemics. Epidemics such as the one caused by the potato blight in Ireland at the beginning of last century will remain in history due to its disastrous consequences, but other plant epidemic events have also caused serious problems to human society through the centuries (Schumann, 1991, Campbell & Madden, 1990, Zadoks & Schein, 1979). Since the birth of agriculture man has had to fight against various types of plant pests and diseases in order to prevent their destruction and this struggle persists in modern farming where the need for production optimisation is greater than ever. Over time an extensive array of crop protection methods has been developed by man, however, despite all the efforts, disease, pests and weeds are still responsible for huge crop losses world-wide (Herwitt, 1998; Pimentel & Greiner, 1997, Clark, 1995, Schumann, 1991).

Plants themselves have evolved to withstand attack from their various natural enemies and although man has explored this ability, for example through the selection of resistant lines, many scientists are of the opinion that better crop protection measures will be developed if we can attain a better understanding of plant defence mechanisms (Heil, 2001, Kombrink & Schmelzer, 2001, Zehnder *et al*, 2001, Ouchi, 1991). These authors and others argue that a more efficient use of agro-chemicals could be made if mechanisms involved in induced systemic resistance (ISR), hypersensitive response (HR) and the release of phyto-alexins, for example, could be better understood and explored on a commercial basis (Kuc', 2001; Métraux, 2001; Tuzun, 2001; Sinha, 1995). It is therefore believed that biotechnology should be able to make an impact in the way intensive farming

is carried out in order to help resolve some of the problems brought about by the pesticide dependent agricultural production of the twentieth century (Harman, 2000; Hokkanen, 1997; Dent, 1995).

## 2.1. Conventional Crop Protection Systems

Conventional or orthodox farming systems, have been criticised for relying on high inputs of agro-chemicals in order to sustain the high returns expected from cultivated land (Harman, 2000, Hodges, 1981, Lairon *et al*, 1981).

For a long time pesticides were generally considered beneficial since they were perceived as essential to the production of food in quantity at a guaranted quality demanded by modern society. Currently, the view on the benefits brought about by pesticides is no longer so positive, nevertheless, they are still relied upon in the vast majority of agricultural systems worldwide (Herwitt, 1998; Perkins & Patterson, 1997). Some of the longer term effects of pesticides on the environment became apparent through the mid to late 1900's and slowly the perception of the general public on their heavy, and often indiscriminate use, has became less favourable. Together with possible detrimental effects of pesticides on the environment and public health, the development of resistance to certain active ingredients and the non-existence of pesticides effective against some plant pests and pathogens emphasises the need for the implementation of alternatives for their control. Soil-borne plant pathogens such as *Gaeumannomyces graminis* var. *tritici*, the cause of take-all in wheat and *Phytophthora fragariae* var. Hickman, the cause of red core of strawberries, are amongst the group of economically important diseases for which no satisfactory control measures exist (Yarham, 1995, Manners, 1993). Rotations have traditionally been employed in order to reduce losses of such soil-borne diseases and these

can often result in lower returns to the farmers as substitute crops are frequently less profitable.

The combined efforts of environmentalists and scientific researchers in response to public requirements, have slowly generated some changes in the way pesticides are used. Some specific chemical compounds have been abandoned in developed countries and a reduction on the overall use of pesticides is generally perceived as the way forward for a safer diet and environment. The changes implemented have become more apparent in developed countries, such the USA, where the concept of integrated pest management (IPM) had its origins as early as the 1940's (Perkins in: Perkins & Patterson, 1997). Studies of sustainable, integrated and biological systems have already produced some examples of viable substitutes for pesticides in specific cases, particularly in glasshouse production systems (Harman, 2000, De Freitas and Germida, 1991). The scientific community has still, however, not attained alternatives to pesticides that might be competitive enough in the more variable environmental conditions of the field, and thus the great majority of farmers continue to resort to the classic (chemical) control strategy (Herwitt, 1998; Perkins & Patterson, 1997, De Freitas and Germida, 1991).

Alternatives to synthetic chemicals for the control of soil-borne diseases have proved hard to attain and this is attributable partly to the difficulties involved in the study of these types of pathogens and the consequent lack of knowledge of their pathogenic abilities (Paulitz, 2000). Other reasons for the reduced examples of non-chemical control methods for plant diseases in general, include the perception of fungicides as less dangerous than other pesticides and the possibility of using various types of compounds to overcome the development of resistance. In intensive conventional farming the application of fungicides in a routine prophylactic manner is still seen as justified if maximum economic yields are to be obtained (Clark, 1995, Jordan & Hutcheon, 1995, Lucas, 1995).

## 2.2. Alternative Crop Protection Systems

Most alternative crop protection methods support the idea that control measures should be applied only when disease severity reaches a certain economically damaging level. Such severity levels have been identified and defined as the threshold levels for specific, common and potentially economically damaging pathogens. The main aim of identifying such levels is to eliminate or reduce the incidence of factors that favour disease establishment and spread in order to ensure that, with minimal fungicide applications a good yield level is still attained (Jordan & Hutcheon, 1995, Strange, 1993, Cammell & Way, 1987). These and other principles are based on the view that more sustainable agricultural practices have to be implemented in order to safeguard the environment and public health (Bailey, 1997).

The development of threshold levels linked with risk assessment strategies has been particularly successful in cereals and other crops traditionally produced in the conventional way as there was an extensive body of studies both on the plants and the pathogens and their inter-relations with environmental factors that allowed for a better forecast of disease epidemics and their management (Bailey, 1997, Pimentel, 1997, Strange, 1993, Cammell & Way, 1987). Risk of disease epidemics is reduced through the adoption of various measures including the use of resistant cultivars, cultivation of alternative crops, implementation of more carefully planned rotations, use of different husbandry methods, use of clean seed and propagation material (Jordan *et al.*, 1990; Nychas & Peter, 1990; Speeding, 1990). In more recent years the development of serological methods has also allowed for the production of more advanced diagnosing techniques such as ELISA (Enzyme Linked Immunosorbent Assay), which is already available in the form of user-

friendly kits for specific pathogens (Jordan & Hutcheon, 1995; Miller & Joaquim, 1993; Strange, 1993; Tait, 1987; Van Emden, 1987; Symons, 1984).

**Biological Control**

In the organic or biological crop production system, various strategies are adopted to control the natural enemies of plants without resorting to synthetic chemical pesticides. Crop protection is attained chiefly through the manipulation of organisms present in the crop environment using cultural means (Campbell, 1989). In plant pathology however, Garrett's definition of biocontrol is more generally adopted (Fry, 1982). Garrett (1965) stated that biocontrol is "*any condition under which or practice whereby, survival or activity of a pathogen is reduced through the agency of any other living organism (except man himself), with the result that there is a reduction in the incidence of the disease caused by the pathogen*". As such, it presents an alternative to conventional farming. Some authors, however, argue that the biological control of plant diseases thus defined should be counted as a component of integrated production (IP) systems (Hokkanen, 1997). They argue that biocontrol should only be used to refer to the control of plant disease by means of antagonistic microorganisms, as defined by Cook & Baker (1983). Aside from the definition adopted, the biocontrol of plant diseases is difficult and, in the opinion of some authors (Paulitz, 2000; Campbell, 1989; Handelsman & Parke, 1989; Mitchell, 1979) it is particularly problematic in the case of soil-borne diseases. According to those authors, only if a very complete knowledge of all factors involved in each particular infection process is attained, can success in controlling disease through biological agencies ever be achieved. Clearly, this is a very ambitious goal that is still very far from being attained.

Biological control of *Phytophthora* species, for example, has been faced with difficulties due to their ability to produce several forms of inoculum: zoospores, sporangia, chlamydospores, oospores and mycelium. That ability is strengthened by the capacity to rapidly reproduce in any of these forms and also to penetrate and infect a host plant within hours (Erwin and Ribeiro, 1996). In addition, some pathogen species can survive at a soil depth where antagonists would struggle to exist. Furthermore, in some cases, a wide range of plants can be used as alternative hosts (Campbell, 1989).

In the rhizosphere three mechanisms of biocontrol may occur singly or simultaneously. The biological control agent might produce volatile and/or non-volatile antibiotics or toxic metabolic products which inhibit pathogen growth (amensalism); an active contact can be established between the microorganisms and degradation of hyphal walls or phagy of whole propagules may occur (parasitism and predation) or two or more microorganisms might demand the same limited resource(s) such as nutrients, oxygen, water and space (competition). Ideally, a biocontrol agent would successfully employ several of these strategies to overpower the pathogen(s) in question (Paulitz, 2000, Finlay & MacCracken, 1991, Campbell, 1989 and Dean, 1983).

Despite the problems involved in the identification of suitable biocontrol agents, over recent years some success has been achieved and products have been developed that have shown promising performances in the field against various types of pathogens (Kurze *et al.*, 2001, Jenkins & Grzywacz, 2000, Lewis & Lumsden, 2000). Organisms with biocontrol ability are varied but *Pseudomonas* and *Trichoderma* are amongst the most promising rhizosphere microorganisms successfully explored so far (Zehnder *et al*, 2001, Mehrotra *et al*, 1987 & Wells, 1987).

Biocontrol agents of plant diseases are normally introduced in high doses to the pathogen environment. The effectiveness of such high dosages, classed as inundative or

augmentative, is often not as high in the field as when they are tested in a controlled environment (Johnson, 1999). This can be due to several factors, thus, some quality control strategies are being enforced to ensure reliability of commercially available products. This has become particularly important as the interest for this type of control agent is growing and the release of successful commercial products is likely to rise as a response to the demands (Jenkins & Grzywacz, 2000).

Some strategies adopted in alternative farming systems are essential for the success of biocontrol agents. It is considered that biological control can be achieved by the introduction, augmentation, inoculation, inundation and conservation of microorganisms, thus, several techniques can be adopted to contribute to its success. By means of fertilisation, husbandry, crop rotations, sanitation, for example, the environment of the crop can be modified in order to foster the growth of the beneficial microorganisms and disfavour pathogenic ones. These techniques are also essential for new or introduced biocontrol agents at the time of their release, during their establishment in the new environment and for their continued action and survival there (Harman, 2000; Dent, 1995; Campbell, 1989).

## Seaweed Extracts as biocontrol and bio-stimulant agents

Seaweed extracts have been used as organic fertilisers for centuries with many favourable effects on plants being reported in different regions of the world. Nevertheless, investigations into the responses of plants to such products have only taken place since the late 1960's (Aitken and Senn in: Cogram, 1994). Investigation of the properties of seaweed extracts has recently become more intense and various research workers have found evidence which explained earlier claims that seaweed amendments enhanced crop growth

and generally improved crop health (Walsh, 1997; Whapham *et al*, 1993; Steveni and Norrington-Davies, 1993; Steveni *et al*, 1992; Nelson and van Staden, 1984). A number of studies have been carried out in order to detect the processes involved in the reported claims, in different plant species and environments. Amongst other effects, it has been shown that Maxicrop seaweed extracts from *Ascophyllum nodosum* reduced fecundity of *Meloidogyne javanica* (root-knot nematode) (Whapham *et al*, 1994), enhanced fungicide activity against *Erysiphe graminis* (cereal mildew) (Steveni and Norrington-Davies, 1993), increased chlorophyll content of plants (Whapham *et al*, 1993) and improved growth of hydroponically grown spring barley (Steveni *et al*, 1992).

The research work most recently developed has indicated that multiple spray treatments with Maxicrop seaweed concentrate could increase resistance to frost in winter barley plants, this being associated with the up-regulation of certain proteins (Burchett, 2000). Applications of different Maxicrop products increased rhizosphere beneficial microorganism populations and activity (Walsh, 1997, Cogram 1994; Pattison, 1994). It was found that seedling and crop growth could be improved and disease infections restrained as a consequence of the stimulus on the beneficial rhizosphere microorganisms (Walsh, 1997, Cogram 1994; Pattison, 1994). In particular, the growth of several *Pseudomonas fluorescens* species and their production of siderophores was increased by applications of Maxicrop concentrate (Walsh, 1997).

**Active substances in seaweed extracts:**

**a) Nutrients**

Seaweeds are rich in major and trace nutrients and as such they are considered suitable for use as soil supplements, especially for sandy soils (Verkleij, 1992). Liquid seaweed extracts at normal application rates, however, are unlikely to provide an adequate level of

nutrition if they are not supplemented with additives as they are normally applied in very low dilutions. The recommended low dilutions seem to refute the hypothesis that these extracts may have a beneficial effect on plant growth through nutrition (Table 1).

Although nutrient content of seaweed extracts may be very small, some authors are of the opinion that they may be sufficient to correct marginal deficiencies (Jeaninnin *et al.*, 1991; Aitken and Senn, 1965). Mineral elements have thus been assumed to be partially responsible for yield enhancing responses in nutrient deficient wheat by extracts of *Ecklonia maxima* prepared by the cell burst technique (Beckett and van Staden, 1990a; 1990b). This assumption can nevertheless be criticised and other authors (Sanderson and Jameson, 1986; Blunden, 1977) have instead hypothesised that because this type of extract has been reported to contain relatively high concentrations of cytokinin this could explain an improvement in yield under stress conditions. Cytokinins are capable of reducing the effects of nutrient deficiency on yield if applied during flowering in barley and wheat (Temple and Bomke, 1989). In view of the fact that seaweed extracts, applied as foliar sprays, supply very limited amounts of nutrients it is thought that their reported beneficial effects may be due to one or a group of organic compounds such as alginates, mannitol, fucoidan, growth regulators and laminarin (Blunden *et al.*, 1968).

Although some seaweed extracts have been marketed as soil conditioning agents, such claims have been questioned by researchers who propose that the alginates present in the extracts are of poor quality and their rate of application is too small to produce significant changes on soil physical properties (Blunden *et al.*, 1992a).

Table 1: Nutrients supplied by two commercial seaweed extracts (Abetz, 1980).

| Nutrients | Maxicrop Concentrate g $l^{-1}$ | Seaol Liquid |
|---|---|---|
| Nitrogen | 3.5 | 1.80% |
| Phosphorus | 1.2 | 0.18% |
| Potassium | 5.5 | 2.55% |
| Calcium | 0.5 | 0.20% |
| Magnesium | 1.4 | 0.16% |
| Sulphur | 1.4 | 0.14% |
| Iron | 1.1 | 24 ppm |
| Iodine | 2.5 | - |
| Boron | 0.011 | 0.5 ppm |
| Copper | 0.05 | 54 ppm |
| Cobalt | 0.014 | - |
| Manganese | 0.044 | 3 ppm |
| Molybdenum | 0.014 | 3 ppm |
| Zinc | 0.18 | 15 ppm |
| Sodium | - | 480 ppm |
| Chloride | - | 0.67 ppm |

**b) Growth Regulators**

Various types of organic growth regulators have been identified in different groups of seaweeds and their extracts. Although the seaweed extracts are generally applied at low concentrations, significant plant growth enhancement has been reported and this effect has been attributed mainly to the existence of growth regulators in their organic fraction (Temple and Bomke, 1989; Abetz, 1980; Blunden *et al.*, 1968).

**Gibberellins**

On freshly prepared extracts of *A. nodosum*, and species of *Laminariaceae* and *Fucaceae*, Williams *et al.*, (1981) detected gibberellin-like activity using the lettuce hypocotyl elongation bioassay. The activity detected was, however, only significant when the extract was fresh and rapidly declined with time. Commercial seaweed extracts remain active after industrial processing and a more or less prolonged storage, therefore, it can be presumed that gibberellins are unlikely to be one of its active compounds.

**Auxins**

Auxins have important roles in plant growth increasing fruit set, improving fruit quality and decreasing fruit drop as well as stimulating cell growth and lateral root induction (Davies, 1995 & Barlow, 1987). Due to the significance of these compounds a body of research has been developed in order to find out whether they could be present in seaweed extracts and thus be responsible for their activity. Indole-3-acetic acid (IAA) was reported to have been identified in green algae *Caulerpa paspaloides* and in brown seaweed *Undaria pinnatifida* using Gas Chromatography-Mass Spectrometry, (GS-MS), (Abe *et al*, 1972).

Auxin-like activity has been detected in extracts of *Ecklonia maxima* (Crouch and van Staden, 1991) and of *A. nodosum* (Mowatt, 1965) employing bioassay techniques. More recently, however, other authors have not been able to detect auxin-like activity in *A. nodosum* extracts using similar bioassay methods (Williams *et al*, 1981). Identification and quantification of auxins has, nevertheless, been obtained by alternative methods.

Kingman and Moore (1982) found magnitudes of 50mg per gram dry weight of IAA in alkaline extracts of *A. nodosum*. More recent quantifications gave rise to lower

values, estimated to be at a level of 6.63 μg ± 0.29 μg $kg^{-1}$ dry weight of dried seaweed extract (Sanderson *et al.* 1987).

Although auxins and other IAA have been identified in brown algae their presence has not been confirmed in extracts. This could be justified by the instability in aqueous media, or due to decomposition during processing of seaweeds. The variety in techniques used and auxin concentrations found in various extracts cause difficulties in making comparisons between studies and assessing significance of results. Hence, it is not possible to decide whether the concentrations of auxins in seaweed extracts can explain part of their reported effects on plant growth. This is clearly an area requiring further investigations if the use of seaweed extracts is to be optimised.

**Cytokinins**

Cytokinins are crucial to plant growth as they stimulate cell division and photosynthesis, are involved in the synthesis of RNA and proteins and the metabolism of carbohydrates and are responsible for delaying senescence in higher plants (Davies, 1995 and Barlow, 1987).

Cytokinins have been identified in various algae species including *Caulerpa, Valoniopsis, Udotea* (Farooqui *et al.*, 1990), in *Porphyra*, *Sargassum* (Zhang *et al.*, 1992) and in the commercial extracts of *Durvillea* spp. (Tay *et al.*, 1985), *Macrocystis pyrifera* (de Nys *et al.*, 1990) and *Ecklonia* (Featonby-Smith and van Staden, 1984a). Cytokinin content of the extracts of *M. pyrifera* varied with time of harvest (de Nys *et al.*, 1990) indicating that seaweed collection should be monitored in order to identify most favourable timing. Some authors (Blunden *et al.*, 1984) have argued that contrary results have been obtained with bioassays employed to study cytokinin activity and that they could have been affected by other growth stimulants. In their view, results should consequently be

interpreted cautiously if not supported by other methods. Thus, although different bioassays have shown that several seaweeds and seaweed extracts have cytokinin-like activity and that concentrations of the phyto-hormone present are sufficient to produce physiological changes, a direct proof by HPLC or GC-MS analysis is still required.

**Betaines**

Betaines are essential to production of chlorophyll thereby improving plant growth and delaying senescence (Davies, 1995). Betaines and/or tertiary sulphonium analogues have been extracted from various marine algae species by Blunden *et al.*, (1992b; 1986; 1984). From *A. nodosum*, the seaweed species most commonly exploited for agricultural extracts, several betaine and analogues were extracted at concentrations that could be considered sufficient to induce plant growth (Blunden *et al*, 1986). Betaines were also found in seaweed extracts at varying concentrations. Alkaline extracts from *A. nodosum* have been demonstrated to significantly increase chlorophyll content using the cucumber cotyledon bioassay (Whapham *et al.*, 1993), such effects were thought to be a response to betaines.

It has been thought that betaines may play important roles in plant disease resistance processes, particularly against biotrophic fungi (Manninger *et al.*, 1992). It has been demonstrated that betaines could reduce disease impact of *Puccinia graminis* (black rust) and *Puccinia recondita tritici* (brown rust) on wheat and *Sphaerotheca fuliginea* (powdery mildew) on cucumber.

Conversely, betaines have been found to stimulate hyphal extension leading to a quicker expansion of fungal colonies of *Fusarium graminearum*. Betaines have thus been implicated in increased susceptibility of wheat to *F. graminearum* (Wiebe *et al.*, 1989). It was demonstrated that this pathogen has a high-affinity transport system for betaine and

choline which could account for a heightened survival capacity (Robson *et al.*, 1994). More investigations should therefore be carried out in order to ascertain whether a similar type of relationship exists for other fungal species, particularly of the soil-borne pathogens.

**Abscisic acid**

In commercial extracts of *A. nodosum* abscisic acid has been identified by Kingman and Moore (1982) using gas chromatography, however, at the concentrations detected no stimulating effect on root extension was considered likely (Finnie and van Staden, 1985).

**Lectins-aldehydes and Ketones**

True lectins and polyphenols have been found in brown algae (*Phaeophyceae*) and although these compounds may not survive industrial extractions they can be broken down into aldehydes and ketones which can influence fungal activity (Rogers and Fish, 1991). Phenolic compounds make up 2-10% (weight per dry weight) of seaweed dry matter. Relative amounts vary depending on season and salinity at the site of seaweed growth (Zavodnik and Jensen, 1989).

**1-aminocyclopropane-1-carboxylic Acid**

Nelson and van Staden (1984) have found that applications of seaweed extracts, industrially prepared using a cell burst technique, increased thickness of wheat culms as a consequence of increased cell size of vascular bundles. Ethylene is considered responsible for this effect. The same researchers (Nelson and van Staden, 1985) have identified 1-aminocyclopropane-1-carboxylic acid, a naturally occurring ethylene-releasing agent, in the seaweed extracts using the thin-layer and gas liquid chromatographic techniques.

**Polyamines**

Some polyamines may have regulatory effect on plant growth, despite not considered as phytohormones, and they can also have fungicidal properties. Although these compounds have not been reported in commercial seaweed extracts polyamine-like substances have been found in unicellular red algae such as *Cyanidium caldarium* (Crouch and van Standen, 1993).

## Aims of this Research Investigation

Empirical observations of beneficial effects of seaweed based products on crop production and health led to an interest in commercializing their extracts and, consequently, it encouraged systematic studies on the reported benefits (Steveni *et al*, 1992, Blunden, 1991, Metting *et al*, 1990 and Pesando, 1990). The literature review indicated that investigations carried out on responses of plants and specific soil microorganisms, provided evidence to suggest that seaweed extracts influenced several of the components of rhizosphere phenomena (Magne, 1993, Metting *et al*, 1990 and Meeting, 1987).

Biological phenomena are complex and it is commonly known that responses of the same organisms may vary under similar environmental conditions even when research is carried out in the laboratory. It is, therefore, often necessary to develop an extensive and detailed study of the same processes in order to arrive at sound conclusions. Previous research into the responses of several plant and microorganism species to Maxicrop seaweed extracts has contributed to enlarge the knowledge of the effects those products have and, consequently, it has assisted in use improvement. Those investigations have, however, frequently been hampered by inconsistency (Cogram, 1994, Pattison, 1994 and Walsh, 1997). Consequently further study allowing for a critical review of concepts already in existence and the development of new hypotheses was required.

Research has so far indicated that Maxicrop seaweed extracts can affect the life cycle of *Phytophthora cinnamomi* inducing morphological changes under *in vitro* conditions (Pattison, 1994). Varying degrees of control were reported for other plant pathogens and pest organisms under various environmental conditions. Those responses were thought to be associated with or dependent upon beneficial microbial activity

implemented by the seaweed extracts (Walsh, 1997, Cogram, 1994 and Pattison, 1994). Although responses reported were promising under strict environmental conditions, the effects became less significant as the control over settings was reduced.

The current reported research aimed firstly to investigate the degree of influence of a Maxicrop seaweed extract – Maxicrop Concentrate - on specific plant and plant pathogen species and their interactions under various environmental conditions. This study focused on wheat and strawberries their important soil-borne pathogens: *Gaeumannomyces graminis* and *Phytophthora fragariae* Hickman, respectively. The objective was to study the responses to the Maxicrop seaweed extract under systems where environmental conditions would be progressively less controllable, from *in vitro* to hydroponics, glasshouse and the field situation. It was hypothesized that previous research results sometimes lacked significance in practical agronomic terms due to the fact that investigations were carried out using controlled artificial systems. This investigation aimed to overcome this criticism by using simple and controlled systems at a first stage and progressively expanding the study to biologically more complex settings.

*Gaeumannomyces graminis* and *Phytophthora fragariae* Hickman are soil-borne plant pathogens of great economic importance worldwide and, since previous researchers have reported significant responses to Maxicrop seaweed extracts by *G. graminis* and *Phytophthora cinnamomi*, another member of *Phytophthora*, it was thought that such responses should be further explored. *Phytophthora fragariae* can be of greater value in investigations of sporangia formation and zoospore release than *Phytophthora cinnamomi* as it can be induced to produce those structures in greater abundance (Grant *et al*, 1985). Responses to different treatments *in planta* can also be simplified as the host of this pathogen has a shorter life span. It was consequently decided to extend and diversify the studies made with *P. cinnamomi* to *P. fragariae*.

## Project Objectives

1- Study the response of *Gaeumannomyces graminis* and *Phytophthora fragariae* to Maxicrop concentrate seaweed extract when grown *in vitro* in agar medium.

2- Determine and analyse the response of *Phytophthora fragariae* to Maxicrop concentrate seaweed extract *in vitro* in liquid medium. Identify and record induced morphological changes if any.

3- Determine and analyse the responses to Maxicrop concentrate seaweed extract on wheat and strawberry plants and the infectivity of *Gaeumannomyces graminis* and *Phytophthora fragariae* Hickman under controlled environment on hydroponics.

4- Study the responses to Maxicrop concentrate seaweed extracts of wheat and strawberry plants and the infectivity of *Gaeumannomyces graminis* and *Phytophthora fragariae* Hickman when the plants were grown in pots in a glasshouse environment.

5- Investigate the response to Maxicrop seaweed extracts of wheat plants and the infectivity of naturally occurring *Gaeumannomyces graminis* in the field environment.

**Note:**

During the course of this work experiments were undertaken on other plant/pathogen systems and whilst the results of these do not form part of the thesis presented, they are included in Appendix 3 for completeness and for the benefit of the sponsors of the project.

# CHAPTER II

## Study of the Influence of *Ascophyllum nodosum* Extracts on the Growth of Wheat and its Interactions with *Gaeumannomyces graminis*

### Take-all - *Gaeumannomyces graminis var. tritici*

*Gaeumannomyces graminis* is a soil-borne pathogen that causes take-all disease in different *Gramineae* being generally severe in intensive cereal farming systems where it is a major contributory factor in yield decline. The inoculum of this disease can survive in the soil, as saprophytic mycelium, for up to 2-3 years using as alternative nutritional substrates roots and stem base debris in the soil, volunteer cereal plants and grass weeds. The amount of inoculum builds up with consecutive cereal cropping. The *var. tritici* Walker is the most important strain and its main hosts are wheat, barley, rye and triticale although it can also infect grasses (Manners, 1993).

Disease infection is favoured by mild soil temperatures (10-23°C) and wet soil conditions in spring. Autumn sowings are most at risk, especially with poor drainage and low fertility soils since these conditions are not favourable for strong root and plant establishment and growth.

The saprophytic mycelium attack of this disease is thought to be stimulated by exudates of young roots. The fungus spreads along the root cell system forming a network of brown runner hyphae. Eventually, roots rot becoming brown or black due to the growth of fungal hyphae and thus, their absorption of water and nutrients can be extremely impaired. Root infection may occur at all growth stages but early attacks frequently reduce seedling survival (Smith *et al*, 1988). Above-ground symptoms caused by this disease are stuntedness of young plants and yellowing of outer leaves. Severely infected mature plants are dwarfed, tillering is reduced and the ears may become infertile showing a grey to white

colour - whitehead symptom. The fertile inflorescences generally produce malformed grain of poor quality. The yield loss can reach 20% in the second and third year during successive cereal cropping seasons (Parry, 1990). Take-all initially occurs in roughly circular patches but it can gradually extend to the whole field.

At present there are no resistant cultivars or direct reliable chemical methods against this disease, therefore, other strategies have to be used to reduce the amount of damage that it can cause. The implementation of break crops together with the adequate use of fertilisers and correction of drainage and soil structure are all useful to help improving plant establishment thereby reducing the scope for disease damage (Parry, 1990).

Farmers may take advantage of the phenomenon called take-all decline in order to try to maximise their cereal production output over time. Take-all decline has been described as a natural reduction in disease expression after 3-4 years of successive wheat or barley production. This decline is not great enough, however, to allow the yield to recover the level achieved by a first year crop (1$^{st}$ wheat) or by a non-infected crop.

Due to the significance of take-all in terms of the agricultural economy intense research work has been developed in order to try to identify more suitable control strategies. Special interest has been placed on the investigation of factors possibly involved in take-all decline. Researchers have shown that it can be associated with antagonistic microbiological activity by *Pseudomonas fluorescens* although their mechanism of action has not yet been found. Work developed by Cogram (1994) has shown that the populations of *Pseudomonas fluorescens* present in the rhizosphere could be increased after applications of Maxicrop liquid seaweed extracts. In that work, a reduction on disease expression was obtained only when *Pseudomonas* were present.

## 1. Effect of Maxicrop Concentrate Seaweed Liquid Extract on the growth of *Gaeumannomyces graminis in vitro*

*In vitro* experiments were conducted to examine the response of *Gaeumannomyces graminis* fungus to treatments with Maxicrop concentrate extract of *Ascophyllum nodosum* seaweed. The responses of the fungus in the treated media were evaluated by carrying out measurements of colony radial growth and comparing this to the growth in standard media.

### Materials and Methods

Inoculation cultures of *Gaeumannomyces graminis* were grown in standard Potato Dextrose Agar medium (PDA, Oxoid) medium at 21° C until mycelia reached the edge of plates. A 1 cm plug was then cut from the advancing edge of the colonies using a flame sterilised cork borer and placed in Petri dishes containing either PDA or PDA media with MLSE at different concentrations. Unless otherwise stated, the MLSE used for all experiments was prepared by diluting 5 ml of the Maxicrop liquid seaweed concentrate in 1000 ml distilled water (DW). Table 2.1. indicates the concentration of some nutrients present in the seaweed extract under study. PDA medium treated with MLSE was prepared by replacing the DW with the appropriate amounts of diluted MLSE solution. Media was autoclaved as standard (120° C for 15 min).

Table 2.1: Nutrients supplied by the Maxicrop Concentrate seaweed liquid extract (Abetz, 1980).

| Nutrients | Maxicrop Concentrate (g $l^{-1}$) |
|---|---|
| Nitrogen | 3.5 |
| Phosphorus | 1.2 |
| Potassium | 5.5 |
| Calcium | 0.5 |
| Magnesium | 1.4 |
| Sulphur | 1.4 |
| Iron | 1.1 |
| Iodine | 2.5 |
| Boron | 0.011 |
| Copper | 0.05 |
| Cobalt | 0.014 |
| Manganese | 0.044 |
| Molybdenum | 0.014 |
| Zinc | 0.18 |

Two measurements of fungal growth over the agar were taken 5 and 9 days after sub-culturing and the experiments were terminated at day 10. The fungal colony diameter was measured along two axes chosen at random at right angles to each other and the mean of the two values taken. The daily growth rate was then calculated using the following formula:

d2 – d1 / number of days between d1 and d2;

d1= colony diameter 6 days after incubation; d2 = colony diameter 2-3 days after d1 (after Erwin & Ribeiro, 1996). Two different experiments were carried out and replicated twice.

All experimental results were analysed using One-way analysis of variance by Excel (Appendix 2 gives an example analysis). Where a statistical significant difference between samples was determined the Least Significant Difference (LSD) was calculated as

follows: LSD = t√(MSError:number replicates per treatment), and used to define significant differences between means.

### 1.1. *Gaeumannomycis graminis* - *In vitro* Experiment 1

The treatments studied were as follows:

1- 100% MLSE

2- 50% MLSE

3- 0% MLSE - Control

**Results**

The results of the experiment showed that *G. graminis* colony growth was not significantly affected by the presence of MLSE in the media although slightly larger colony sizes were found in the plates treated with the seaweed extract (Table 2.2). Observations of mycelium through out the experiment suggested, however, that mycelial growth was less dense in the MLSE treated media. This could have accounted for the initial faster growth observed indicating that MLSE might have interfered with the normal colony expansion over the media. It was also observed that fungal mycelial coloration was distinct in MLSE rich media. Where MLSE was present, mycelium was predominantly dark grey and distinct black strands were observed (Plate 1). These changes could imply that negative component(s) was/were present in the media and the fungus grew quicker in order to overcome such harmful element(s).

Table 2.2- *Gaeumannomycis graminis* colony Daily Growth Rate in standard PDA and MLSE treated PDA between the 6th and 9th d of incubation.

| Media Treatment | Average Daily Growth Rate (mm/day) |
|---|---|
| 100 % MLSE | 1.03 |
| 50% MLSE | 0.8 |
| 0% MLSE | 0.9 |

**1.2. *Gaeumannomyces graminis* - *In vitro* Experiment 2**

A second *in vitro* experiment was conducted in order to study further the responses of *G. graminis* to Maxicrop concentrate liquid seaweed extract *in vitro* to assess whether the responses of the pathogen to a range of concentrations of MLSE. This would also allow the determination of the critical concentration of the seaweed extract which could alter the growth of the fungus. An assessment of any quantitative response to the MLSE concentration could also be made. There were 4 replicates per treatment and the experiment was repeated twice.

The methods used were as for Experiment 1 and the treatments studied were:

1- 100% MLSE

2- 75% MLSE

3- 50% MLSE

4- 25% MLSE

5- 0% MLSE - Control

**Results**

Data from this experiment confirmed the results obtained in Experiment 1 and no significant difference in fungal colony sizes were found in any of the treatments although they were slightly larger in MLSE treated media (Table 2.3). The observations made of colony thickness and colour during Experiment 1, were also confirmed here (Plate 1). Colony density was also progressively reduced with the increasing MLSE concentrations. Darkening of mycelia was more evident as the concentrations of MLSE in the medium increased. These results confirmed that the seaweed extract induced some changes in *G. graminis* mycelial growth but it did not prove to be fungicidal or fungistatic.

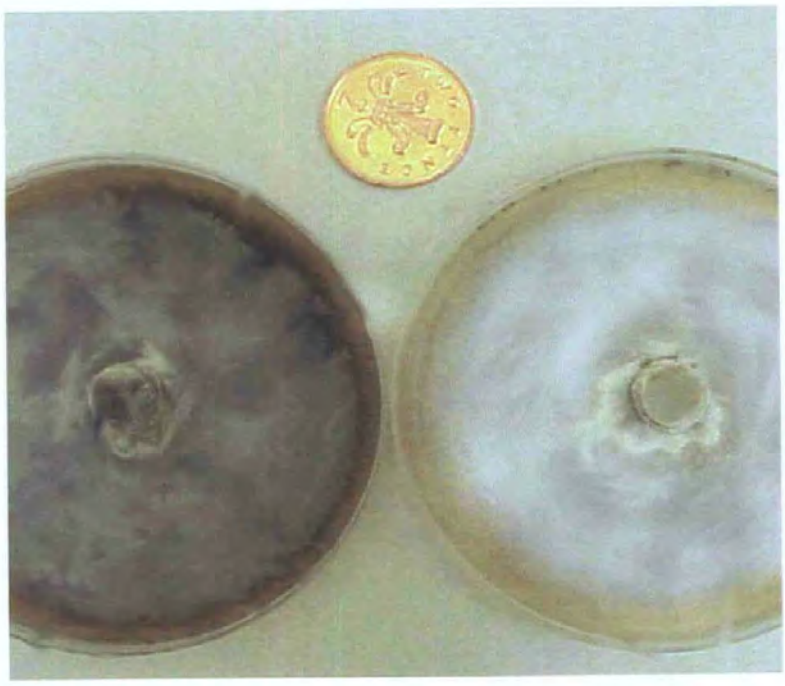


Plate 1 – Growth of *Gaeumannomycis graminis* over PDA amended with 100% MLSE (left Petri dish) and Control (right Petri dish).

Table 2.3- *Gaeumannomycis graminis* colony Daily Growth Rate in standard PDA and MLSE treated PDA between the 6th and 9th d of incubation.

| Media Treatment | Average Daily Growth Rate (mm/day) |
|---|---|
| 100 % MLSE | 0.85 |
| 75% MLSE | 0.88 |
| 50% MLSE | 0.9 |
| 25 % MLSE | 0.8 |
| 0% MLSE | 0.9 |

**Conclusions/Discussion**

Although *G. graminis* cultures grown in standard PDA may vary in colour from white to dark grey, the former is normally dominant. Some researchers claim that *G. graminis* cultures' pathogenicity varies not only with consecutive sub-culturing of original inoculum but also within the same colony. It has also been argued that mycelial inoculum of some particular colours can be more pathogenic (Dr. Pitt, personal communication). It was therefore hypothesised that although *G. graminis* colony growth was not significantly affected when MLSE was added to the PDA, its pathogenicity could have been affected. Experiments were conducted using a hydroponic system to explore this hypothesis.

## 2. Effect of Maxicrop Concentrate Seaweed Extract Liquid on *Gaeumannomyces graminis* infection and wheat plant growth in hydroponics

Hydroponics is the term generally applied to the production of plants without using soil as a substrate and feeding them on solutions of water and nutrient supplements (Jensen & Collins 1985; Harris, 1974 and Douglas, 1972). Allegedly, this system has some advantages over the growth of plants in soil such as facilitating the correct surplus of nutrients, maximising fertilisers use and increasing the degree of control over environmental abiotic factors which can affect plant growth. Another relevant advantage of the system is that soilborne plant diseases can more easily be prevented or even totally eradicated if the system and the plant material employed are uncontaminated (Harris, 1974). In plant pathological studies, particularly in the area of soil-borne diseases, this system can be of great use facilitating investigations into the effects of specific factors on the levels of infection caused by pathogens. Following the analysis of results obtained through *in vitro* experiments the responses of the pathogen to the Maxicrop Concentrate in the presence of plants was investigated and a hydroponic system was chosen for this purpose.

### General Materials and Method

Although several experiments were conducted using this system and the treatments investigated varied, the method and materials used were the same wherever possible and the preparation of growth substrates and other materials used is described below.

Two layers of blotting paper were placed to cover the walls of 250 ml glass beakers and the internal cavity was filled with Perlite (Horticulture Medium grade) and 100 ml of a nutrient solution (Phostrogen – 0.325 $gl^{-1}$). All the outer surfaces of the beakers were

covered with aluminium foil and then autoclaved for 15 min at 121°C to sterilise the system.

Four plugs (1 cm) of take-all cultures (10 -12 d old) were placed equidistantly from each other, between the beakers walls and the paper layer at approximately 5 cm from the base. Pre-germinated wheat seeds (variety Brigadier) were then placed approximately 1 cm above each fungal inoculum source. Seeds were pre-germinated after surface sterilisation for 15 min with 10% bleach solution prior to pre-germination and then incubated for in water soaked paper in a plastic container for 48 h until the radicle had emerged. A solution (15 ml MLSE or nutrient solution) was then added to each beaker according to the treatment under study. Nutrient solution was supplied twice a week and Maxicrop Concentrate solutions were applied once a week unless otherwise stated. Previous investigations (Cogram, 1994) have shown that stimulatory effects of Maxicrop seaweed extracts were not affected by autoclaving, therefore, all materials and liquid solutions applied were sterilised (120° C for 15 min) prior to use.

Experiment 1 was conducted in a walk-in growth chamber at approximately 20°C with a 16h day length. All other experiments were conducted in a phytotron (Fi-totron PG 660, Gallenkamp) with a 16h day length, at approximately 20°C day and 16°C night and with a relative humidity of 80%. The phytotron has frequently been used for physiological studies since its invention (Chourd, 1972) and presented some advantages for the present investigation when compared to the growth chamber. It allowed for more reliable control over environmental conditions such as temperature and relative humidity.

The duration of all experiments was 3 weeks, after which seedlings were harvested and disease symptoms assessed in seminal roots. Disease severity was assessed in each individual seminal root using the scale indicated in Appendix 1. Agronomic characters,

fresh and dry weight and number of leaves, were measured to assess plant growth response to the treatments applied.

**Preparation of soil inoculum**

The responses to a fresh soil inoculum were studied in some experiments as previous research by Cogram (1994) indicated that take-all disease symptoms were only reduced by amendments of Maxicrop seaweed extracts in presence of a soil microflora (principally *Pseudomonas* species). The soil samples were all collected from the same area of a permanent grass field. Soil was air dried at room temperature for 24 h and 1g was added to 90 ml DW and mixed thoroughly for 1 h. One ml aliquots were then applied in the appropriate pots.

**Preparation of *Pseudomonas fluorescens* solution**

In experiment 3 the effects of a cultured *Pseudomonas fluorescens* solution on the infections caused by take-all were studied. Pure cultures were grown in Nutrient Broth for 48 h at 22° C. Aliquots (1 ml) containing approximately $10^8$ bacterial cells $ml^{-1}$ were subsequently added to the nutrient solution in the pots on the first day of the experiment.

**Experiment 1**

The following treatments were studied:

1- NS + inoculum (Control inoculated)

2- NS + 0.75 ml MLSE $l^{-1}$

3– NS + 1 ml MLSE $l^{-1}$

4– NS + 2.5 ml MLSE $l^{-1}$

5– NS + 5 ml MLSE $l^{-1}$

6– NS + 7.5 ml MLSE $l^{-1}$

**Results**

Results of this experiment showed that the system allowed for normal wheat seedling growth and that the onset of disease had occurred in such a way that at the time of harvest varying levels of infection were found (Plates 2 & 3). In MLSE treated plants disease symptoms were less severe than in control plants, except for treatment with 0.75 ml MLSE where severity of infection was significantly higher than in the control. Analysis of levels of disease showed that plants treated with 5 ml MLSE $l^{-1}$ had significantly lighter take-all infections (Fig. 1.1). Data also showed that plants amended with MLSE showed increased mean plants' fresh and dry weights and this was more evident for treatment 5 (not statistically significant).

Fig. 1.1- Effect of MLSE on Take-all of Wheat grown in hydroponics. Columns with the same letter are not statistically significantly different from each other (P>0.05).

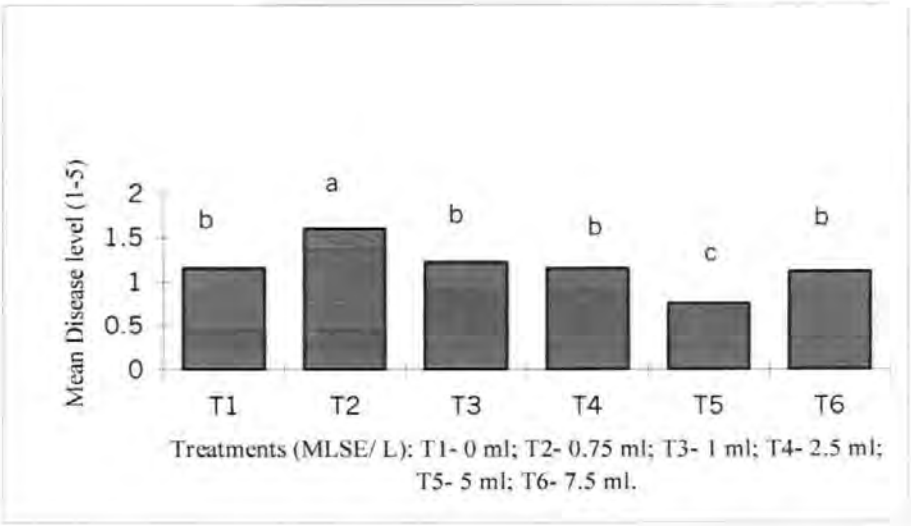


Plate 2- Wheat seedlings grown in hydroponics in phytotron. From left to right, plants submitted to treatments 1 to 6.

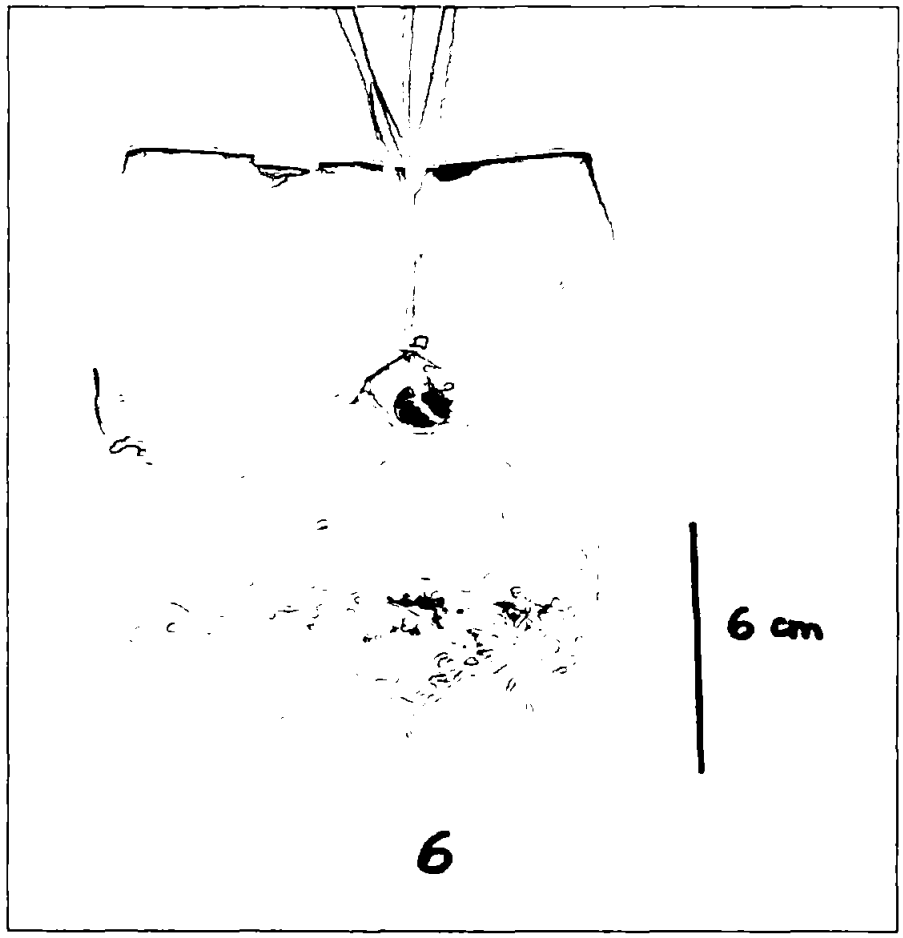



Plate 3- Detail of root system of seedlings submitted to treatment 6 showing light Take-all symptoms.

## 2.2. Effect of Maxicrop Concentrate Liquid Seaweed Extract on *Gaeumannomyces graminis* infection and Wheat Plant Growth in hydroponics - Experiment 2

Investigations by Cogram (1994) showed that Maxicrop extracts depended on the presence of a soil microflora to exert a suppressive effect on the infectivity of take-all on wheat plants. Following the results of the previous experiment it was thought that it was necessary to investigate whether the responses obtained could be influenced by additions of a soil solution that would provide an undefined microflora. Experiment 1 was therefore repeated with two additional treatments with soil solution and only one control - the inoculated control.

The following treatments were studied:

1 – Nutrient solution (NS) + inoculum (Control)

2- NS + 0.75 ml MLSE $l^{-1}$

3 – NS + 1 ml MLSE $l^{-1}$

4 – NS + 2.5 ml MLSE $l^{-1}$

5 – NS + 5 ml MLSE $l^{-1}$

6 – NS + 7.5 ml MLSE $l^{-1}$

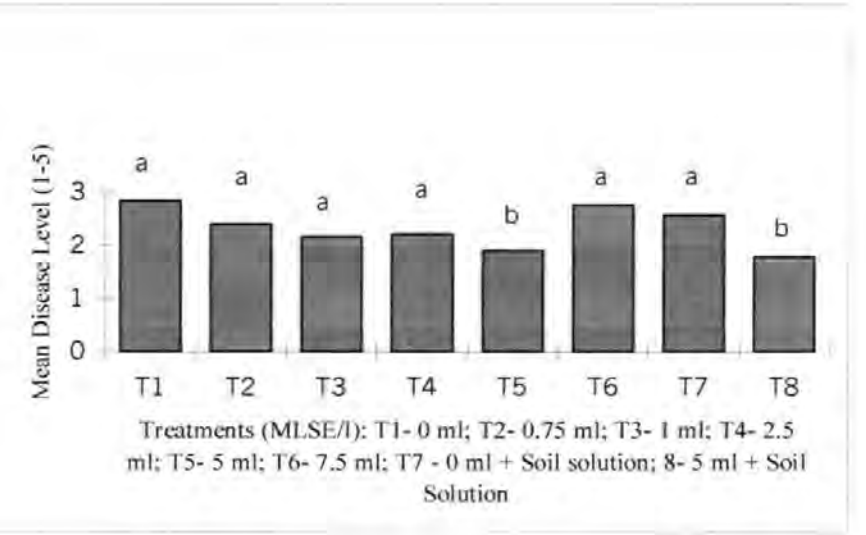
7 - NS + 1 ml Soil Solution (SS)

8 – NS + 5 ml MLSE $l^{-1}$ + SS

**Results**

A reduction of the severity of take-all disease infection of seedlings was observed in plants treated with MLSE (5ml $l^{-1}$) alone and MLSE and soil solution although this was not statistically significant (Fig. 1.2). Addition of the soil microflora alone (treatment 7) did not result in reduced take-all disease infection but when applied in conjunction with MLSE at the recommended rate (Treatment 8) infection was statistically significantly lighter than control. The response to that treatment was not, nevertheless, different from the treatment with MLSE (5ml $l^{-1}$) alone.

Fig. 1.2- Effect of MLSE on Take-all of Wheat grown in hydroponics. Columns with the same letter are not statistically significantly different from each other (P>0.05).



## 2.3. Effect of Maxicrop Concentrate Liquid Seaweed Extract on *Gaeumannomyces graminis* infection and Wheat Plant Growth in hydroponics - Experiment 3

There have been extensive investigations into the factors that can foster the phenomenon entitled take-all decline in consecutive cereal cropping. Different naturally occurring microorganisms, including *Pseudomonas* and *Actinomyces*, have been found to act as essential components of that process. Populations of *Pseudomonas fluorescens*, in particular, have been implicated as one of the most important take-all antagonists in the rhizosphere. An experiment was conducted to investigate whether *P. fluorescens* would have an effect on take-all infection when applied in solo and whether it would influence the response of plants to Maxicrop Concentrate seaweed extracts. The experiment also compared the performance of the bacterial solution to the soil solution previously used.

In the previous two experiments plants showed some signs of water stress, particularly over the last week of the trial. This stress could have affected the responses both to the pathogen and to the treatments applied and as a consequence, some measures were introduced to ensure that the amount of water present in the substrate was more constant. In this experiment, and all subsequent experiments, flasks were weighed at day 0 and at regular intervals of 2d and nutrient solution was added to maintain a constant weight through-out.

The following treatments were studied:

1 – Nutrient Solution (NS) – Control

2- NS + inoculum

3 – NS + 1ml Soil Solution

4 – NS + 1 ml of *P. fluorescens* Solution

5 – NS + 1ml Soil Solution + MLSE ($5ml l^{-1}$)

6 – NS + 1ml *P. fluorescens* solution + MLSE ($5ml l^{-1}$)

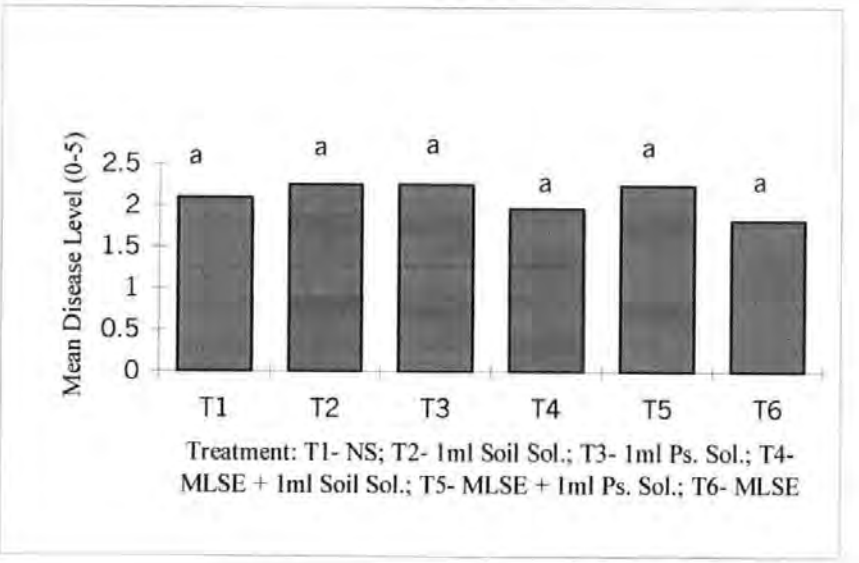
7 – NS + MLSE ($5ml l^{-1}$)

**Results**

Disease severity on MLSE treated plants was either equal or slightly lighter than in non-treated plants but these reductions were not statistically significant (Fig. 1.3). The results of the experiment also showed that neither *P. fluorescens* nor the soil solution when applied singly suppressed take-all disease. Disease infections found in plants treated with the soil solution and MLSE were slightly less severe than in non-treated plants. The infections found in plants amended with *P. fluorescens* solution, applied singly or in conjunction with MLSE, were slightly more severe than in control plants. The responses to both types of solutions were not, nevertheless, statistically significant.

The inoculum used caused moderate lesions and although plants treated with MLSE had a better general appearance throughout the trial, this did not translate into a better performance for any of the characters assessed (Number of Leaves, Fresh and Dry Weight) at the time of harvest.

Fig. 1.3- Effect of MLSE applied with soil and *Pseudomonas fluorescens* solutions on Take-all of Wheat grown in hydroponics. Columns with the same letter are not statistically significantly different from each other (P>0.05).



## 2.4. Effect of Maxicrop Concentrate Liquid Seaweed Extract on *Gaeumannomyces graminis* infection and Wheat Plant Growth in hydroponics - Experiment 4

This experiment was conducted to assess whether take-all inoculum was still pathogenically active after being grown on PDA containing Maxicrop Concentrate seaweed extract (as described for *in vitro* experiments 1 and 2). The method followed was the one described for the previous experiments and the inoculum sources and treatments used were the following:

1- Inoculum grown in PDA containing 5 ml $l^{-1}$ MLSE + nutrient solution (NS)

2- Inoculum grown in PDA containing 3.75 ml $l^{-1}$ MLSE + NS

3- Inoculum grown in PDA containing 2.5 ml $l^{-1}$ MLSE + NS

4- Inoculum grown in PDA containing 1.25 ml $l^{-1}$ MLSE + NS

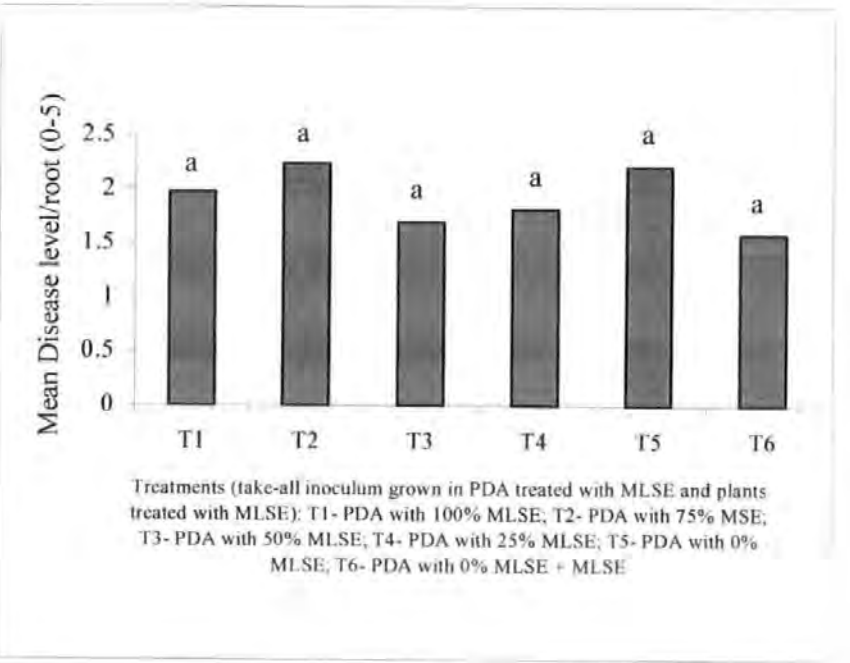
5- Inoculum grown in standard PDA + NS (Control)

6- Inoculum grown in standard PDA + MLSE (5ml$l^{-1}$) + NS

### Results

At harvest, take-all lesions on roots were present in all plants, indicating that all inoculum sources used were active. Hence, the results of the experiment imply that cultures of *G. graminis* were not affected in their capacity to infect the host plant when previously grown in PDA amended with MLSE (Fig.1.4). As in previous experiments, the addition of MLSE to the nutrient solution reduced disease symptoms slightly but the effects were not statistically significant here.

Fig. 1.4- Effect of MLSE on Take-all of wheat grown in hydroponics. Columns with the same letter are not statistically significantly different from each other (P>0.05).



## 2.5. Effect of Maxicrop Concentrate Liquid Seaweed Extract on *Gaeumannomyces graminis* infection and Wheat Plant Grown in Hydroponics – Experiment 5

In the previous experiment *G. graminis* inoculum grown in PDA amended with 5ml $l^{-1}$ and 2.5 ml $l^{-1}$ MLSE induced slightly less severe disease symptoms than inoculum grown in standard PDA medium (not statistically significant). A second experiment was conducted to investigate further the effect of these treatments in the presence and absence of a soil microflora. The following treatments were studied:

1- Inoculum grown in standard PDA + Soil Solution (SS) + NS

2- Inoculum grown in PDA containing 2.5 ml $l^{-1}$ MLSE + SS + NS

1- Inoculum grown in PDA containing 5 ml $l^{-1}$ MLSE + SS + NS

2- Inoculum grown in standard PDA + MLSE (5ml $l^{-1}$) + SS + NS

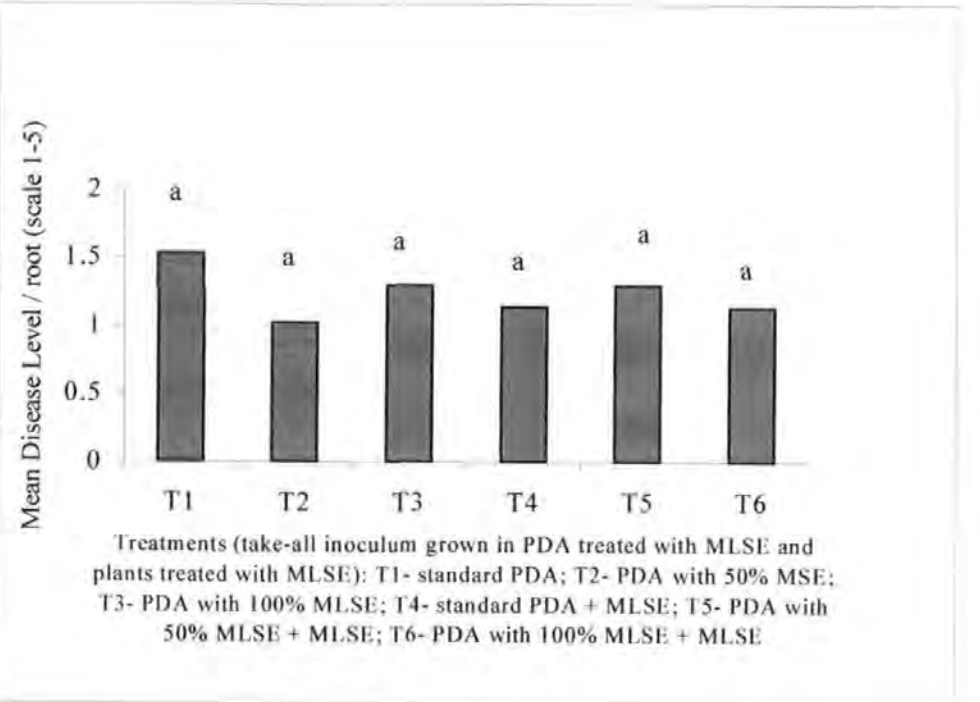
3- Inoculum grown in PDA containing 2.5 ml $l^{-1}$ MLSE + MLSE (5ml$l^{-1}$) + SS + NS

4- Inoculum grown in PDA containing 5 ml $l^{-1}$ MSLE + MLSE (5ml$l^{-1}$) + SS + NS

**Results**

Results of this experiment, as shown in Fig. 1.5, indicated that *G. graminis* inoculum grown in standard PDA was active but it's infectivity was low as indicated by the severity of lesions found. The results indicated, nevertheless, that the infectivity of the inoculum grown in PDA treated with different concentrations of MLSE was not affected. As in previous experiments, wheat plants inoculated with take-all grown in standard PDA (Treatment 4) and treated with MLSE showed reduced disease severity although this was not statistically significant.

Fig.1.5- Effect of treatments with MLSE on *G. graminis* of wheat grown in hydroponics. Columns with the same letter are not statistically significantly different from each other (P>0.05).



**Discussion and Conclusions**

Despite some inconsistency, the experimental work using the hydroponic system indicated that MLSE amendments could have a suppressive effect on the infectivity of take-all of wheat seedlings. The responses to the MLSE at the recommended rate (5 ml $l^{-1}$) were small but consistently positive and, in some instances, had a significant impact on take-all symptoms. The presence of a soil microflora had no effect on observed results neither affecting the control in the absence of MLSE nor enhancing or suppressing the MLSE effect at 5 ml $l^{-1}$. These results contrasted the previous findings by Cogram (1994 and Walsh (1997). Since the soil solution applied was an unknown bacterial mix further investigation with a known population was thought to be desirable. The treatment with a solution containing *P. fluorescens* did not, however, have an effect on the onset of the disease. Further to that, these experiments demonstrated that neither *P. fluorescens* nor the soil microflora treatments significantly affected the growth of wheat seedlings.

Taking into consideration these results and since the results of the in vitro work demonstrated that the MLSE was not fungicidal to take-all it can be hypothesised that it may be eliciting a defence response in the plants.

## 3. Effect of Maxicrop Concentrate Seaweed Liquid Extract on *Gaeumannomyces graminis* infection and Wheat Plant Growth in the Glasshouse

In this section work developed in order to study the growth responses to Maxicrop seaweed extracts (Maxicrop Concentrate and Maxicrop Extruded Granules) by wheat plants grown in pots in a glasshouse environment are presented. The other main aim of this research was to assess whether the responses obtained in hydroponic assays for take-all infected wheat would be apparent in the glasshouse environment.

### METHOD AND MATERIALS

#### Preparation of inocula

Inoculum of *G. graminis* was grown as sand-cornmeal cultures comprising 100g washed sea-sand, 2 g maize-meal and 30ml distilled water. Sterilised sand-cornmeal medium was inoculated with plugs of actively growing mycelium taken from 2 week old cultures grown on PDA. The fungus was allowed to colonize the media for 4 to 5 weeks until the mycelia had covered all the available surface. Experiments were conducted in 11 cm diameter plastic pots (300 ml volume). Growing compost was inoculated by mixing 10g of the *G. graminis* sand-cornmeal culture with 250g of John Innes compost and mixing thoroughly. For control pots un-inoculated sand cornmeal medium (10g) was applied and mixed.

**Preparation of soil solution**

A fresh soil solution was prepared as described for the hydroponics experiments (page 45) and in some experiments 1 ml aliquots were added as appropriate.

**Preparation of bacterial solution**

A solution of *Ps. fluorescens* was applied in some experiments and was prepared and applied as described in section 2.

**Planting and growth conditions**

Six wheat seeds (variety Brigadier) were sown per pot approximately at 5 cm depth. A standard germination test performed prior to the trial revealed that 86% of seeds had germinated after 48 h which was acceptable for the experiments. It was aimed to maintain day temperature below 25-27°C and night temperature over 10°C.

**Application of Seaweed Extracts**

Where Maxicrop granulated seaweed extracts were applied, these were mixed with the compost and sand-cornmeal media at potting. The liquid seaweed extract (5ml $l^{-1}$) was sprayed until run off from the leaves was observed.

**Sampling and assessment techniques**

The parameters analysed were: disease level, plant growth stage, plant height, shoot dry weight and leaf area. This last parameter was collated only in the first glasshouse experiment since the procedure adopted revealed to be very time consuming and was considered to be of questionable relevance.

After the appropriate times plants were harvested and the roots washed prior to analysis of root/shoot base infections according to assessment keys (Appendix 1). Symptoms were categorised, with 0 representing a healthy plant and 5 the most severely infected plants.

### 3.2.1. Experiment 1

The following treatments were applied using a fully randomised-block design with six replicates per treatment Two repeats of this experiment were conducted.

Table 3.1- Treatments investigated.

| Treatment | Corn meal | Soil Solution | + pathogen | 10 g EMG | 20 g EMG | MLSE Spray 5 ml $l^{-1}$ | MLSE Spray 10 ml $l^{-1}$ |
|---|---|---|---|---|---|---|---|
| Control 1 | - | - | - | - | - | - | - |
| Control 2 | √ | √ | - | - | - | - | - |
| Control 3 | √ | √ | √ | - | - | - | - |
| T1 | √ | √ | √ | √ | - | - | - |
| T2 | √ | √ | - | √ | - | - | - |
| T3 | √ | √ | √ | - | √ | - | - |
| T4 | √ | √ | - | - | √ | - | - |
| T5 | √ | √ | √ | - | - | √ | - |
| T6 | √ | √ | - | - | - | √ | - |
| T7 | √ | √ | √ | - | - | - | √ |
| T8 | √ | √ | - | - | - | - | √ |

EMG = Maxicrop extruded granules

MLSE = Maxicrop Concentrate Liquid Seaweed Extract

Sprays of MLSE were applied at two different growth stages: one week after sowing when plants had reached GS 11 (1$^{st}$ leaf unfolded) and at 4$^{th}$ week after sowing when plants had reached GS 22 (2$^{nd}$ tiller visible). The experiments were terminated at the 6$^{th}$ week after sowing and disease symptoms assessed according to the assessment scale in Appendix I. Individual plants' scores in each pot were used to calculate separate disease indices for each treatment unit (treatment replicate) according to the following formula:

$$\text{Disease Index} = \frac{(0 \times a) + (1 \times b) + (2 \times c) + (3 \times d) + \ldots}{(a + b + c + d + \ldots)} \quad \frac{100}{n\text{-}1}$$

Where n-1 was the number of points in any assessment key; a, b, c and d was the number of plants examined which fell into the disease levels 0, 1, 2, 3, respectively (after Parry, 1990).

**Results**

At harvest control non-inoculated plants and non-inoculated plants treated with a soil solution were disease free indicating that that solution was not detrimental. The comparison between treatment C1 and C2 indicated that treatment with the soil solution did not have any effects on plant growth.

The inoculum used induced very light levels of disease (in the lower quartile of the disease index range) in this experiment (Fig. 1.6.1). Plants submitted to treatments with EMG showed higher levels of disease infection, particularly T3 (20g EMG) which showed the highest level of disease infection in the whole experiment. However, the amount of granules applied caused a brown tinted colour on the roots which made healthy roots look similar to take-all infected roots thus making accurate identification and quantification of symptoms difficult. It was also found that plants presented a very extensive root system which impaired observation and scoring of disease symptoms.

All of the spray treatments, T5-T7, showed lighter levels of disease infection than the inoculated control plants (Control 3). Furthermore, plants treated with the lowest rate of MLSE (5 ml $l^{-1}$ – T5) showed significantly lower levels of disease than the inoculated control (C3).

In terms of plant growth, there was no significant difference between plants treated with MLSE and the controls. Plants treated with granules, however, were at lower growth

stages with T3 and T4 showing significantly lower growth stages compared with Control 3 and MLSE treated plants (Fig. 1.6.2). Similar results were obtained for the other two parameters analysed, plant height and shoot dry weight (Fig. 1.6.3 and 1.6.4).

These results indicate that the MLSE (spray) caused a reduction of disease but did not produce alterations in terms of plant growth or development. The presence of EMG in the compost however was ineffective against disease while showing a detrimental effect in terms of plant growth. It also suggested that the detrimental effect was stronger with the higher dose applied. The apparent opposing results of the two formulations of the same product was intriguing and demanded further investigation and this is presented later.

Fig. 1.6.1- Effect of Maxicrop seaweed extracts on Take-all of wheat. Columns with the same letter are not statistically significantly different from each other (P>0.05).

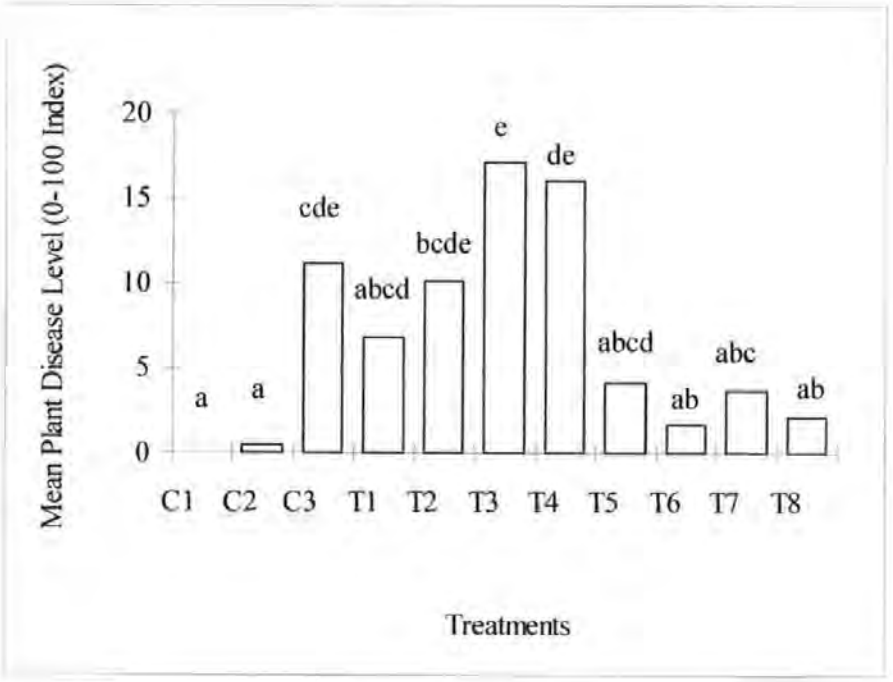



Fig. 1.6.2- Effect of Maxicrop seaweed extracts on wheat plants. Columns with the same letter are not statistically significantly different from each other (P>0.05).

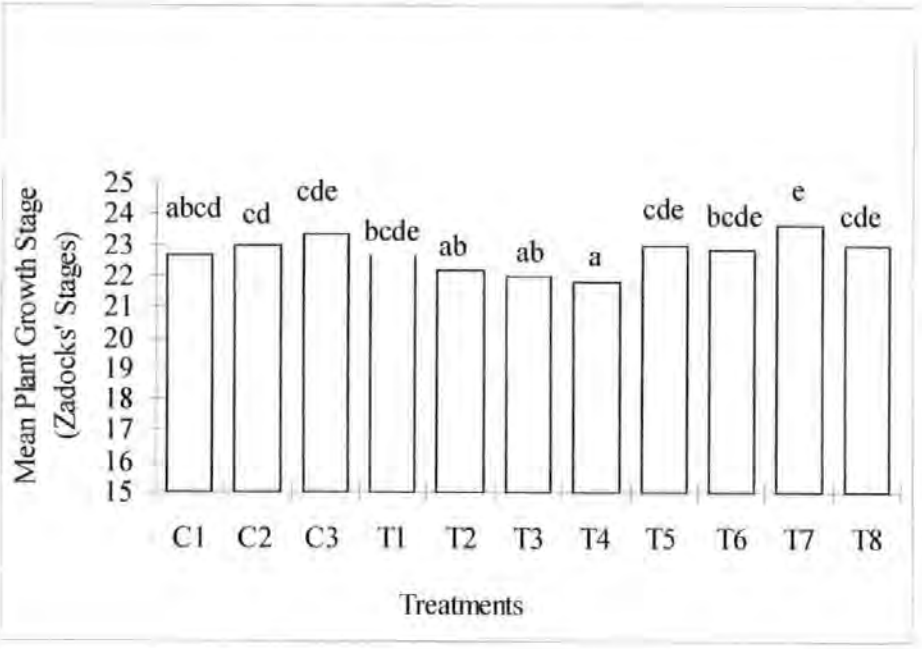


Fig. 1.6.3- Effect of Maxicrop seaweed extracts on wheat plants. Columns with the same letter are not statistically significantly different from each other (P>0.05).

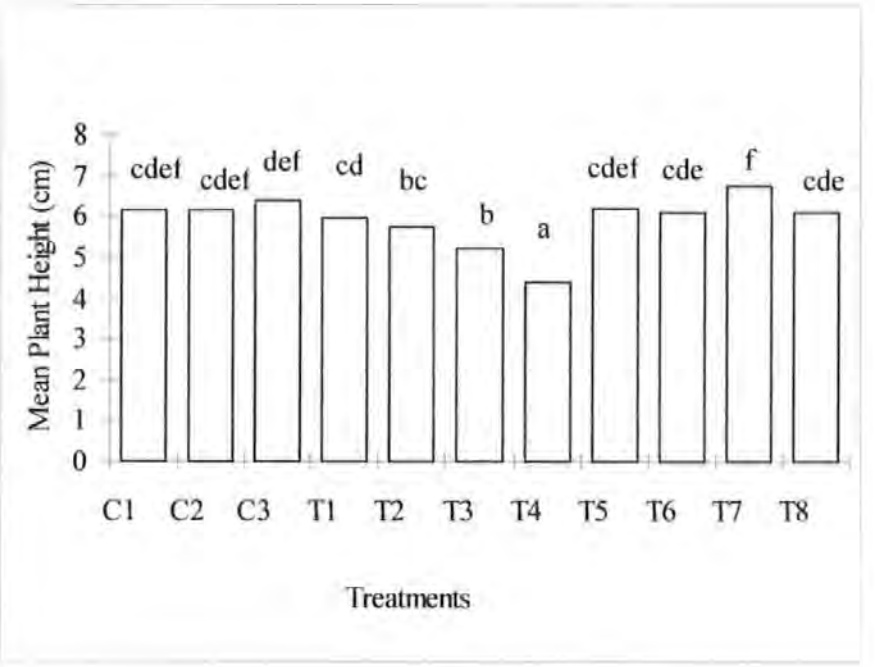
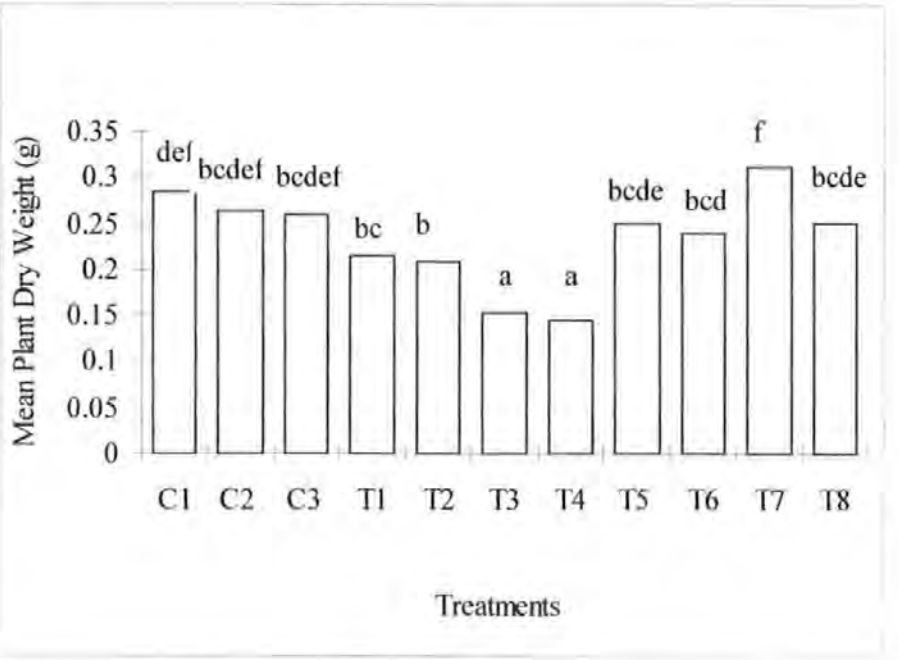



Fig. 1.6.4- Effect of Maxicrop seaweed extracts on wheat plants. Columns with the same letter are not statistically significantly different from each other (P>0.05).



### 3.2.2. INVESTIGATION OF DISEASE SUPPRESSION AND PLANT GROWTH – GLASSHOUSE & GROWTH CHAMBER EXPERIMENTS

In this section experiments conducted in the glasshouse and growth chamber are described. This work aimed to study in greater detail the responses to MLSE by wheat and its interactions with take-all.

Experiments described in the previous sections showed that, contrary to findings by Cogram (1994), a soil solution or populations of *P. fluorescens* were not essential for a suppression of the soil-borne disease under study to occur. One possible explanation for the negligible responses to treatments with MLSE and EMG combined with a soil solution, could be that the beneficial microorganisms were not present in the soil solution used or their concentrations were not adequate.

An analysis was carried out to determine whether the soil used contained *P. fluorescens* and if so, to determine the average number of colony forming units (CFU). Six samples of the soil were collected and cultured in a *Pseudomonas* selective media. *P. fluorescens* were found in 4 of the samples with an average of $6.35 \times 10^5$ CFU.

Since *Pseudomonas* have been implicated in the take-all decline process and there have been various reports providing evidence of their antagonistic effect and against this pathogen (Cogram, 1994, Pattison, 1997, Raaijmakers & Weller, 1998, Roberts *et al.*, 1998, Slininger *et al*, 1998), it was decided that the responses to soil solution and *P. fluorescens* obtained previously should be explored further in the glasshouse environment. An experiment was carried out to determine the effects on wheat plant growth and take-all disease expression. As a control treatments were incorporated with a solution containing a known CFU of *P. fluorescens* bacteria originated from pure cultures not isolated from soil.

Wheat seeds were pre-germinated on water soaked paper in a plastic container for 2 days, when the radicle had emerged, and placed at approximately 1.5 cm deep in the compost. Solutions (2 ml) containing soil solution or the dilution of pure *P. fluorescens* cultures were then added to the compost spreading the liquid over the seeds. The general method and materials described for the previous wheat experiment were applied.

Table 3.2 - There were four replicates of the following treatments:

| Treatment | Un-inoculated Corn Meal | MLSE (1ml $l^{-1}$) | MLSE (5ml $l^{-1}$) | Inoculated Corn Meal | Soil Solution | *P. fluorescens* |
|---|---|---|---|---|---|---|
| T1 | √ | - | - | - | - | - |
| T2 | √ | √ | - | - | - | - |
| T3 | √ | - | √ | - | - | - |
| T4 | - | - | - | √ | - | - |
| T5 | - | - | - | √ | √ | - |
| T6 | - | - | - | √ | - | √ |
| T7 | - | √ | - | √ | - | - |
| T8 | - | √ | - | √ | √ | - |
| T9 | - | √ | - | √ | - | √ |
| T10 | - | - | √ | √ | - | - |
| T11 | - | - | √ | √ | √ | - |
| T12 | - | - | √ | √ | - | √ |

The experiment was terminated 4 weeks after sowing and the plants assessed as described in the previous experiments.

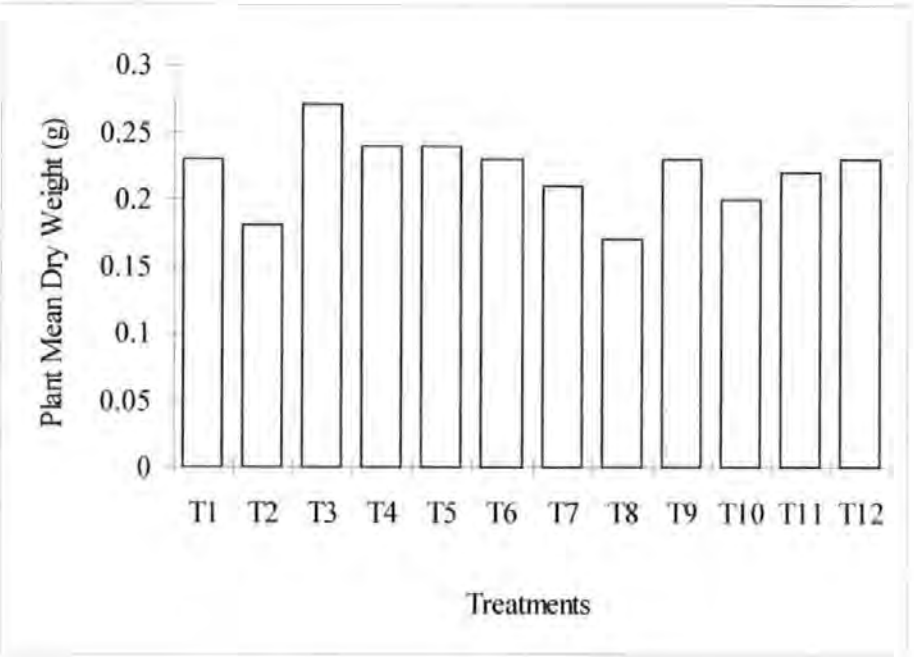
**Results**

Symptoms of disease found at harvest in inoculated control plants (T4) were very light, on average, not reaching category 1 of the disease scale adopted (Appendix I). The lesions observed were very small, light brown and were only found in a small number of roots. It was thought that these results did not allow for a reliable comparison between treatments in respect with their effects on take-all infection.

Statistical analysis of the results of this experiment, concerning the agronomic characters under study, showed that none of the treatments applied attained significant improvements of the plants' growth. Despite the fact that the disease infections were very light, disease inoculated plants exhibit poorer growth than non-inoculated plants (Fig. 1.7). Although plants treated with the soil solution seemed to have grown better than plants

treated with the *P. fluorescens* solution this was not significant for any of the characters under study. Results also indicated that the two MLSE treatments applied (comparison between T1, T2 and T3) did not significantly improve plant growth compared with untreated plants.

Fig. 1.7 - Effect of MLSE on the growth of wheat. Columns with the same letter are not statistically significantly different from each other (P>0.05).



### 3.2.2.a. Effect of Maxicrop Concentrate Liquid Seaweed Extract on *G. graminis* infection and Wheat Plant Growth

The low levels of disease infection found in inoculated plants of the experiment 2 were considered to have been a consequence of the unexpected rise in temperature in the glasshouse at the start of spring season. In an attempt to minimise this effect subsequent experiments were conducted in a growth chamber where temperature was controlled more accurately at 20 ± 2°C with a 16 h photoperiod. No other alterations were made to the general method previously described and there were 6 plants per replicate.

Four replicates of the following treatments were applied in a fully randomised block design:

T1 - non-inoculated corn meal (CM)

T2 – CM + Soil Solution (SS)

T3 - SS + *G. graminis* inoculated CM (Ggl)

T4 - SS + Ggl + 2 sprays MLSE (5 ml $l^{-1}$)

T5 - Ggl + 2 sprays MLSE (5 ml $l^{-1}$)

T6 - SS + Ggl + 3 sprays MLSE (5 ml $l^{-1}$)

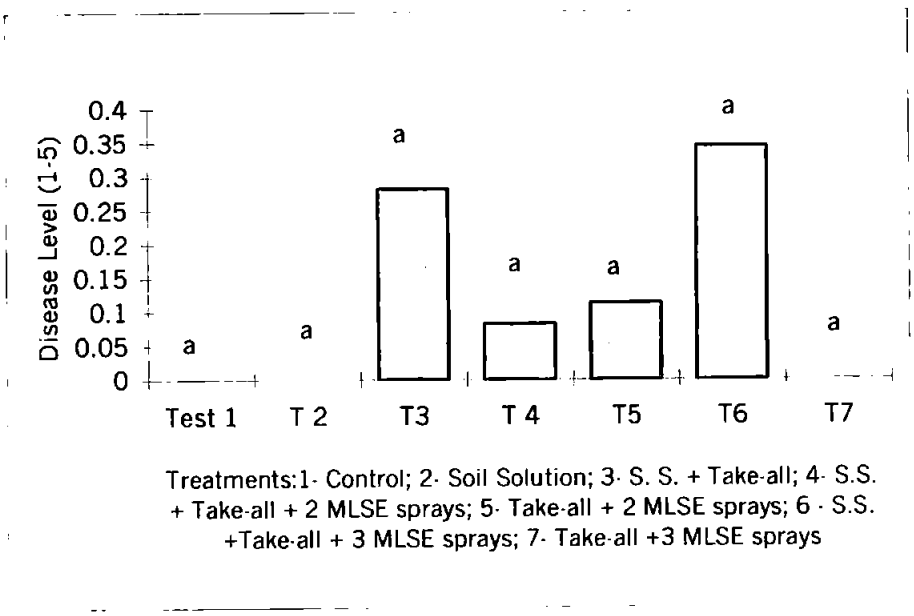
T7 – Ggl + 3 × sprays MLSE (5 ml $l^{-1}$)

**Results – Experiment 3.2.2.a**

Despite the measures taken, levels of infection found at the time of harvest were still very light (Fig. 1.8.1). Data collected for all other characters under study (Fresh and Dry Weight, Number of Tillers and Leaves) indicated that applications of both the soil

solution and the MLSE or their combination did not cause significant changes in plant growth.

Fig. 1.8.1- Effect of MLSE on Take-all of wheat. Columns with the same letter are not statistically significantly different from each other (P>0.05).



### 3.2.2.b. Effect of Maxicrop Concentrate Liquid Seaweed Extract on *G. graminis* infection and Wheat Plant Growth

A second experiment was conducted in the growth chamber to confirm or disprove the results obtained with the preceding experiment. Two repeats of this experiment were conducted where the following treatments were applied:

T1 - un-inoculated Corn Meal (CM)

T2 – CM + Soil Solution (SS)

T3 - 2 sprays MLSE (5 ml $l^{-1}$)

T4 – CM + *G. graminis* (GgI)

T5 - SS + GgI

T6 - SS + GgI + 2 sprays MLSE (5 ml $l^{-1}$)

T7 – GgI + 2 sprays MLSE (5 ml $l^{-1}$)

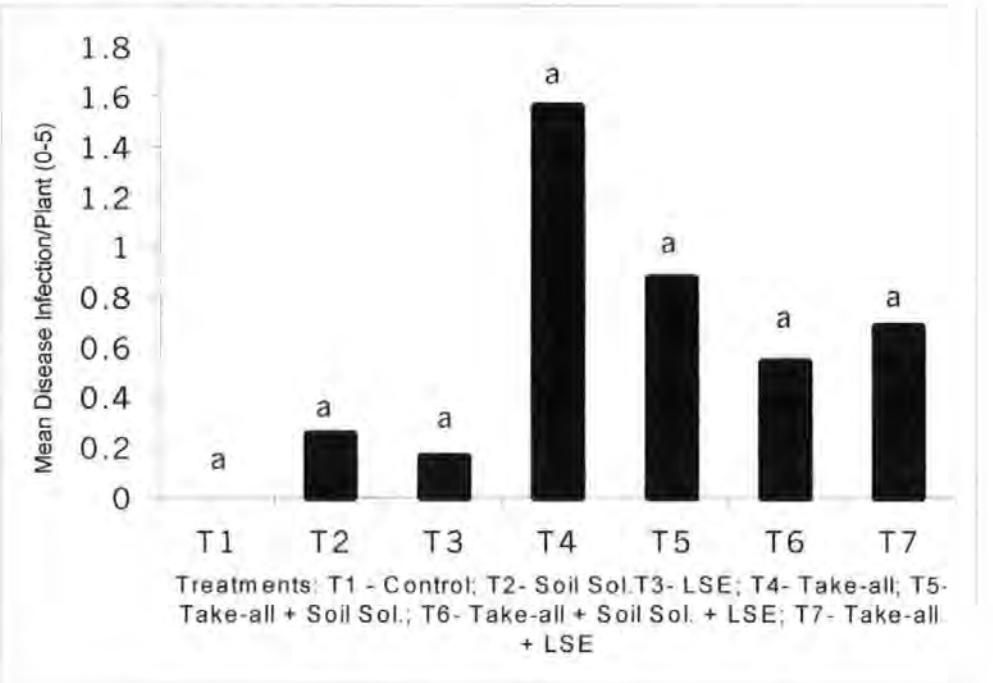
**Results – Experiment 3.2.2.b.1.**

Despite the measures taken to ensure the environmental conditions were appropriate for disease establishment, no disease infection was found, therefore the experiment was considered invalid. In view of the low infection levels caused by the inoculum applied, it was decided that a new isolate of the pathogen should be isolated from plant roots and used to repeat this experiment.

**Results - Experiment 3.2.2.b.2.**

Infections found at harvest showed that the freshly isolated inoculum was active but it still caused only light disease symptoms. In response to MLSE applications lighter levels of disease damage were found (not statistically significant). Reduction of take-all infection was further intensified in plants treated with MLSE in conjunction with the Soil Solution. Plants of this treatment (T6) showed a three fold reduction in disease levels when compared to inoculated control plants (not statistically significant – Fig. 1.8.2). Evaluation of all other parameters, (Fresh and Dry Weight, Number of Tillers and Leaves), suggested that neither MLSE nor the soil solution or their combination significantly affected plant growth.

Fig. 1.8.2 - Effect of MLSE on Take-all of wheat. Columns with the same letter are not statistically significantly different from each other (P>0.05).



## 4. Investigation of Wheat growth responses to Maxicrop Seaweed Extracts – Glasshouse Experiments

Following the analysis of the results obtained with the glasshouse experiments it was necessary to study further the wheat plant responses obtained following the amendments of the seaweed extracts under the two types of formulations. It was also important to obtain more information on the seaweed extruded granules that might allow an interpretation of the responses observed. Work was developed in order to obtain more information about the EMG with respect to their chemical composition, their effect on compost conductivity and on wheat plant growth. A glasshouse/pot experiment was conducted to investigate the response of wheat plants to applications of different amounts of EMG to the compost.

### 4.1. Conductivity and Chemical Analysis of Seaweed Extruded Maxicrop Granules

#### 4.1.a. Electric Conductivity Analysis of Seaweed Extruded Maxicrop Granules

Previous research work (Walsh, 1997) registered detrimental effects following amendments with Maxicrop seaweed granules and suggested that this might be due to a rise in EC in the compost. Since the type of responses observed in the glasshouse experiments described earlier were similar to the ones depicted by Walsh (1997), an investigation into the EC of compost treated with EMG was undertaken.

**Method and Materials**

Solutions containing 450g of compost (John Innes as used for pot experiments) and different amounts of EMG were studied. The solutions were prepared by thoroughly mixing 2:1 parts of water and solids respectively for approximately five minutes. The solution was then filtered through gauze to remove the larger sized debris and approximately 50 ml were used for the measurement of the electrical conductivity. Four replicates were prepared for each solution. The electrical conductivity was measured using a Walder WP4 precision conductivity meter. Table 4.1 indicates the treatments studied.

Table 4.1- Solutions of Compost treated with EMG analysed for EC.

| Solution | Granules (g/450g compost) | Water (ml) |
|---|---|---|
| 1 | 0 | 900 |
| 2 | 5 | 910 |
| 3 | 10 | 920 |
| 4 | 20 | 940 |
| 5 | 30 | 960 |
| 6 | 40 | 980 |

**Results: Electrical Conductivity Analysis**

Results presented in Table 4.2 show that EC of the solutions increased with the increasing amounts of EMG present. This increase was statistically significant for solutions 5 and 6 (30 and 40g respectively) compared with the control. The EC measured for solution 2 (5g of EMG) was significantly lower than the one measured in solutions 5 and 6 (30 and 40g respectively).

Table 4.2- Mean EC of Compost treated with EMG.

| Solution | EMG (g/ 450g compost) | Mean Electric Conductivity (μS) # |
|---|---|---|
| 1 | 0 | $1.7^{a}$ |
| 2 | 5 | $2.2^{ab}$ |
| 3 | 10 | $2.5^{abc}$ |
| 4 | 20 | $3.0^{abc}$ |
| 5 | 30 | $3.8^{c}$ |
| 6 | 40 | $3.8^{c}$ |

#Means with the same letters are not statistically significantly different.

### 4.1.b. Chemical Analysis of Seaweed Extruded Maxicrop Granules

Seaweeds have a high mineral content and, for example, they are richer in potassium than farm manure (Round, 1973). In 4.1.a. the analysis carried out in MLSE was described showing that high levels of potassium were present in the extract and that this correlated with an increase in the EC of the solution. The results of the analysis described in 1.a. of this Section showed that a significant increase in EC was obtained with the highest amounts of EMG present in the compost. Consequently an analysis into the mineral constituents of the EMG was undertaken.

#### Method and Materials

Solutions containing EMG were prepared by adding 1g of the granules to 100 ml distilled water in 250 ml plastic conical flasks. The flasks were placed in a shaker for 10

min (until all the granules were completely dissolved). One ml of the solution obtained was then poured into 100 ml volumetric flask and 99 ml of distilled water was added. Six replicates were prepared and analysed for levels of K and Mg using an atomic spectroscopy system (AA).

**Results: Chemical Analysis of Extruded Maxicrop Granules**

The chemical analysis carried-out revealed that, on average, 1 g of EMG provides 146.8 mg of K and 1.85 mg of Mg. Values for each sample analysed are shown on Table 4.3.

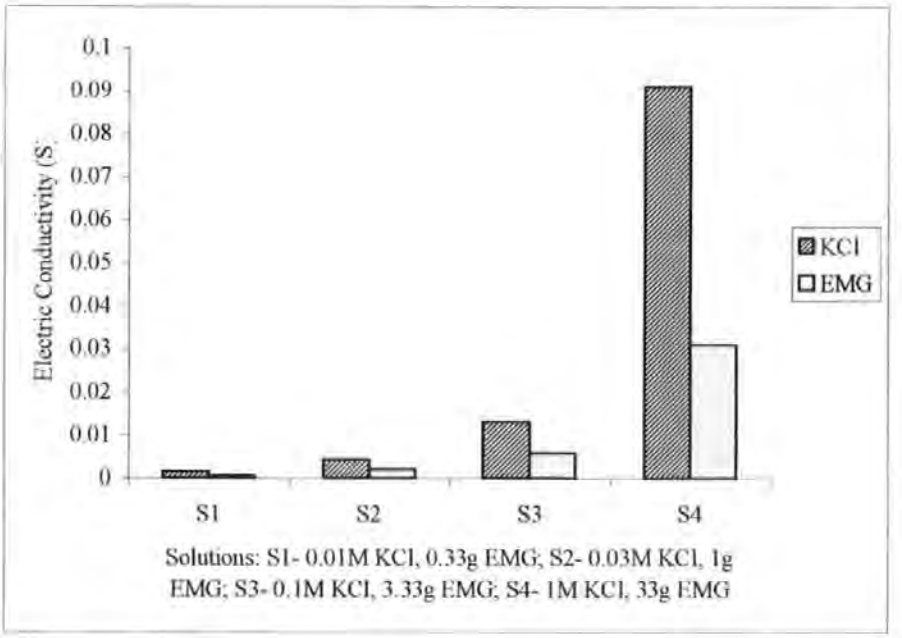
Table 4.3- Content of K and Mg on Extruded Maxicrop Granules (EMG).

| Replicate Sample | K (mg/g EMG) | Mg (mg/g EMG) |
|---|---|---|
| 1 | 165.6 | 1.68 |
| 2 | 125.9 | 1.76 |
| 3 | 136.8 | 1.73 |
| 4 | 143.9 | 1.89 |
| 5 | 124.8 | 2.42 |
| 6 | 183.9 | 1.59 |
| Mean | 146.8 | 1.85 |
| St. Dev. | 21.4 | 0.27 |

Variability was quite high between replicates with no apparent correlation in variability between K and Mg.

KCl solutions were prepared with the same molar concentration of K to a series of EMG solutions and their EC measured. Results showed that equivalent KCl solutions expressed an EC almost three times higher than EMG solutions (Fig. 1.9). This indicates that not all the potassium present in the granules is in an ionic form and is either bound or in organic complexes and as such is probably not immediately available to plants.

Fig. 1.9 – Comparison between electrical conductivity of KCl and EMG at equivalent K concentrations.



## 4.2. Responses of Wheat Plants to Seaweed Extruded Maxicrop Granules

An experiment was conducted to analyse the responses of wheat plants grown in the glasshouse to amendments of compost with increasing amounts of EMG. The method, materials and environmental conditions used in this experiment were the ones employed in the glasshouse experiments with wheat plants described in Section IV. Different rates of EMG were added to the compost at the time of potting/seeding.

The experiment comprised four fully randomised replicates of eight treatments with the following treatments.

T1- 0g - Control

T2- 0.2g

T3- 1.0g

T4- 2.0g

T5- 5g

T6- 10g

T7- 20g

T8- 30g

### Results: Responses of Wheat Plants to Extruded Maxicrop Granules

Results showed that small amendments with EMG may cause a slight stimulation of plant growth but this was not significant (Fig. 1.10.a. to 1.10.d). The higher amounts (20 and 30g) however were detrimental to the plants causing significant reductions in Fresh and Dry Weight. It is possible that the discrepancies between plant performances

would be heightened if the plants were submitted to environmental stresses and pathogen infections.

Fig. 1.10.a- Response of Wheat to increasing amounts EMG.

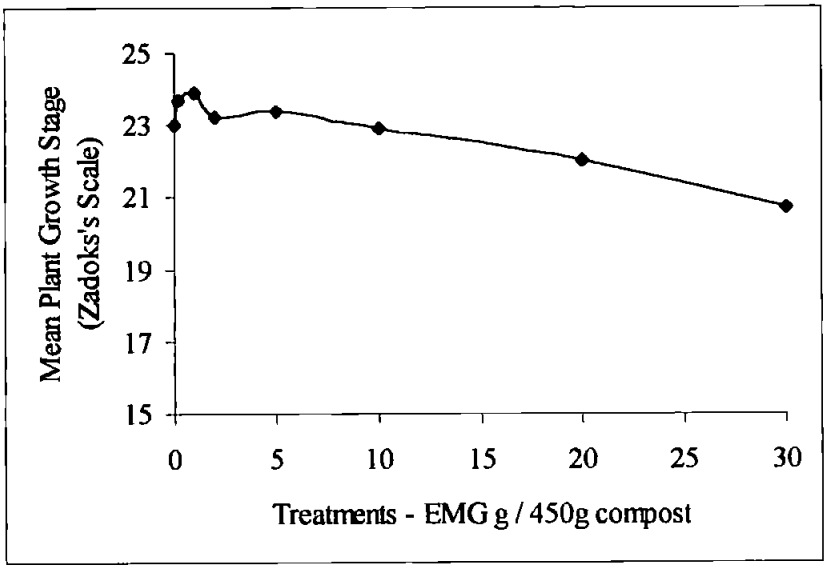


Fig. 1.10.b – Response of wheat to increasing amounts of EMG.

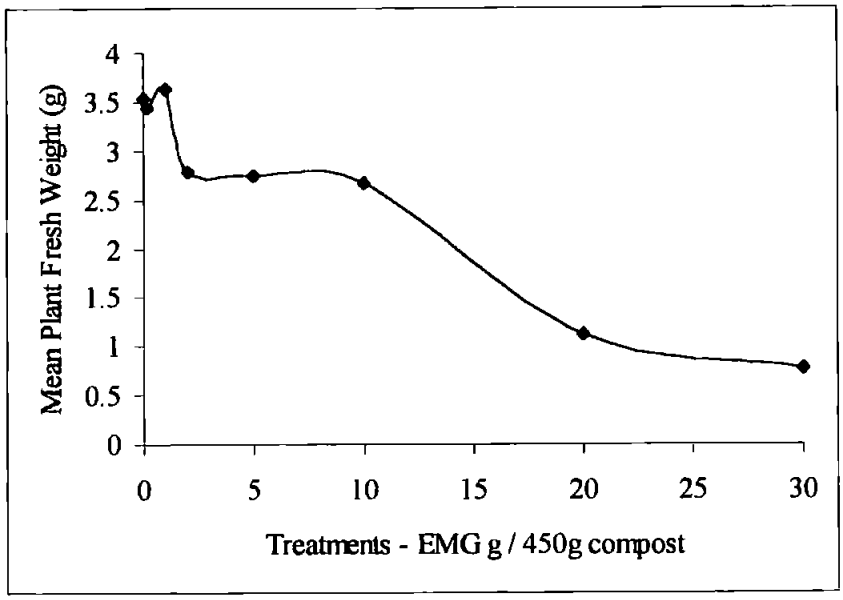



Fig. 1.10.c – Response of wheat to increasing amounts of EMG.

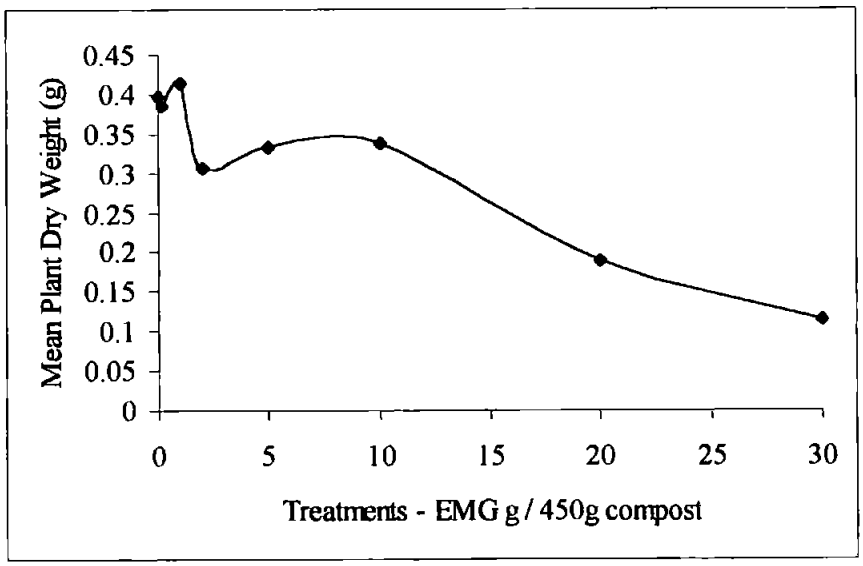
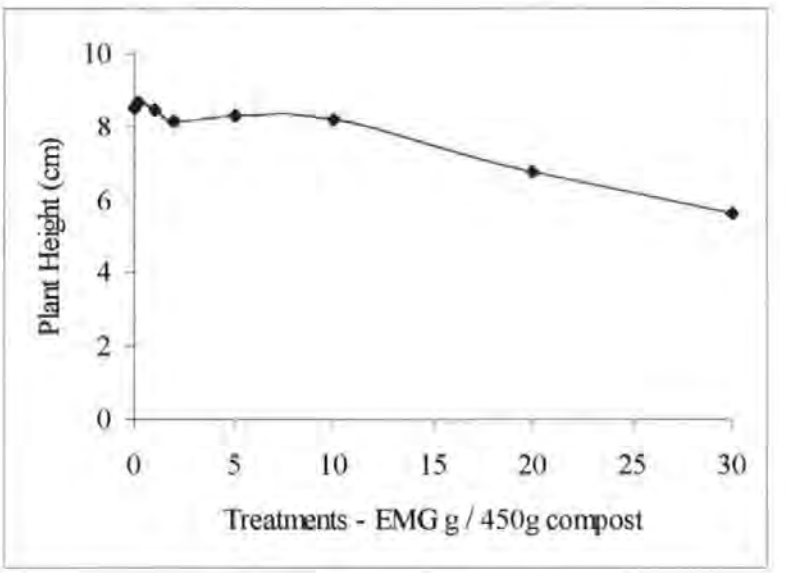


Fig. 1.10.d – Response of wheat to increasing amounts of EMG.




**Discussion and Conclusions**

Results of the glasshouse experiment showed that EMG has little effect on wheat plant growth if used in very small amounts, such as 1g / 450g compost but a significant depressive effect when used at high incorporations rates. The chemical analysis carried out to the EMG treated compost and the granules themselves suggest that the positive responses observed could have been promoted by a slight elevation on the gradients of macronutrients, such as potassium and magnesium, released from the granules.

The experiment indicated, however, that there is a risk of causing detrimental effects on wheat plants when the extracts are applied at high rates (30g / 450g compost). The results of chemical analysis suggest that a possible explanation for the negative growth responses observed is the rise in the compost electrical conductivity (EC) caused by the granules. It has been shown that potassium plays an important part in the ionic fluxes established in the rhizosphere which can in turn be measured as EC (Rowell, 1994).

Chemical analysis showed that EMG had a high content in potassium, thus it is reasonable to presuppose that this component of the seaweed extract could promote the rise in EC measured in the compost. The potassium content could also directly contribute to the detrimental effect on plant growth observed for high rate treatments. There is evidence (Finck, 1982, Archer, 1988 and Gair, 1990) that potassium is a nutrient that, when present in high levels in the rhizosphere, is absorbed in excess of the plants needs in detriment of the absorption of other nutrients such as magnesium, calcium and sodium. Such luxury absorption can cause an unbalanced nutritional status that will express itself in poor plant growth even when the other nutrients are present at the required level in the substrate (Marschner, 1995). The poorer wheat plant growth observed for high rate EMG treatments could thus potentially be explained by luxury potassium absorption and a consequent obstruction of the absorption of other essential nutrients.

It should be taken into consideration that EC is unlikely to remain constant through-out a pot experiment as, for example, the granules will not all dissolve simultaneously in that environment. The effect of applications of EMG in EC of a pot rhizosphere or soil is, therefore, likely to be less dramatic than the one obtained in the laboratory experiment. The study of the effects of EMG over a compost or soil rhizosphere EC throughout the plants' life cycle would probably provide useful information in this respect. The experimental data showed, however, that the highest rates of EMG applied prompted clear negative plant growth responses. If commercialization of this product is to be pursued a careful study of the rates of application should be carried-out to ensure its optimal use in different plant production systems.

## 4.3. Effect of Maxicrop Concentrate Seaweed Liquid Extract on Wheat Plant Growth and Protein Production

Previous work (Burchett, 2000, Cogram, 1994) has found that healthy wheat and barley plants treated with multiple applications of MLSE showed increased tiller production, plant protein and chlorophyll contents and greater wheat culm diameter amongst other advantageous traits. Earlier indications of the work presented here was unable to confirm these beneficial effects. Experiments were therefore undertaken in order to try to find the reason for this divergence in results.

### Method and Materials

#### Growing Conditions

Two experiments were conducted following the method previously described for Take-all glasshouse experiments. The wheat seeds were pre-germinated for 24 h as described in Section 3.

#### Experiment 1

In the first experiment there were five replicates in a randomised block design of the following treatments:

T1 - 0 MLSE sprays

T2 - 1 MLSE (5ml $l^{-1}$) spray

T3 - 2 MLSE (5ml $l^{-1}$) sprays

T4 - 3 MLSE (5ml $l^{-1}$) sprays

Sprays were applied at weekly intervals, during the 2nd, 3rd and 4th weeks (first spray was applied when plants had 2/3 leaves and the last spray when the first leave of the first tiller had emerged). Plants were harvested at the end of the 5th week.

**Experiment 2**

A second experiment was carried out to study further the responses of the plants to application of MLSE at different times and at different frequencies. The treatments below were studied each being replicated 4 times in a randomised block design.

T1 - 0 MLSE sprays

T2 - 1 MLSE (5ml $l^{-1}$) spray at week 1

T3- 1 MLSE (5ml $l^{-1}$) spray at week 5

T4 - 2 MLSE (5ml $l^{-1}$) sprays at weeks 1 and 5

T5 - 3 MLSE (5ml $l^{-1}$) sprays at weeks 1, 3 and 5

The experiment was terminated at the 5th week two days after the last spray applied in an attempt to collect the plant material at the most active response stage. Work developed by Burchett (2000) indicated that responses to spray applications were subtle and became more difficult to assess when longer time lapses between application and measurements occurred.

## Protocol for the extraction of total protein on wheat

### Preparation of Buffer solution and Gels

Tris buffer (15.142 g) was diluted in 250 ml of double distilled water (ddw). This solution (10 ml) was then diluted in 90 ml of ddw to give a 50 mM Sol. Phenylmethyl-sulfonyl fluoride (0.1742 g) was dissolved and serially diluted to obtain a 1mM solution

that was then mixed with the Tris buffer. The final buffer thus obtained was adjusted to pH 7 using HCl.

**Extraction Procedure**

Wheat plants were harvested 2 days after the last MLSE spray had been applied. Whole plants were washed and surface sterilised for 10 min using a 1% bleach solution. Leaves were cut in order to obtain material for analysis made up of stems and leaves only. Fresh material (3g) was finely cut and placed into a pestle and liquid nitrogen ($N_2$) poured over and allowed to boil off before crushing to a fine dust. Tris-HCl buffer (6 ml) was then added and the material was crushed once again until a fine slurry was obtained. This solution was centrifuged at 15000 rpm at 4°C for 5 min, the supernatant decanted and filtered through a Whatman no 1 filter paper and stored in eppendorf tubes at -80°C until required.

**Separation of Proteins**

A separation of total protein was achieved using a mini electrophoresis unit (Techne, Grant USA, with a Bio-Rad Power supply). This is a vertical slab gel unit that obtains rapid electrophoresis of small samples of proteins, separating them according to their size, shape and electrical charge. The sample mixture was loaded into the wells of the stacking gel, the power was set at 115mV and gels were left to run for 1h 45min. After electrophoresis, the gels were stained for 1-2 hours in coomassie blue and destained overnight. The bands obtained were subsequently scanned using a densitometer (Enhanced Laser Densitometer LKB Bromma - Ultrascan XL) using the following settings:

X width 3

Y step 2 or 1

Smoothing 2

Peak width 1

**Photography**

Gels were photographed using either a Polaroid camera with white back lighting supplied by a transilluminator, with the aperture F32, shutter speed 1/125 seconds with a 45 second development time at room temperature; or a digital camera (Sony Mavica MP3).

**Analysis of Gels**

Gels were interpreted and data was analysed using the following steps:

- measure the number of bands detectable by densitometer and cross reference gel by eye.
- observe any reproducible patterns detected by densitometer
- construct molecular weight calibration curve
- construct analysis of variance test using the data obtained by densitometer

**Chemical analysis**

Dried whole plants were ground to obtain a fine powder that was subsequently used to perform a series of chemical analysis in order to obtain levels of N, C, $PO_4$ and K.

**Determination of N and C**

Levels of N and C were determined on 1.5 g samples of plant material using a LECO elemental analyser (LECO FP - 2000). Four samples were analysed for each treatment.

**Determination of K and $PO_4$**

The same preparation procedure was followed to determine the levels of potassium and phosphorous. The plant samples (0.50g) were ashed at approximately 550°C until a white or light grey ash was formed. After cooling, 5 ml of concentrated hydrochloric acid (HCl) were added to the crucibles containing the ashes and the mixture was boiled for 5 min on a hot plate in a fume cupboard, adding HCl as necessary to maintain the initial volume. The solution was then transferred into a beaker and the crucible washed with distilled water (DW). The volume was adjusted to about 40 ml with DW and the solution was boiled for 10 min. After cooling, the solution was filtered through glass wool into 100 ml volumetric flasks. The volume was made up to 100 ml adding DW and this solution was used for the determination of $PO_4$ and K. Levels of potassium were determined using an atomic absorption spectrophotometer (SpectrAA-200, Varian). Levels of total phosphorus were determined using a flow injection analyser (FIAstar 5023 Spectrophotometer).

## RESULTS

### Effect of Maxicrop Concentrate Liquid Seaweed Extract on Wheat Plant Growth and Protein Production – Experiment 1

Applications of MLSE had no significant effect on wheat plants in terms of their fresh and dry weight and number of leaves and tillers. Treated plants showed an average fresh weight similar to non-treated plants, however, there was a reduction on average dry weight with all MLSE treatments (not statistically significant). Higher number of leaves and tillers was produced by plants treated with 1 and 3 MLSE sprays, while the opposite was found on plants treated with 2 LSE sprays although these results were not statistically significantly different ($P>0.005$).

#### Separation of Proteins

Observation of gels and the curves obtained by densitometry indicated that with MLSE treatments some new bands appeared while the concentration of others was up or down regulated (Plate 4). In particular, a high molecular weight protein band was up-regulated in plants treated with multiple MLSE sprays (T4).

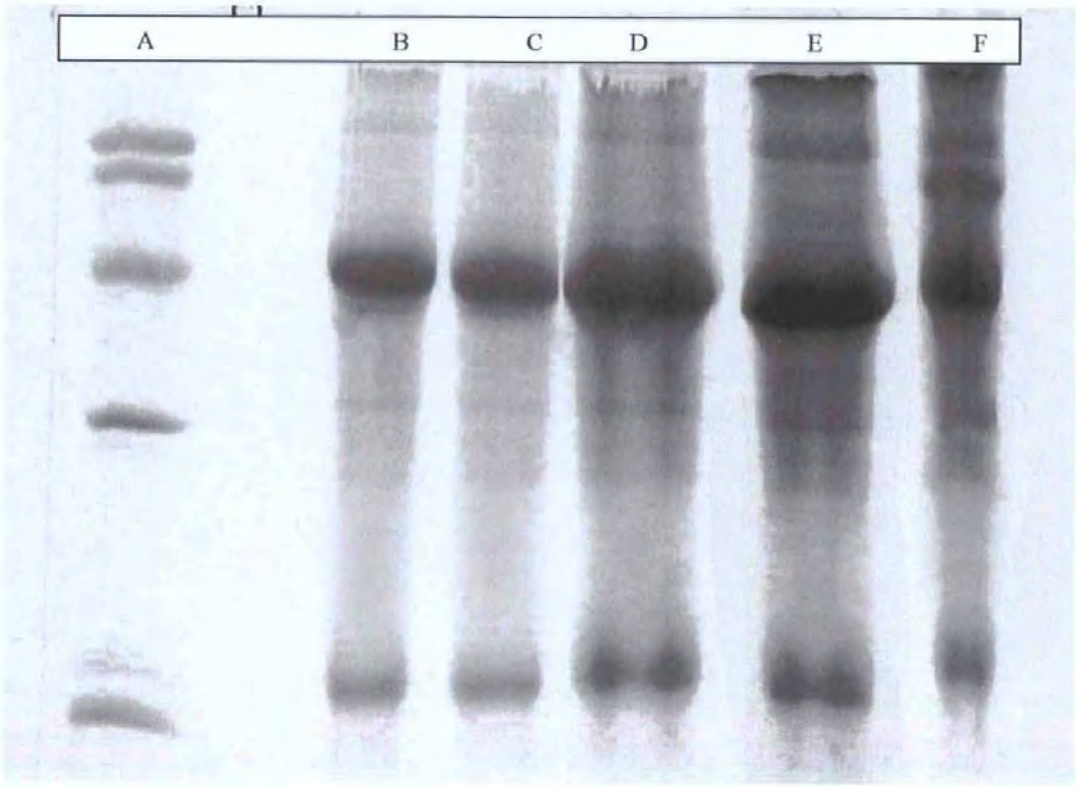
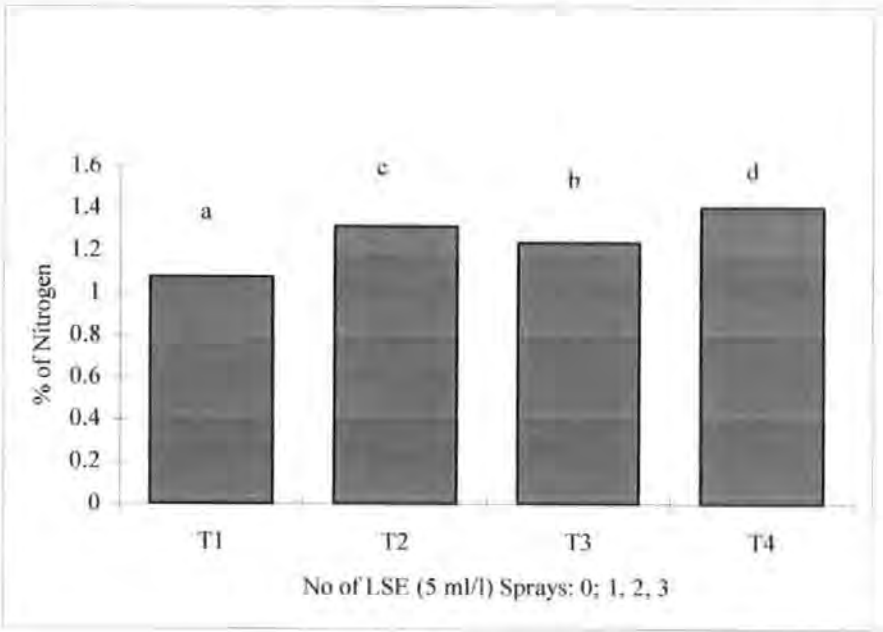



Plate 4- Protein bands, from left to right: A- standards, B- 0 MLSE, C- 0 MLSE, D- 1 MLSE spray, E- 2 MLSE sprays, F- 3 MLSE sprays.

**Determination of N and C**

Plants treated with MLSE showed small but significant increases ($p<0.001$) in percentage of total nitrogen and the 3 spray treatment induced the highest response (Fig.1.11.a). The percentage of total nitrogen found in plants treated with 2 MLSE sprays was significantly lower than in plants treated with 1 and 3 sprays. Carbon percentage obtained in plants treated with 2 sprays was also significantly lower than in control plants and plants treated with one spray. There were no significant differences in percentage of carbon in plants of the other treatments. Accordingly, the C/N ratio was higher in control than in MLSE treated plants being lowest with the 3 LSE spray treatment.

Fig. 1.11.a – Nitrogen content of Wheat plants treated with MLSE. Columns with the same letter are not statistically significantly different from each other ($P>0.05$).



**K and $PO_4$ Content**

A very significant increase ($p<0.001$) in levels of potassium was found in all MLSE treated plants, reaching a maximum with the multiple spray treatments (Fig. 1.11.b). Treated plants also presented higher levels of phosphorous (non-significant) than control plants, with the 2 MLSE sprays showing the highest mean value despite the fact that these results were more variable and not significant (Fig. 1.11.c).

Fig. 1.11.b – Potassium content of Wheat plants treated with MLSE. Columns with the same letter are not statistically significantly different from each other ($P>0.05$).

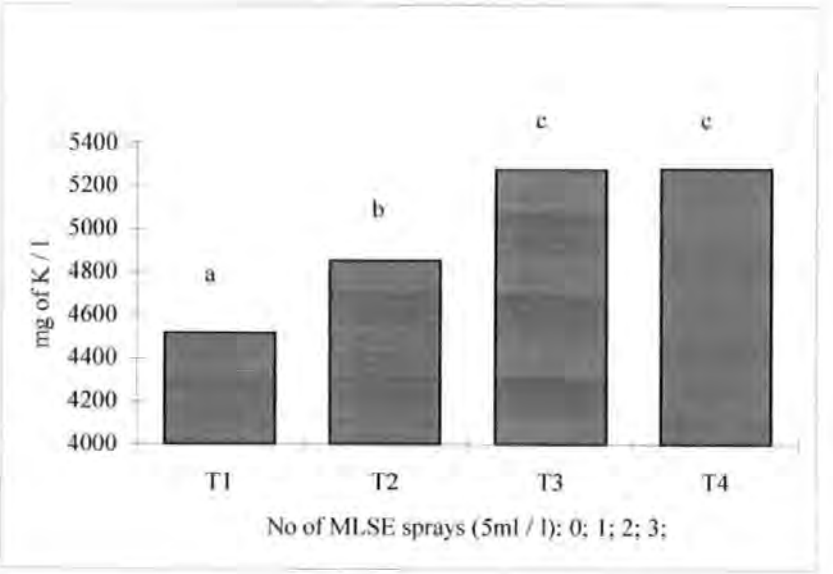


Fig.1.11.d – Effect of one MLSE treatment on Protein band curves of wheat as registered by densitometry.

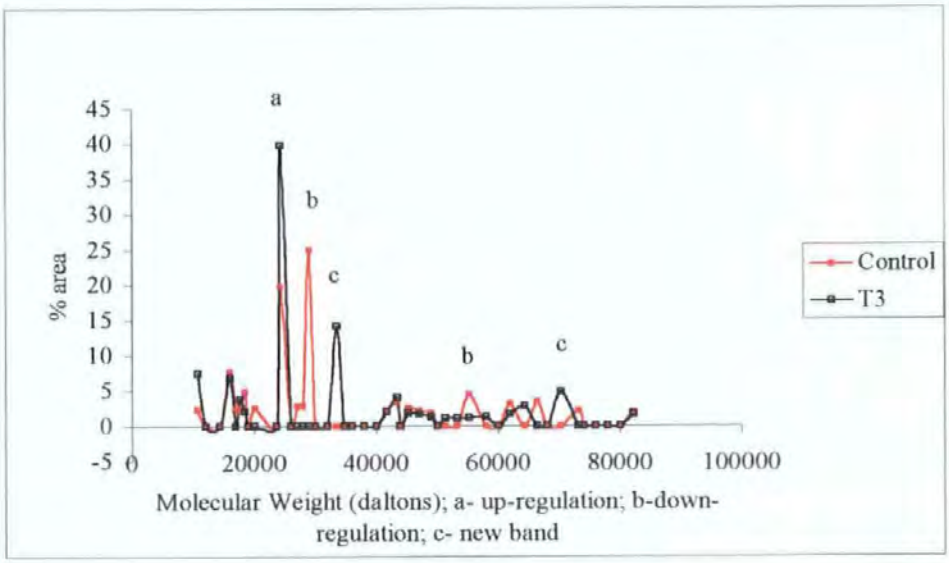



Fig. 1.11.e – Effect of multiple MLSE treatments on Protein band curves of wheat as registered by densitometry.

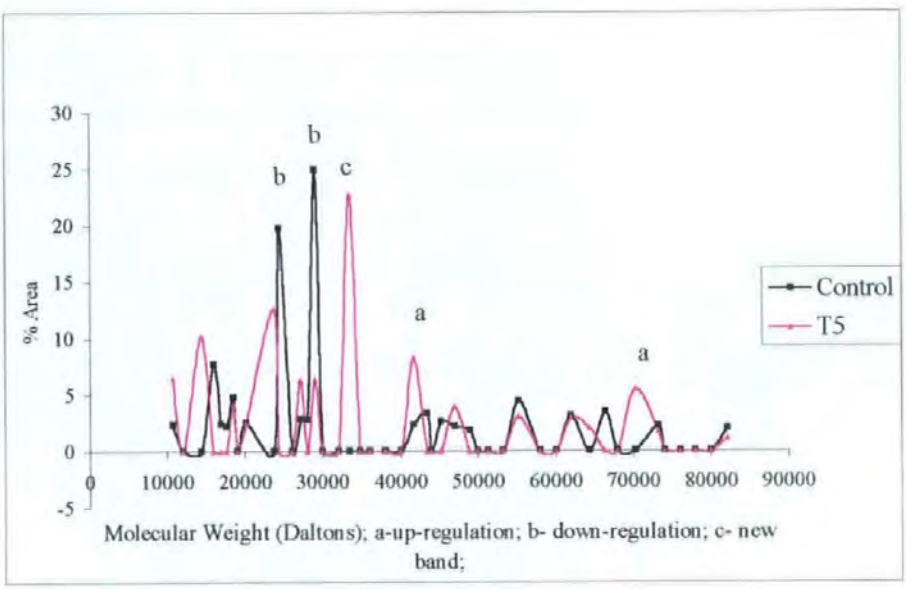
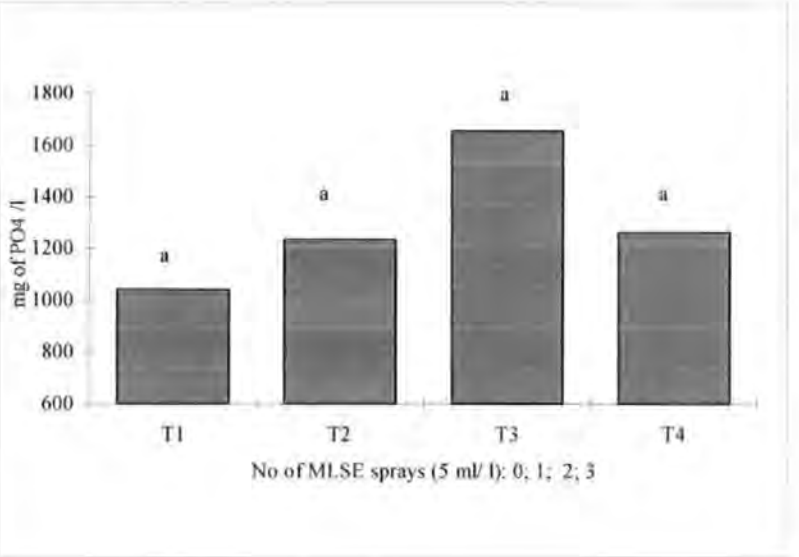


Fig. 1.11.c – Phosphorous content of Wheat plants treated with MLSE. Columns with the same letter are not statistically significantly different from each other (P>0.05).




**Results - Effect of Maxicrop Concentrate Liquid Seaweed Extract on Wheat Plant Growth and Protein Production. Experiment 2.**

**Separation of Proteins**

Observation of gels and the curves obtained by densitometry indicated that, although the treatments applied were different, the responses induced by the MLSE treatments were similar to those observed in experiment 1. There was an indication that an up-regulation of a group of proteins in the high molecular region occurred in MLSE treated plants (Plate 5 and Figs. 1.11.d & 1.11.e). This response was more noticeable for the multiple spray treatment. The gel also showed that a new protein band was present in the same region for all MLSE treatment and that its concentration was higher in multiple spray plants. A reduction on concentration of two bands of low molecular weight occurred in all MLSE treatments and was more noticeable for single spray treatments.

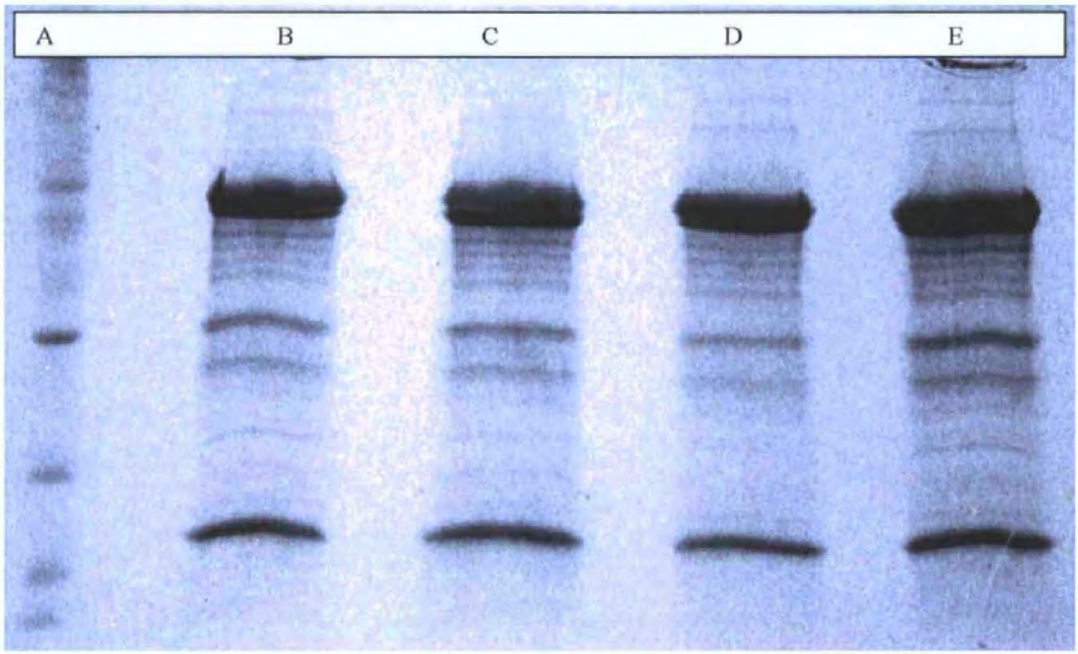



Plate 5- Protein bands, from left to right: A- standards, B- 0 MLSE, C- 1 MLSE spray, D- 2 MLSE sprays, E- 3 MLSE sprays.

**Determination of N, C, K and $PO_4$**

In this experiment the average levels of N and $PO_4$ and C found in MLSE amended plants were equally or only marginally higher and not statistically significantly different from the controls. The seaweed extract treated plants had higher average K content of plants but this was not significant.

**Discussion and Conclusions**

In both experiments the MLSE caused no significant changes in the plant growth and development characters measured.

Electrophoresis of leaf tissue showed that in MLSE treated plants, from both experiments, a band of high molecular weight proteins was up-regulated. Burchett (2000) studying the responses of barley plants to MLSE treatments found that multiple applications could significantly stimulate the production of proteins. His investigation showed that bands of high molecular weight proteins, and a band that he identified as a sub-unit of RUBISCO, were up-regulated in MLSE amended plants. This correlated with treated plants showing increased ability to withstand frost. He concluded that the protein stimulation observed could form part of a mechanism of action of the MLSE that would induce plant defence mechanisms that would allow them to respond better to the stress caused by low temperatures. The experiments described here suggest that wheat plants might respond in a similar way to the seaweed extract, therefore, similar mechanisms of action might be in place in the plants response to fungal infection.

Chemical analysis showed that higher levels of total nitrogen were present in treated plants, which would be expected if a stimulation on protein levels was induced as seen in electrophoresis. Phosphorous and potassium were also present at higher rates in

treated plants and the increase of potassium was highly statistically significant in experiment 1. The discrepancy of results between these two experiments could be due to the fact that plants were grown in different seasons, the second experiment being conducted in late spring. Although efforts were made to ensure that environmental conditions were as similar as possible in both trials, during the second trial it was difficult to maintain constant temperatures in the glasshouse, as the external environment was warmer. To compensate for water lost by plants during this trial, watering had to be supplied more often, thus, irrigation was applied in the days immediately after MLSE treatments. It was shown in the work carried out by Burchett (2000) that the beneficial effects of MLSE in barley plants were decreased when rain occurred within the two days following sprays. It was suggested that the precipitation could dilute and/or leach the MLSE from the soil thus reducing its uptake by the plants and their dependent responses. It is possible that a similar phenomenon could be operating in the second experiment explaining the reduction in response to MLSE observed.

The results described indicate that the MLSE has the potential to alter wheat plants' content in total nitrogen, phosphorous and potassium. This could in turn form part of a signalling strategy that might allow them to respond better to stress challenges of abiotic or biological nature. The responses to MLSE described above could help to explain the suppression of plant diseases by Maxicrop extracts presented here and elsewhere (Cogram, 1994, Pattison, 1994, Walsh, 1997). Further studies of this hypothesis using other plant species would be valuable so that a mechanism of action of the Maxicrop liquid seaweed can be proposed.

## 5. Investigation of Wheat and Take-all responses to Maxicrop Seaweed Extracts - Field Experiments

Early in the research period of this work an opportunity to study take-all responses in the field was available. A parallel research programme had already established a take-all susceptible field trial. This was taken advantage of to study the effects of Maxicrop seaweed extracts in the field.

### 5.1. Field Experiment 1

A field trial was conducted to assess the response of winter wheat plants and take-all inoculum to applications of Maxicrop seaweed extracts. The performances of different application rates of Maxicrop Liquid Seaweed Extract (MLSE) and Maxicrop Extruded Granules (EMG) were assessed in this experiment.

Winter wheat cv. Estica was sown at a seed rate 180 kg/ha, and a row width of 0.125m. Standard husbandry operations for winter wheat were used except for fungicides and growth regulators that were not used as these may interact with the seaweed extract treatments. Plots size was 2×4m and there were six replicates of each treatment in a completely randomised block design.

The following treatments were applied:

1- Control (no Seaweed Extracts)

2- 1 MLSE spray at week 3 of October (2$^{nd}$ leaf emerging - 3 to 5 cm long)

3- 1 MLSE spray at week 4 of October (3 $^{rd}$ leaf starting to emerge)

4- 1 MLSE spray at week 1 of November (3 leaves fully expanded)

5- 2 MLSE sprays at weeks 3 of October and 1 of November

6- 2 MLSE sprays at week 3 of October and 2 of November

7- 4 MLSE sprays at weeks 3 and 4 of October and 1 and 2 of November

8- 2 applications of EMG at weeks 4 of October and 2 of November

The recommended dose rate of MLSE applied was 1l/ha (25 ml of MLSE concentrate per 5 l of water). The rate of application of EMG was 400 g /plot (50 $g/m^2$ = 5 kg/ha).

The plant population was estimated by counting the number of plants along 2x1 m row lengths to establish whether seedling emergence and initial plant population were homogenous throughout the field trial area. This was assessed in the 3rd week of October, when the plants were at growth stage 11 (Zadocks *in* Tottman & Makepeace, 1979) when the 2nd leaf was emerging and plants were 2-5 cm tall. Statistical analysis revealed no significant differences between the plots for plant population. Any differences in terms of the levels of disease and plant growth found could therefore be assumed not to be related to the plant population.

A first assessment of the levels of take-all was carried out in the first week of December (9 weeks after sowing). For this assessment 10 plants from each plot were randomly harvested and washed (care was taken to ensure integrity of the root system throughout the procedure). The parameters assessed were: disease severity, plant growth stage and shoot fresh weight.

A second assessment was carried out in the last week of April (30 weeks after sowing). For this assessment 6 plants from each plot were randomly harvested and scored. The parameters assessed were: disease severity, plant growth stage, plant height and shoot dry weight.

**Results: Field Experiment 1**

None of the factors assessed in either of the two samples from this trial showed statistically significant results partly attributable to a high degree of variability. Some trends were found however, figs 1.12.a and 1.12.b show that the disease levels found were lower for several of the MLSE treatments (Treatments 5, 6, 7 and 8) when compared to control plants. This response was more marked for Treatment 5 (1 MLSE spray at weeks 3 of Oct and 1 of Nov) for which the lowest levels of disease infection in both assessments were found. This could indicate a trend to a reduced plant disease expression with this treatment. Plants treated with EMG showed lesser disease infections than controls and this response was more marked at the second assessment. However, for both harvests, none of the differences between treatments was statistically significant.

Results in Fig. 1.12.b show that the disease symptoms found at the time of the second assessment showed an increase since harvest 1 indicating that the pathogen had developed. On average, plants submitted to treatments 4, 5, 6, 7 and 8 had less severe disease infections than plants not treated with Maxicrop. It was found that plants treated with one MLSE spray at week 3 and 4 of October (Test 2 and 3 respectively), had slightly higher levels of disease infection than plants of control plots. A similar result had been obtained, at the time of the first assessment, for Test 3 and 4 but, contrary to this, plants submitted to Test 2 had shown lower levels of disease than control.

In the first assessment, although all the plants harvested were at the same average growth stage, GS 22 (main shoot and two tillers), the mean plant shoot fresh weight was slightly higher for all plants treated with Maxicrop. The heaviest plants were the ones treated with 4 MLSE sprays (Treatment 7). Figure 1.12.d shows that, in the Spring, plants

of all treatments had very similar mean dry weights and only for Treatment 8 (EMG application) did plants show a slightly higher dry weight.

By the second assessment the differences between treatments, in terms of plant growth stage, were still not significant. All plants had started stem elongation and although plants submitted to Tests 1, 2 and 5 where at GS 31 ($1^{st}$ node detectable) and all other plants were on average at GS 32 ($2^{nd}$ node detectable) this was not statistically significant.

Plant Height was only assessed in the Spring and, as Fig. 1.12e shows, all the plants that received a Maxicrop treatment were taller than control plants. This increase was more obvious for plants submitted to 4 MLSE sprays and to EMG applications but the differences were not statistically significant.

Work proposed for a summer collection of data from the take-all field trial in order to check any effects on ear formation was not carried out. This was decided after analysing the results obtained for the Spring sampling that showed no significant differences between plants of any of the treatments, for either the disease symptoms or the agronomic parameters assessed. Since no further Maxicrop extracts were applied, it was thought that if any differences could be found at a later growth stage these could not positively be connected with the treatments applied in winter.

Fig. 1.12.a – Effect of Maxicrop seaweed extracts on take-all of Wheat grown in the field; Winter assessment. Columns with the same letter are not statistically significantly different from each other (P>0.05).

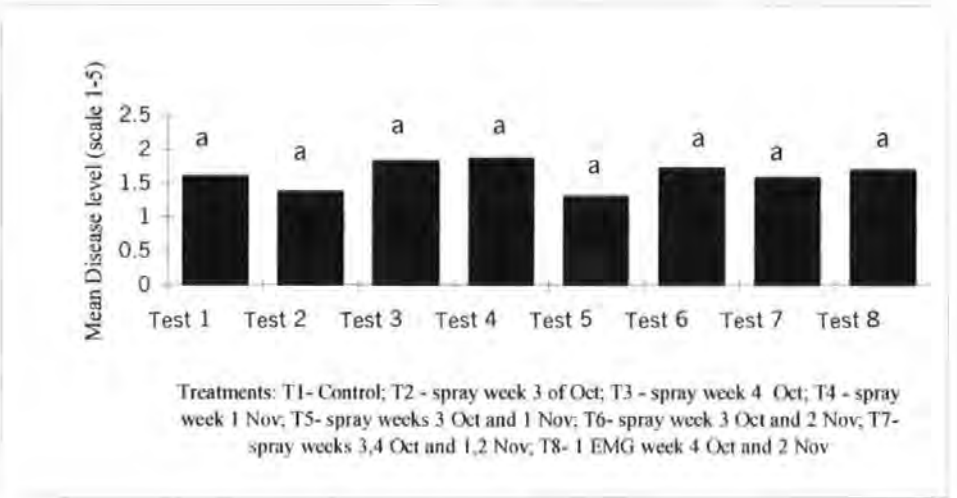



Fig. 1.12.b - Effect of Maxicrop seaweed extracts on take-all of Wheat grown in the field: Spring assessment. Columns with the same letter are not statistically significantly different from each other (P>0.05).

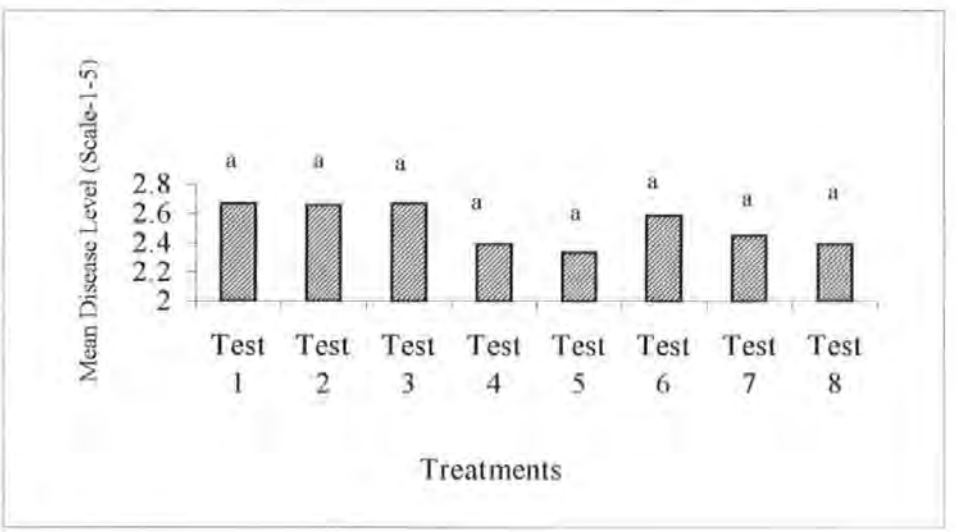


Fig. 1.12.c - Response of Wheat grown in the field to Maxicrop seaweed extracts; Winter assessment. Columns with the same letter are not statistically significantly different from each other (P>0.05).

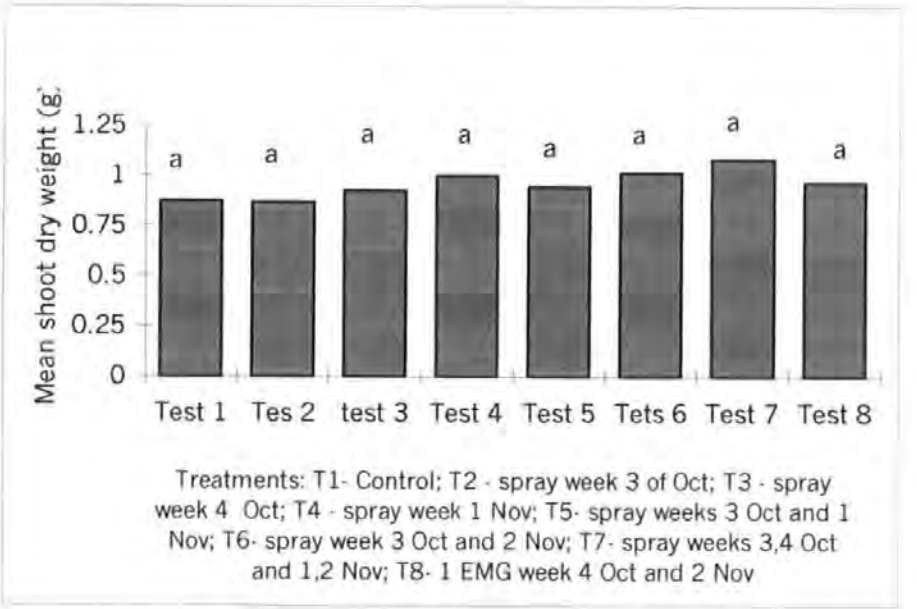



Fig. 1.12.d- Response of Wheat grown in the field to Maxicrop seaweed extracts; Winter assessment. Columns with the same letter are not statistically significantly different from each other (P>0.05).

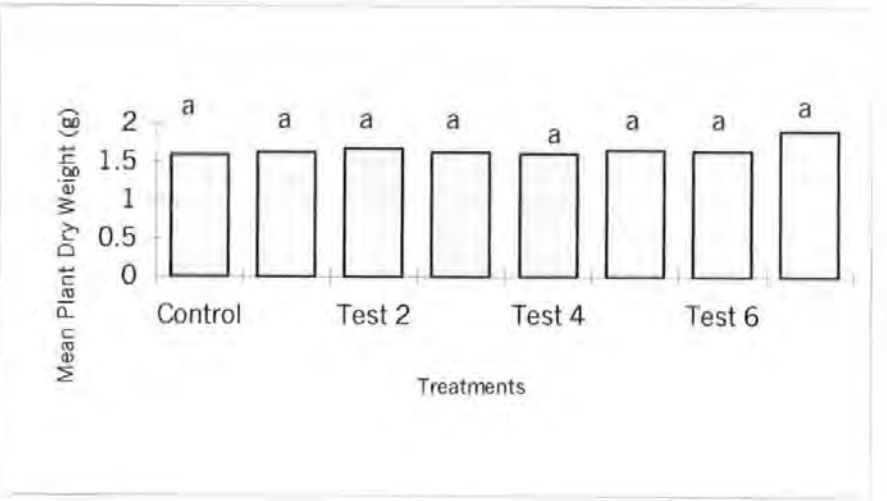
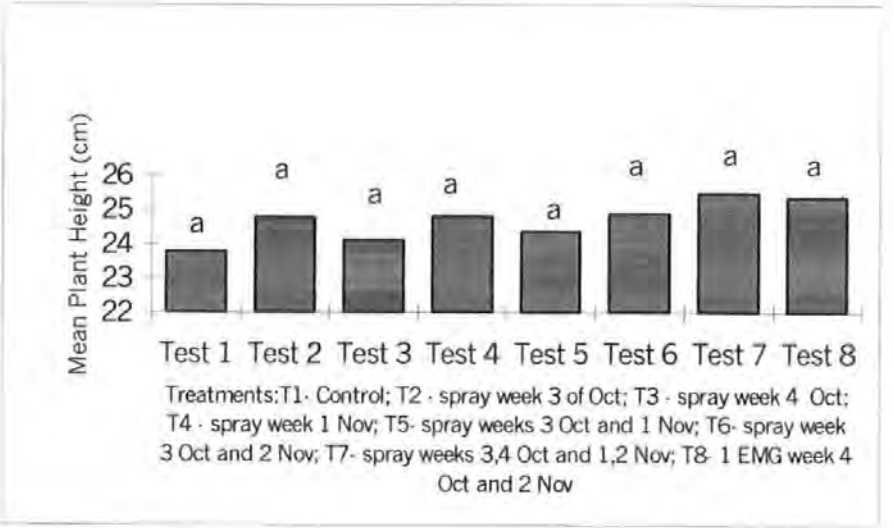


Fig. 1.12.e. – Response of Wheat grown in the field to Maxicrop seaweed extracts; Spring assessment. Columns with the same letter are not statistically significantly different from each other (P>0.05).



## 5.2. Field Experiment 2

Following the analysis of the results obtained in the first field trial a new experiment was planned to ascertain whether similar results would be obtained with consecutive wheat cropping in the same area. Glasshouse and laboratory experiments conducted after the first field experiment indicated that EMG could be detrimental to wheat plant growth when application rate was high. Information on the Maxicrop Extruded Granules product concerning its performance in the field was still limited and it was consequently decided that responses to smaller rates should also be investigated. Two of the plots were treated with EMG at smaller rates than the one studied in the first experiment aiming to obtain more data on the effects of this extract.

The general guidelines applied for the first year field trial were followed to prepare the plots for each treatment. There were six replicates for each treatment in a completely randomised block design.

The following treatments were applied:

1. Control - no Maxicrop Seaweed Extracts
2. 1 MLSE spray at week 1 of December
3. 1 MLSE spray at week 3 of December (3/4 leaves)
4. 1 MLSE spray at week 1 and 3 of December
5. 1 MLSE spray at week 1 of December and week 1 of January
6. 1 MLSE spray at week 1 and 3 of December plus week 1 of January
7. 200 g EMG / plot
8. 400 g EMG / plot

The dose rate of MLSE applied was the one recommended by the manufacturer: 1l/ha (25 ml of LSE per 5 l of water). Both treatments with EMG were applied prior to sowing.

**Results: Field Experiment 2**

Analysis of results of this trial showed no significant effect of any of the Maxicrop Seaweed Extracts applied on take-all severity. The levels of disease found on plants treated with 200 g of EMG (Treatment 3) and 1 MLSE spray at the 3 rd week of December (Treatment 4) were, nevertheless, less severe than in non-treated plants (Fig. 1.13.a). Plants submitted to all other treatments showed equal or slightly higher levels of infection than Control plants.

The agronomic characters studied in this experiment (No of Tillers, Fresh and Dry Weight) revealed that no significant responses were elicited by any of the seaweed extract treatments applied. A slight increase on fresh and dry weight was observed for multiple sprays and both granules treatments (Fig.1.13.b & 1.13.c). Also, a nearly significant (P-value = 0.058) increase on average number of tillers produced was observed in plants submitted to those treatments (Fig. 1.13.d).

Fig. 1.13.a – Effect of Maxicrop seaweed extracts on take-all of Wheat grown in the field; Second Field Trial. Columns with the same letter are not statistically significantly different from each other (P>0.05).

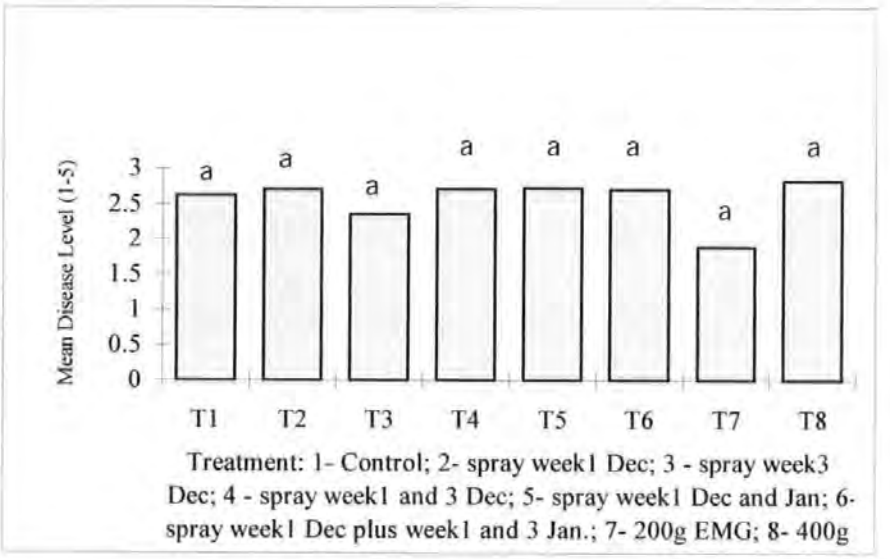



Fig. 1.13.b - Response of Wheat grown in the field to Maxicrop seaweed extracts; Second Field Trial.

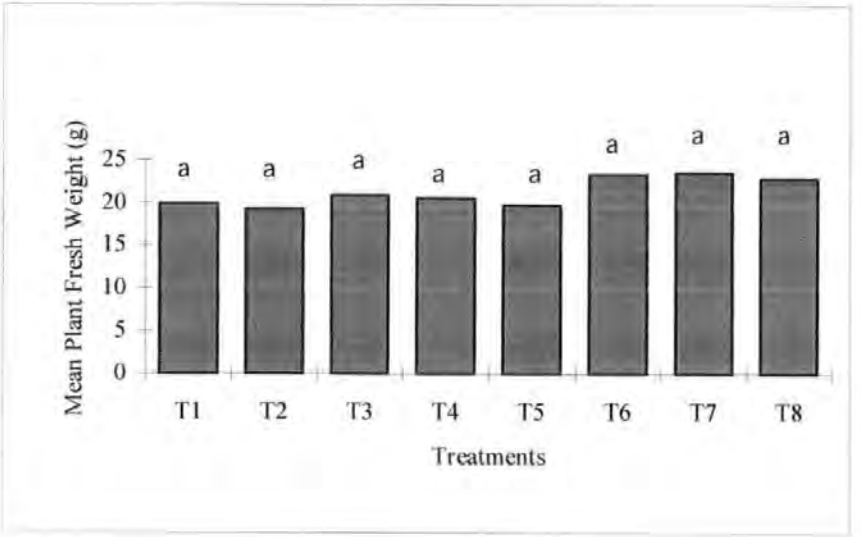


Fig. 1.13.c - Response of Wheat grown in the field to Maxicrop seaweed extracts; Second Field Trial. Columns with same letter are not statistically significantly different from each other (P>0.05).

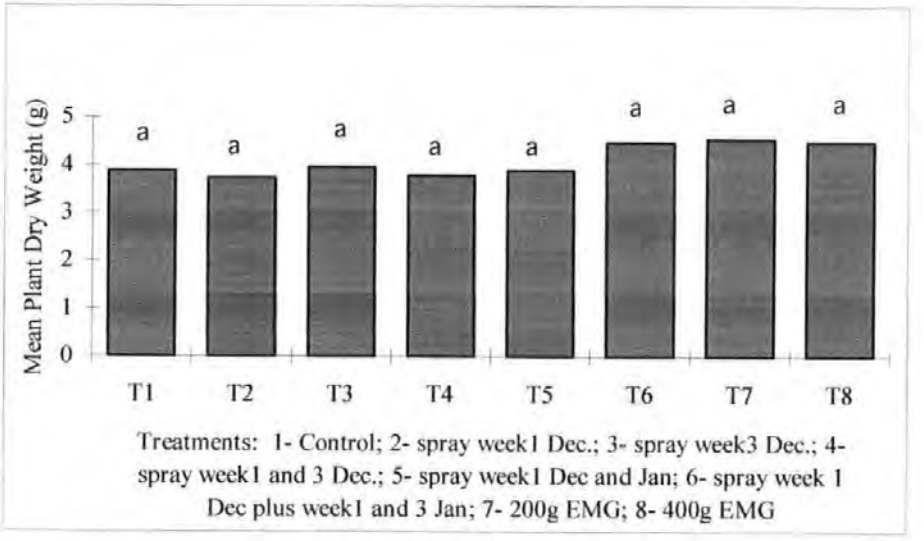
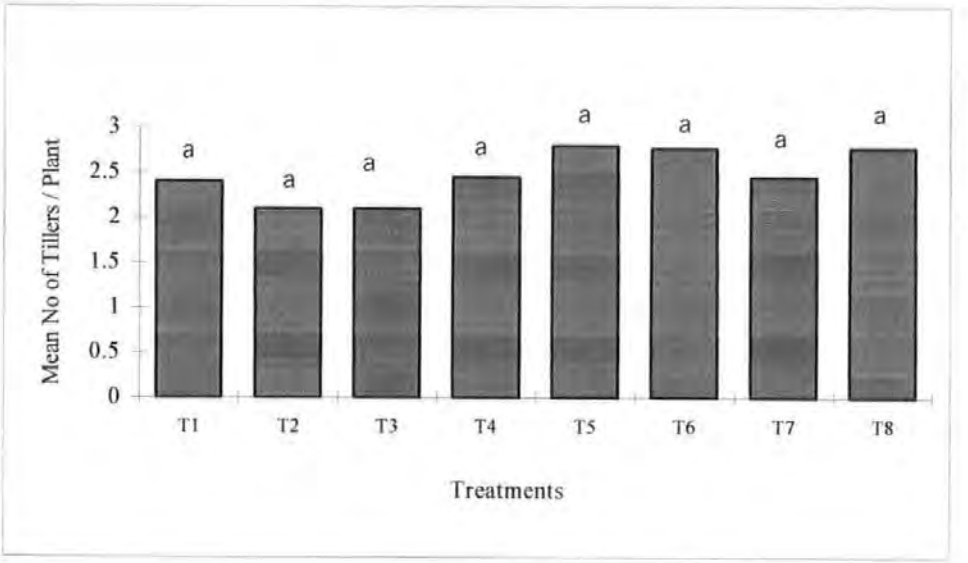



Fig. 1.13.d- Response of Wheat grown in the field to Maxicrop seaweed extracts; Second Field Trial.



**Discussion and Conclusions**

Field trial results did not demonstrate that any significant differences in either plant growth and development or take-all severity occurred due to the Maxicrop seaweed extract treatments applied. Data collected showed great variability of results despite the planning of the experimental set up and the number of replicates of each treatment. Further to that, through out Experiment 2 the weather conditions were very adverse affecting plant establishment and growth, therefore affecting the reliability of results which were analysed with reservations. The results could indicate a trend for reduced disease severity with spray treatments applied at early wheat growth stages, however, further trials should be conducted to ascertain whether a significant response would be obtained under different environmental conditions.

Measurement of agronomic characters in both experiments showed that plant responses to the seaweed extracts were not consistent. Although plant height in Experiment 1 was increased by all seaweed extract treatments and growth was improved with some of the MLSE treatments this not statistically significant and was not observed in Experiment 2. As observed for the disease assessment, it could be argued that there was a trend indicating that multiple sprays and granule treatments may improve wheat plant growth, however, further research is required in order to corroborate this.

In conclusion, other research workers have found that Maxicrop seaweed extracts could significantly reduce take-all disease infections and improve wheat and barley plant growth under controlled environmental conditions (Cogram, 1994 and Burchett, 2000). Our investigations carried out to study the responses to Maxicrop Concentrate seaweed extract, using hydroponic and pot-glasshouse systems, confirmed those findings only in

some instances due to the fact that our results were not consistently statistically significant. Results of both field experiments showed that there was only a very marginal trend of positive responses to the seaweed extracts applied. It is possible that the responses observed could be optimised, therefore, further research should be carried out in order to establish best rates and times of application of the products.

# CHAPTER III

## Study of the Influence of *Ascophylum nodosum* Extracts on Plant Growth and *Phytophthora fragariae* Disease of Strawberries

### Red Core – *Phytophthora fragariae*

The genus *Phytophthora* is included in the Pythiaceae family being closely related to the genus *Pythium*. In the genus *Phytophthora* are included a vast number of fungi species most of which are plant pathogens (Erwin and Ribeiro, 1996). The name of this genus is derived from Greek (phyton, plant; phthora, destruction) and has been first attributed by Anton de Bary in 1876 when describing potato late blight fungus, *Phtytophthora infestans*, which, as the name implies, has a very destructive nature (Zentmyer, 1983). After that first classification many other species belonging to this genus were described, including *Phytophthora fragariae* Hickman, which was identified in 1940.

The number of plant species which can be infected by *Phytophthora sp.* is wide and it includes some of the most serious soil-borne diseases. *Phytophthora* first became famous for the devastation caused in potato crops in Ireland (*P. infestans*) at the end of the 19$^{th}$ and beginning of the 20$^{th}$ century. *P. fragariae, P. infestans* and *P. phaseoli* have narrow host ranges compared to other species, such as *P. cinnamomi* and *P. parasitica. P. megasperma* f.sp.*glycinea* which causes root rot on soybean, has host specificity therefore being classed as *forma especialis*.

Amongst the plant pathogenic species belonging to this genus, there is a wide preference of the plant organs attacked. Some species infect primarily the foliage and other aerial parts while others affect mainly roots and other underground organs. There

are, nevertheless, some examples of the species that invade both aerial and subterranean plant parts.

Some of the unique features of the organisms belonging to this genus can be summarized:

- Production of motile zoospores which have the ability to synthesise a cyst cell wall within minutes of encystment. Complete differentiation of zoospores occurs before release from zoosporangia (=sporangia). Zoospores have whiplash and tinselated flagella.
- Several sporangia are produced at the end of each sporangiophore.
- Cell walls are primarily composed of a cellulose microfibril skeleton and β-1,3-glucans unlike most fungi which are rich in chitin. Mycolaminarin, a β-1,3-glucan, is the usual storage carbohydrate
- Homo and Heterothallic species frequently occur. Single oospores are usually formed in each oogonium and antheridia are usually single.
- They have a diploid nature with meiosis occurring in the gametangia.
- They lack epoxidation of squalene to sterols, therefore, although they do not require sterols for vegetative growth, an external source is essential for sporulation to occur. This property makes plyene antibiotics, like pimaricin, ineffective against *Phytophthora* species as sterol is not present.

Taxonomic studies carried-out within the last decade highlighted the variation within and among species. Variability occurs in morphology, cultural and physiological characteristics, pathogenecity and resistance to fungicides (Erwin *et al*, 1988 and Erwin & Ribeiro, 1996). The group oomycetes, to which *Phytophthora* belongs, is diploid during the vegetative stage whereas almost all other fungi are haploid. These and other peculiar aspects of this group have been the cause of controversy amongst taxonomists, and it is

generally thought that the group should be differentiated from other organisms and that it should no longer be classed as true fungi (Erwin and Ribeiro, 1996).

### *Phytophthora fragariae* Hickman

This species was first associated with red core in strawberry plants by Alcock in the 1920's but it was only two decades later that it was fully described by Hickman (Erwin and Ribeiro, 1996). It has a limited host range as it appears in the field only on strawberry although it is capable of infecting other plant species after artificial inoculation. In all strawberry growing regions in the world this root rot causes considerable economic damage in the field and it is considered by some as the most important fungal disease of this crop. A complete loss of crop often occurs after the first bearing year in contaminated fields.

*Phytophthora fragariae* shows its optimum growth at relatively low temperatures ranging from 10 to 15°C, nevertheless, it is capable of growth within a larger scope of temperatures. In culture it has slow growth and is more nutritionally demanding than other species.

#### Disease Symptoms

Red core (UK common name) or red stele (USA common name) is usually detectable in spring or early summer when severe stunting can be found as, with warm temperatures, outer leaves or entire plants can suddenly wilt. Leaf production on infected plants is affected and the new shoots are smaller, blue green and borne on short petioles. Old leaves senesce prematurely taking various shades of yellow, red and brown, then wilting and drying. At the later stages of infection fruits are either not produced or they

shrivel up before their growth is completed. Intermediate levels of infection are often undetectable but as the disease progresses plant growth becomes increasingly retarded.

Symptoms at the root level are characterised by the reduction or absence of lateral roots and a dark brown or black coloration of larger roots. The stele is distinctively red or brown reddish in sharp contrast to the white cortical tissue. This coloration may extend to the whole root up to the crown, thus, the disease is commonly known as red core. As infection is not favoured by high temperatures, root growth over the summer may be unaffected providing an opportunity for partial or total recovery, depending on the severity of prior infection. Isolation of *P. fragariae* and diagnosis from field plants should be made in spring as diseased roots decay during the summer and evidence of the disease is hard to find (Erwin and Ribeiro, 1996).

**Disease Life Cycle**

Epidemiologicaly, this disease can be characterised as a multi-cycle type, unlike most other non-oomycetous root diseases. It has a short generation time and great reproductive capacity as new inoculum is continuously produced within the crop year after an initial infection. This feature of this pathogen gives it the potential to cause severe epidemics when the environmental factors are favourable. The presence of water in the soil is essential for disease infection to spread and its development is not common if soil water-holding capacity is below 50%. Expansion is typically triggered by soil temperatures below 18°C and free water during autumn, winter and spring. Slightly infected plants are often undetected, thus, becoming sources of pathogen for new sites (Erwin and Ribeiro, 1996).

This pathogen produces, in its' sexual phase, copious numbers of oospores in infected steles which can survive 3 or more years in the field (Duncan) even in adverse

conditions such as drought. In some hosts, oospores were found also in leaves, petioles and stolons. In culture, however, they do not develop in abundance. Oospores have the ability to germinate in the soil and infect the plant through the germ tubes or by producing sporangia which then release zoospores. It is possible that oospores are stimulated to germinate by exposure to combination of light, a pH near neutral (6.5-7.0) and a change in external temperatures.

The assexual sporangium, since it contains zoospores is also known as a zoosporangium. The sporangia of this species have a characteristic lemon shape and they appear hyaline to light yellow by transmitted light microscopy measuring approximately 40μm in diameter. When matured its plasma membrane ruptures releasing the zoospores. Zoospores are reniform in shape with two flagella, one of which has a long whiplash form. Zoospores can swim for hours and when they eventually stop they loose the flagella, round up and develop a cell wall within minutes. This type of encystment is induced artificially by shaking the zoospores and naturally when they encounter a solid surface. Cysts germinate through a germ tube and development of mycelia and stimulation of this process is triggered by various types of chemical substances, most of which can be found in or near the root surface (Hardham *et al*, 1991).

The production of zoospores and their release from sporangia is considered the most important part of the life cycle of *Phytophthora* since it sustains a rapid population increase and wide dispersion in the presence of free water (Erwin and Ribeiro, 1996). In culture, sporangia production is optimal on new mycelial growth only when the culture is changed from a relatively rich medium to near starvation regime. Sporangium formation seems to require an exhaustion of the sugars in the medium, while oospore production demands an adequate level of carbohydates. Production of sporangia also demands free water, therefore, in laboratory experiments aqueous salt solutions and soil extracts are

often used for the study of this phenomenon. It has also been demonstrated that, both in soil and laboratory, concentrations of oxygen and carbon dioxide must be similar to those normally found in the atmosphere. Combinations of $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, and $K^{+}$ are also reported as essential elements for sporangia formation (Erwin and Ribeiro, 1996). KMnO4 at 0.025% killed mycelial fragments and chlamydospores but stimulated oospore germination. Trolldenier (1979) found that plants grown in K deficient medium had greater exudation rates than plants treated with higher K, these exudates may attract more pathogens.

It has been reported that plants respond to infection through phytoalexin accumulators. Studies with *P. infestans*, *P. megasperma* f.sp. *glycinea*, amongst others, revealed that phytoalexin elicitors originally produced or present in the pathogens are probably glycoproteins or of a lipidic nature. β-1,3-glucans or mycolaminarins isolated from the fungi were amongst the carbohydrate-containing elicitors capable of enhancing that phytoalexin response (Friend, 1991 and Hohl, 1991). There is also some evidence indicating that phytoalexin responses involve $Ca^{2+}$ and $H^{+}$ uptake, $K^{+}$ efflux and $Ca^{2+}$ - dependent protein phosphorylation (Parker, 1991).

**Disease Control**

The most effective disease control measure is by preventing sources of inoculum reaching production sites by using planting material that is pathogen free. This measure is of extreme importance as the disease can spread at great speed when conditions are wet and cool. Various methods of disease detection have been employed with varying degrees of success. Baiting with susceptible roots and the use of serological enzyme-linked immunosorbent assay (ELISA) are two methods that have helped to eliminate stocks that were contaminated with some success (Duncan *in*: Walsh, 1997).

Once the disease is found in the field the most common control measure is soil fumigation using, for example, a mixture of methyl bromide and chloropicrin. This is a costly measure that has to be applied each year before new plants are put into the soil. Root drenches with chemicals, such as metalaxyl, prior to planting followed by sprays of, for example, fosetyl-al has shown only limited and temporary success as most chemicals are site-specific and field resistance rapidly develops. Some authors also argue that because most fungicides cannot kill oospores, the use of root dips has only limited value. The other problem concerning the use of such chemicals is the development of pathogen tolerant or resistant strains in the field. Metalaxyl is a phenylamide that has already shown reduced effectiveness in the field, especially in the protection of potato late blight (Cooke, 1991 and Davidse *et al*, 1991). A synergistic chemical protection seems to give some advantages when compared to the use of a single fungicide. This approach can explore the fact that resistance is slower to build up when different sites in the metabolic pathways and in the life cycle of the pathogen are targeted (Gisi, 1991).

Methyl bromide emits halogen residues which are considered detrimental to the ozone layer in the atmosphere, therefore, its large scale use as a soil fumigante in agricultural areas will probably be discontinued. If this is implemented, since rotations are of limited value in intensive production areas because of the long term inoculum survival capacity, more emphasis will have to be placed on detection, sanitation measures, host resistance and possibly biocontrol measures. Several biocontrol strategies have been employed against *Phytophthora* pathogens. Finlay & Macracken (1991) indicated the following methods as the most promising:

- use of microbial antagonism to reduce pathogen inoculum either favouring the growth of existing species or through the introduction of new agents;
- isolation of plant surfaces against infection;

- exploring incompatibilities between host and pathogen.

Several biocontrol agents have been investigated against different species of *Phytophthora* with varying degrees of success. The actinomycete group has some of the most promising microorganisms applied either singly or in mixtures (Erwin and Ribeiro, 1996).

Past research has indicated that Maxicrop seaweed extracts can affect the life cycle of *Phytophthora cinnamomi* inducing morphological changes under *in vitro* environment. Varying degrees of control of the disease *in planta* were reported with applications of the seaweed extracts under a glasshouse environment (Walsh, 1997; Pattison, 1994). Although the responses reported sometimes lacked statistical significance it was thought that it would be valuable to explore further the responses to the seaweed extracts by another member of *Phytophthora* species. It was thought that *Phytophthora fragariae* could be of greater value in investigations of sporangia formation and zoospore release than *Phytophthora cinnamomi* as it can be induced to produce those structures in greater abundance (Grant *et al*, 1985). Responses to different treatments *in planta* can also be simplified as the host of this pathogen has a shorter life span. We have consequently decided to extend and diversify the studies made with *P. cinnamomi* to *P. fragariae*. The main aims of this research were divided into the following stages:

1- Study the response of *Phytophthora fragariae* to Maxicrop concentrate seaweed extract when grown *in vitro* in solid medium.

2- Determine and analyse the response of *Phytophthora fragariae* to Maxicrop concentrate seaweed extract *in vitro* in liquid medium. Identify and record induced morphological changes if any.

- exploring incompatibilities between host and pathogen.

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3- Determine and analyse the responses to Maxicrop concentrate seaweed extract on wheat and strawberry plants and the infectivity of *Phytophthora fragariae* Hickman under controlled environment on hydroponics.

5- Study the responses to Maxicrop concentrate seaweed extracts of wheat and strawberry plants and the infectivity of *Phytophthora fragariae* Hickman when the plants were grown in pots in a glasshouse environment.

## 1. Effect of Maxicrop Concentrate Seaweed Liquid Extract on the growth of *Phytophthora fragariae in vitro* in Solid Medium

In parallel to experiments conducted to assess the responses of *Gaeumannomycis graminis* to MLSE *in vitro*, experiments were conducted to assess whether *P. fragariae* colony growth would be affected by the addition of the Maxicrop seaweed extract to its growth media.

**Materials and Methods**

*P. fragariae* cultures were grown in Red Bean Agar (RBA) at 15° C until mycelia approached the edge of the plates. A 1 cm plug was cut from the advancing edge of the colony using a flame sterilised cork borer and placed in Petri dishes containing either standard RBA or RBA amended with MLSE.

Standard RBA medium ingredients:

- 17g Agar No 1 (Sigma)
- 35g Ground Red Beans
- 1 l Distilled Water

RBA + MLSE ingredients:

- 17g Agar No 1 (Sigma)
- 35g Ground Red Beans
- 1 l MLSE solution (5ml MLSE concentrate $l^{-1}$ DW)

The fungal colony diameter was measured along two axes chosen at random at right angles to each other and the mean of the two values was taken. Measurements were taken on day 5, 10 and 15 after sub-culturing, there were 6 replicates per treatment and the experiment was conducted twice. The daily growth rate was calculated using the following formula:

d2 – d1 / number of days between d1 and d2;

where d1 = colony diameter 5 days after incubation; d2 = colony diameter 4-5 days after d1 (after Erwin & Ribeiro, 1996).

**Results**

*P. fragariae* growth in solid media is slow (Erwin & Ribeiro, 1996), however, the assays showed that the growth of the fungus over RBA was not altered when the MLSE was added to the media. There was no significant difference (P>0.05) between colony sizes even though in MLSE treated media the average area covered by the colonies was slightly smaller than in standard RBA medium. The results showed that colony daily growth rate slowed down as the colony expanded over both types of media (Table 5.1). The reduction in growth was slightly faster in standard RBA than where MLSE was present but this was not significant (P>0.05). Observations throughout the assays suggested that no changes in mycelia characteristics were triggered by the MLSE.

Table 5.1– Daily growth rate (mm/d) of *Phytophthora fragariae* cultures grown in standard RBA and RBA amended with MLSE.

| | Assay 1 | | Assay 2 | |
|---|---|---|---|---|
| Incubation Period (days) | RBA | RBA + MLSE | RBA | RBA + MLSE |
| 5-10 | 6.2 | 5.3 | 5 | 5.1 |
| 10-15 | 3.6 | 3.6 | 1.2 | 1.5 |
| Significance | n.s. P>0.05 | | n.s. P>0.05 | |

## 2. Response of *Phytophthora fragariae* to treatments with Maxicrop Concentrate Seaweed Liquid Extract in Liquid Medium

The release and dispersion of zoospores, the most relevant infective structure of *Phytophthora*, requires free water to be available in the rhizosphere (Erwin & Ribeiro, 1996). Previous research studies have revealed that Maxicrop extracts could alter morphology of other *Phytophthora in vitro* in a liquid medium (Walsh, 1997; Pattison, 1994). The aim of this investigation therefore was to assess how *P. fragariae* responded to applications of MLSE under zoospore inductive conditions. This could effectively be achieved if the mycelial growth, formation of sporangia and release of zoospores could be studied in a liquid medium that would allow identification of morphological changes induced as a response to the seaweed extract.

In total a series of nine sequential experiments were conducted each replicated four times. Initial materials and methods for the establishment of cultures were identical.

### Materials and Methods for the establishment of cultures

*Phytophthora fragariae* cultures were grown in Red Bean Agar (RBA) solid medium at 13-15°C until the mycelia occupied approximately two thirds of the plate. Plugs (1cm diameter) were then cut from the advancing edges of the colonies and placed in Petri dishes containing 15 ml of a liquid solution. This solution was made up of standard soilless leachate or soilless leachate containing MLSE. The MLSE was prepared as described before (5 ml $l^{-1}$ MLSE in DW).

The Petri dishes were kept at 15°C through out and the solutions were changed daily. At the fourth day of the experiment microscope observations (100× magnification) were made in order to identify and record induced fungal morphological changes if any.

Morphological responses were quantified by counting the number of sporangia at the edge of each plug. Three random counts were made where all sporangia were counted in the field of view. In part of the experiments (2, 3, 4 and 5) the response to the MLSE treatments was further assessed by evaluating zoospore presence and locomotion patterns using the following classes:

1- No zoospores observed in the medium

2- Zoospores observed in the medium - abnormal locomotion patterns

3- Zoospores observed in the medium - normal locomotion patterns

Soilless leachate is one of the media routinely employed for the production of zoospores of *Phytophthora* (Erwin and Ribeiro, 1996). It was prepared by pouring 150 ml of distilled water over 15g of soil-free compost (Bulrush & Bowers peat based compost), contained in a plastic filter funnel. The solution was filtered through Whatman No. 4 filter paper and stored in a refrigerator at 5°C until needed (never stored longer than 10 days).

## 2.a. *In vitro* Experiment 1 – Initial screen for the response of *Phytophthora fragariae* to treatments with Maxicrop Concentrate Liquid Seaweed Extract in Liquid Medium

This experiment was conducted as an initial screen in order to determine whether the liquid media would be appropriate for the growth of *Phytophthora fragariae* and the study of its responses to MLSE *in vitro*. It aimed to investigate whether the Maxicrop concentrate seaweed extract had any effect on the growth or morphology of the pathogen. Microscopic observations were compared with descriptions and illustrations published by Erwin (1988) and Erwin & Ribeiro (1996). The treatments under study were the following:

1- MLSE (control)

2- + MLSE (5ml $l^{-1}$)

### Results

Observations made at the end of the experiment showed that fungal growth was normal in control plates with abundant hyphal growth and normal morphology (Plate 6). A large number of sporangia, the structure where zoospores are produced, was found in all control plates. Sporangia were distinctly limoniforme in shape characteristic of this species (Plate 7). Some of these structures had matured and the release of zoospores into the liquid media was observed. This process would occur suddenly and as the outer membranes opened the zoospores swiftly abandoned the sporangial sac and spread throughout the liquid medium exhibiting normal swimming pattern and then encysting (Plate 8).

Fungal growth in plates amended with the seaweed extract was very small or absent. Only a few sparse hyphal strands could be observed and sporangia could only be

found on one of the plugs observed. Zoospores were not observed in any of the plates amended with the seaweed extract. These observations indicated, therefore, that a fungicide/fungistatic-like activity was exerted by the Maxicrop seaweed extract and merited further investigations into the responses obtained.

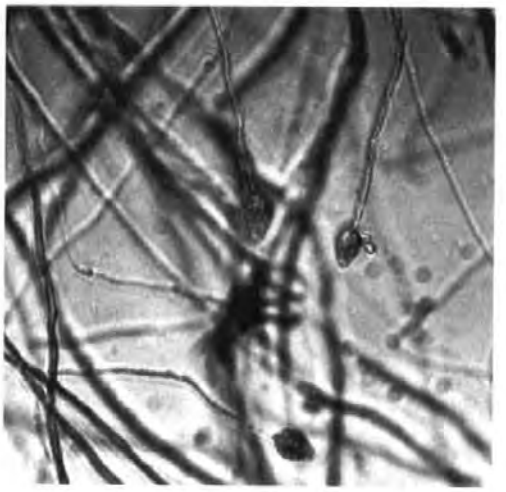


Plate 6– Sporangia formed by abundant normal mycelium of *Phytophthora fragariae* in control solution.

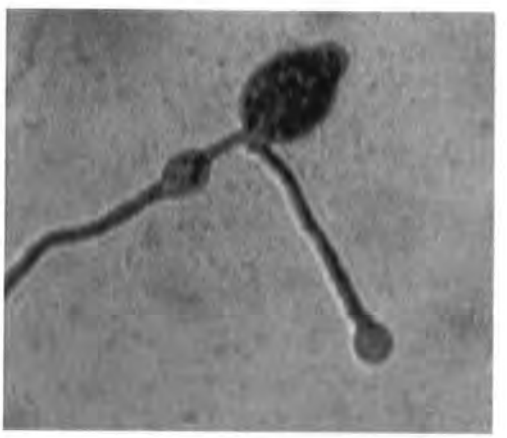


Plate 7– *Phytophthora fragariae* sporangium formed in control solution.

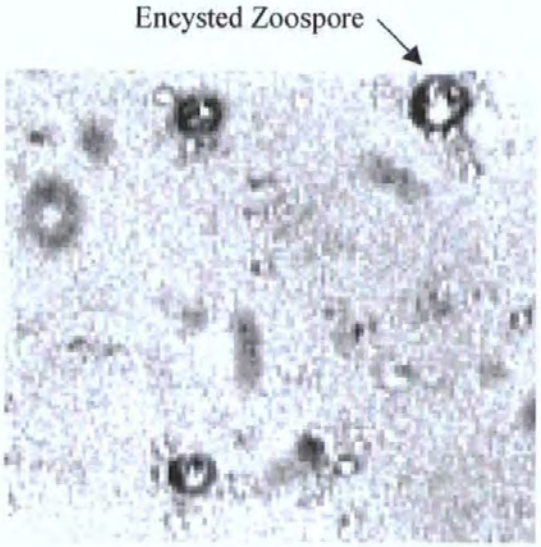



Plate 8 – Encysted zoospores of *Phytophthora fragariae* observed in control solution.

**2.b. *In vitro* Experiment 2 - Response of *Phytophthora fragariae* to treatments with varying concentrations of Maxicrop Concentrate Liquid Seaweed Extract in Liquid Medium**

This experiment was conducted to assess whether responses of *P. fragariae* to Maxicrop concentrate liquid seaweed extract observed in Experiment 1 were repeatable and, if so, to analyse them further. Assessments of the numbers of sporangia formed at the edge of each plug were carried out. Responses of zoospores were also further analysed by assessing their abundance and their locomotion patterns in the liquid medium.

Five treatments were studied:

1- 0% MLSE (Control)

2- 25% MLSE

3- 50% MLSE

4- 75% MLSE

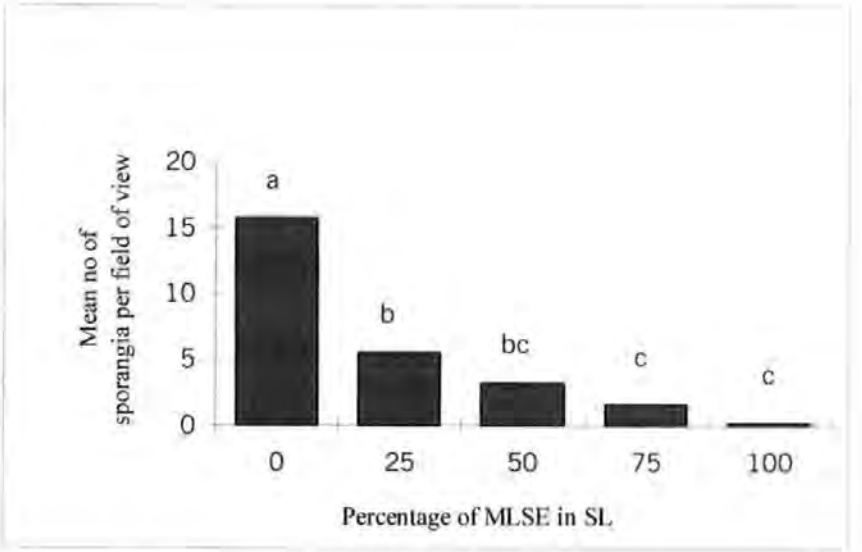
5- 100% MLSE (5ml $l^{-1}$)

**Results**

Observations made throughout this experiment indicated that *P. fragariae* responded negatively to all MLSE treatments and clear disruptions on the growth of the fungus were observed. All treatments of MLSE had a marked suppressive effect on the number of sporangia formed by the *P. fragariae* culture plugs (Fig. 2.1). The effect observed was significant ($P<0.05$) for 25% MLSE and very highly significant ($p<0.001$) for the other concentrations applied. Evaluation of zoospore production and their locomotion patterns revealed that where they were present in MLSE treated media they

exhibited abnormal movement patterns. No zoospores could be found in liquid medium made up of 100% MLSE concentrations.

Fig. 2.1 – Effect of treatment with MLSE on *Phytophthora fragariae* production of sporangia in soilless leachate (SL). Columns with the same letter are not statistically significantly different from each other (P>0.05).



## 2.c. *In vitro* Experiment 3 - Response of *Phytophthora fragariae* to treatments with Maxicrop Concentrate Liquid Seaweed Extract in Liquid Medium

A criticism that could be made of Experiments 1 and 2 is that the concentration of the MLSE solution was confounded with the concentration of soilless solution present in the plates such that, for example in Petri dishes treated with 100%, no soilless was present. An experiment was therefore necessary to determine the response of *P. fragariae* to different concentrations of soilless leachate. The general method previously described was followed and the soilless leachate was progressively diluted with distilled water.

1- 0% DW (100% soilless leachate)

2- 25% DW

3- 50% DW

4- 75% DW

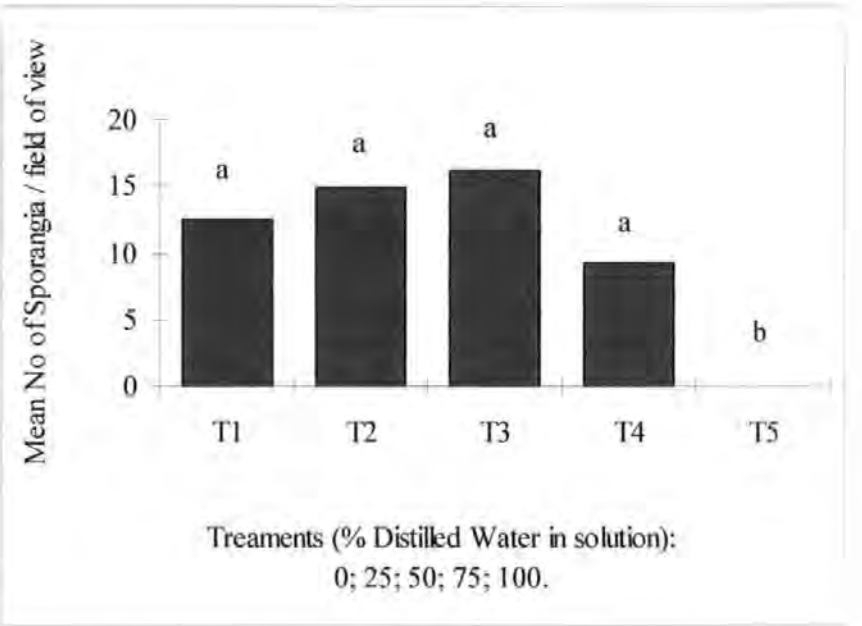
5 – 100% DW (0% soilless leachate)

### Results

Numbers of sporangia observed in controls were of the same magnitude as observed in Experiments 1 & 2. A slight to moderate dilution of the soilless solution was not detrimental to the production of sporangia and the numbers even increased slightly when compared to control (Fig. 2.2) although not significantly ($P>0.05$). A significant reduction in sporangia production was observed when media was made up of 75% DW and no mycelium growth or sporangia production occurred in 100% DW (Treatment 5). Zoospores were present in all plates except 100% DW and their locomotion behavior was unaffected by the progressive dilution of the medium.

These results suggest therefore that the responses of *P. fragariae* observed in Experiment 1 and 2 could only partially be attributed to the dilution of the medium. In experiments 1 and 2, the abnormal behavior of the zoospores observed in MLSE treatments, for example, was not observed here. In highly diluted soilless leachate (75 % DW) zoospores were still found and their locomotion pattern was normal.

Fig. 2.2 – Effect of dilution of Soilless leachate (SL) with DW on the production of sporangia by *Phytophthora fragariae*. Columns with the same letter are not statistically significantly different from each other (P>0.05).



## 2.d. *In vitro* Experiment 4 - Response of *Phytophthora fragariae* to treatments with Maxicrop Concentrate Liquid Seaweed Extract in Liquid Medium

This experiment repeated experiment 2 but the MLSE was dissolved directly into soilless leachate medium. Treatments were:

1- 0% MLSE 100% soilless leachate (SL)

2- 25% MLSE 100% soilless leachate (SL)

3- 50% MLSE 100% soilless leachate (SL)

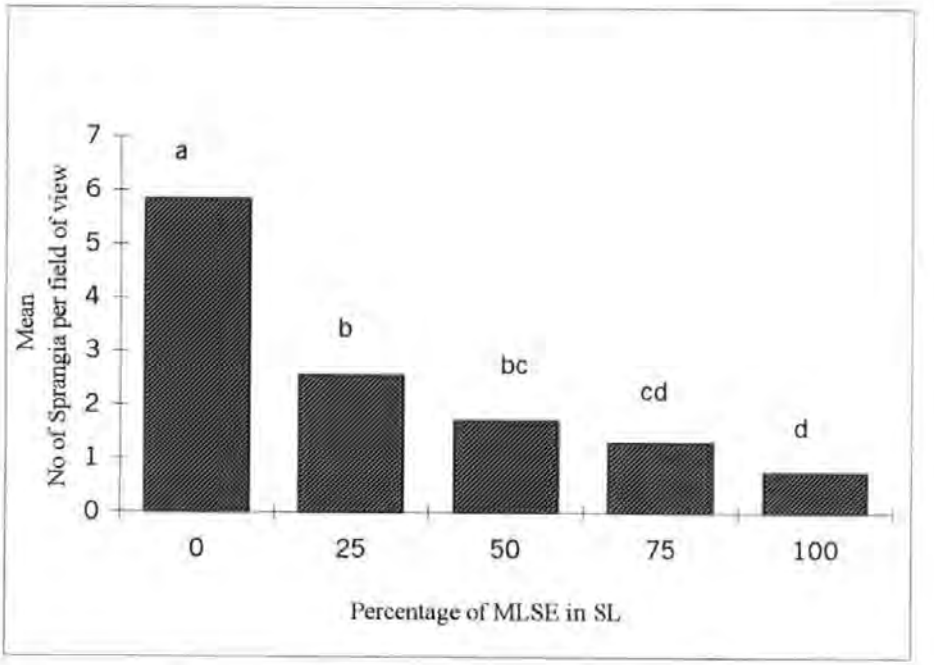
4- 75% MLSE 100% soilless leachate (SL)

5- 100% MLSE 100% soilless leachate (SL)

### Results

Sporangia numbers observed in this experiment were approximately half of those observed previously but a quantitative response was still evident in response to MLSE concentration as in experiment 2. Results confirmed that the presence of the MLSE in the mediium was sufficient to impair the normal fungal growth of *P. fragariae* (Fig. 2.3). A significant ($P<0.05$) suppression of mycelium growth with a consequent reduction in sporangia formed was observed with even the most dilute MLSE concentration and was highly significant ($P<0.001$) with the highest MLSE concentrations. Despite a small number of sporangia being produced in MLSE amended plates, zoospores were only found in plates of 25 and 50% MLSE. The numbers of zoospores present in 50% MLSE was, however, much reduced when compared to 25% MLSE and their locomotion patterns were abnormal with the loss of ability to swim in a normal random zigzag like pattern but instead swam in a circular fashion at a reduced speed.

Fig. 2.3 – Effect of MLSE on sporangia production by *Phytophthora fragariae* in non-diluted soilless leachate (SL). Columns with the same letter are not statistically significantly different from each other (P>0.05).



## 2.e. *In vitro* Experiment 5 - Response of *Phytophthora fragariae* to various concentrations of Maxicrop Concentrate Liquid Seaweed Extract and Distilled Water in Liquid Medium

Following the previous experimental results this experiment was conducted to study the responses to both medium dilution with DW and amendment with MLSE in parallel. The solutions investigated were prepared as described for Experiments 3 and 4.

In this experiment the treatments studied were:

1- 0% MLSE - Standard Soilless leachate (SL)

2- 0% DW - SL

3- 25% MLSE

4- 50% MLSE

5- 75% MLSE

6- 100% MLSE

7- 25% DW

8- 50% DW

9- 75% DW

10- 100% DW

**Results**

Sporangia numbers were higher in control plates in this experiment than had previously been observed. As a consequence this experiment can be considered to be more sensitive than previous experiments. Where DW was added to the soilless leachate a more sparse mycelium growth occurred and the numbers of sporangia formed dropped (Fig. 2.4).

The drop in number of sporangia was progressive and proportional to the dilution of the media. This was in contrast to the responses previously observed in Experiment 3, where a reduction in fungal growth and numbers of sporangia formed only occurred with the 75% DW treatment but this may be a function of the lower numbers of sporangia produced in that assay. Effects of water dilutions observed here were, nevertheless, less drastic than the effects of MLSE. There was a significant difference in numbers of sporangia produced in plates diluted with DW and plates treated with MLSE. Thus, these results indicated that MLSE had a more dramatic negative impact on the fungus. The difference in response to the two types of treatments suggested that additions of DW to the media affected the fungus in so far as it caused a reduction of nutrients and/or stimulatory substances in the environment. As in Experiment 3 fungal growth did not occur in 100% distilled water, and consequently sporangia were not formed, probably due to the total absence of nutrients and/or stimulatory substances in the media.

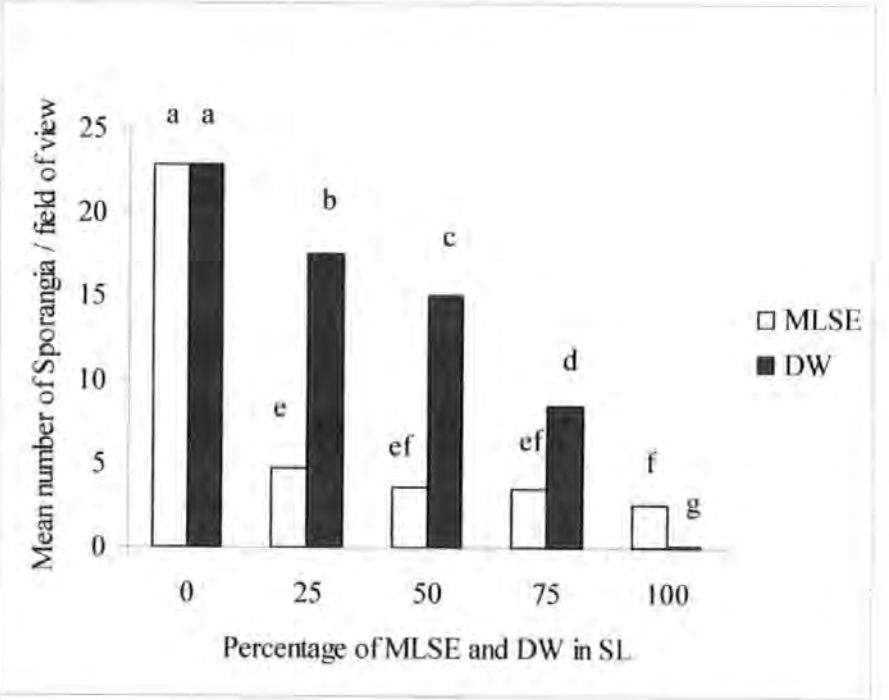
The results obtained here suggested that the suppressive effect of MLSE was not directly proportional to the increase in MLSE concentrations in the medium. There was a significant reduction in the numbers of sporangia formed where 25% MLSE was applied. The level of suppression of sporangia formation observed for treatments with 25, 50 and 75% MLSE was similar ($P>0.05$) and not significantly different from each other. In common with the previous experiments 100% DW completely suppressed sporangia production whilst 100% MLSE did allow a small amount of sporangia.

As in the previous experiments although sporangia were formed the zoospores released from them did not show a normal behavior. The response of zoospores both to dilution of media and amendments with MLSE confirmed observations previously made in Experiments 2, 3 and 4. The zoospores seen in any of the DW treated plates evidenced

normal swimming patterns while zoospores in MLSE treated plates showed ineffective movements.

It can be concluded from these results that MLSE can consistently significantly reduce the production of sporangia by *P. fragariae* and disrupt zoospore locomotion. It was therefore hypothesized that one or several of the seaweed extract compounds may have a fungicide-like or fungistatic-like activity.

Fig. 2.4- Effect of treatments with MLSE and DW on *Phytophthora fragariae* sporangia production in soilless leachate. Columns with the same letter are not statistically significantly different from each other (P>0.05).



**2.f. *In vitro* Experiment 6 - Response of *Phytophthora fragariae* to treatments with Maxicrop Concentrate Liquid Seaweed Extract and Potassium Chloride in Liquid Medium**

Previous experiments demonstrated that MLSE was consistently detrimental to *P. fragariae* growth *in vitro* in liquid medium. To further understand the responses observed it would be important to determine which components or group of components of the concentrate were responsible for the changes in the fungus behavior. Previous reports have suggested that that various cations can have detrimental effects on fungal pathogens and potassium has been identified as one of those cations capable of suppressing plant diseases, including *Phytophthora* species (Bécot *et al*, 2000, Erwin & Ribeiro, 1996, Urs *et al*, 1997, Zhang *et al* 1990, Irving & Grant, 1984).

Since potassium is a major constituent of the mineral fraction of Maxicrop seaweed extracts, it was speculated that it could be involved in the MLSE inhibitory effect on *P. fragariae*. This experiment was designed to compare the responses of *P. fragariae* to MLSE and potassium.

The treatments studied were:

1- Soilless leachate (SL)

2- 25% MLSE

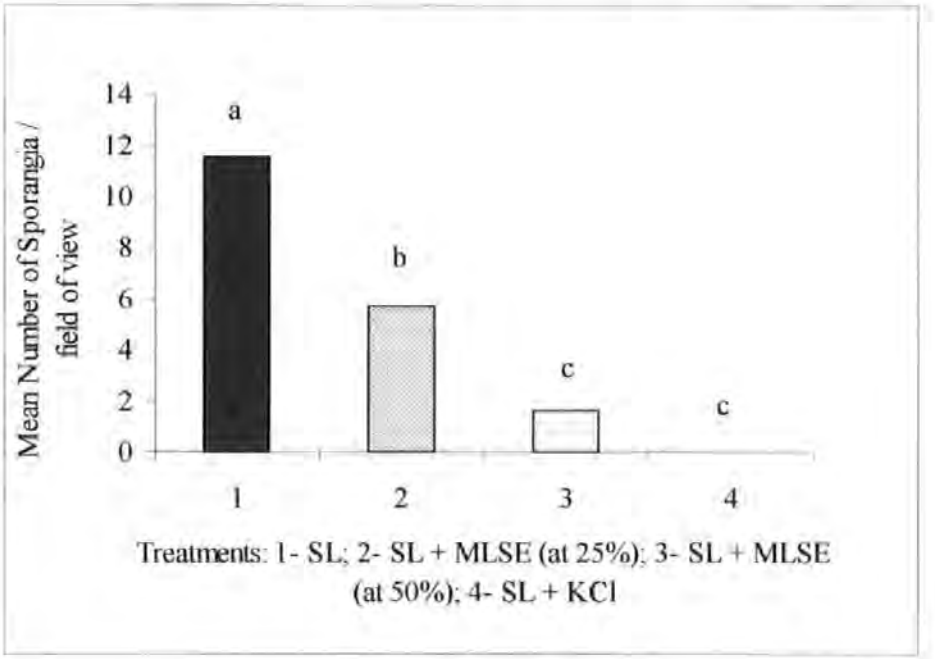
3- 50% MLSE

4- SL + 250 mM KCl (9.78 $gl^{-1}$)

**Results**

The responses to MLSE treatments and the control were similar to that previously observed with sporangia formation significantly reduced ($P<0.001$) with both concentrations used (Fig. 2.5). KCl had a drastic fungicide-like effect on *P. fragariae* with no mycelium growth observed on plates amended with KCl and, consequently, no sporangia formed. It is possible that this could have been due to the high concentration of KCl used that would caused a sharp rise in EC and osmotic pressure. By comparison the concentration of seawater is approximately 500mM NaCl.

Fig. 2.5- Effect of treatments with MLSE and Potassium Chloride (KCl) on *Phytophthora fragariae* sporangia production in soilless leachate (SL). Columns with the same letter are not statistically significantly different from each other ($P>0.05$).



**2.g. *In vitro* Experiment 7 - Response of *Phytophthora fragariae* to treatments with Maxicrop Concentrate Liquid Seaweed Extract and varying concentrations of Potassium in Liquid Medium**

Following the results for Experiment 6, the response of *P. fragariae* to potassium was further investigated. Two sources of Potassium were investigated, KCl and $K_2HPO_4$ at a range of concentrations. Solution conductivity (EC) and pH, were measured in all media combinations under study. The MLSE solution was also analysed in order to determine its Potassium content, pH and EC.

Treatments studied were:

1- Soilless Leachate (SL) (Control)

2- SL + 125 mM KCl

3- SL + 65 mM KCl

4- SL + 30 mM KCl

5- SL + 15 mM KCl

6- SL + 5 mM KCl

7- SL + 15 mM $K_2HPO_4$

8- SL + 5 mM $K_2HPO_4$

9- 100% MLSE (5 ml $l^{-1}$ SL)

**Results**

Results of chemical analyses carried out for all solutions are given in Table 5.2.

Table 5.2 - Analysis of solutions investigated in Experiment 6.

| Solutions | Potassium content ($g\ l^{-1}$) | Electrical conductivity (S) | pH |
|---|---|---|---|
| Soiless Leachate | 0.46 | $0.23 \times 10^{-3}$ | 6.8 |
| MLSE | 5.3 | $10.9\times 10^{-3}$ | 8.0 |
| 125 mM KCl | 4.89 | $6.6\times 10^{-3}$ | 6.8 |
| 65 mM KCl | 2.54 | $3.3\times 10^{-3}$ | 6.8 |
| 30 mM KCl | 1.18 | $1.85\times 10^{-3}$ | 6.8 |
| 15 mM KCl | 0.59 | $0.83\times 10^{-3}$ | 6.8 |
| 5 mM KCl | 0.19 | $0.21\times 10^{-3}$ | 6.8 |
| 15 mM $K_2HPO_4$ | 1.17 | $0.94\times 10^{-3}$ | 8.0 |
| 5 mM $K_2HPO_4$ | 0.39 | $0.84\times 10^{-3}$ | 7.9 |

While solutions of soilless leachate treated with Maxicrop seaweed extract and potassium phosphate were slightly alcaline, the soilless leachate and the solutions treated with potassium chloride were slightly acidic. Although variations in pH may disturb *P. fragariae* growth *in vitro*, it has been reported that higher variations than the ones observed in these solutions would be required to cause significant deleterious effects on the fungus (Erwin & Ribeiro, 1996). It was, therefore, assumed that pH was not responsible for the responses observed.

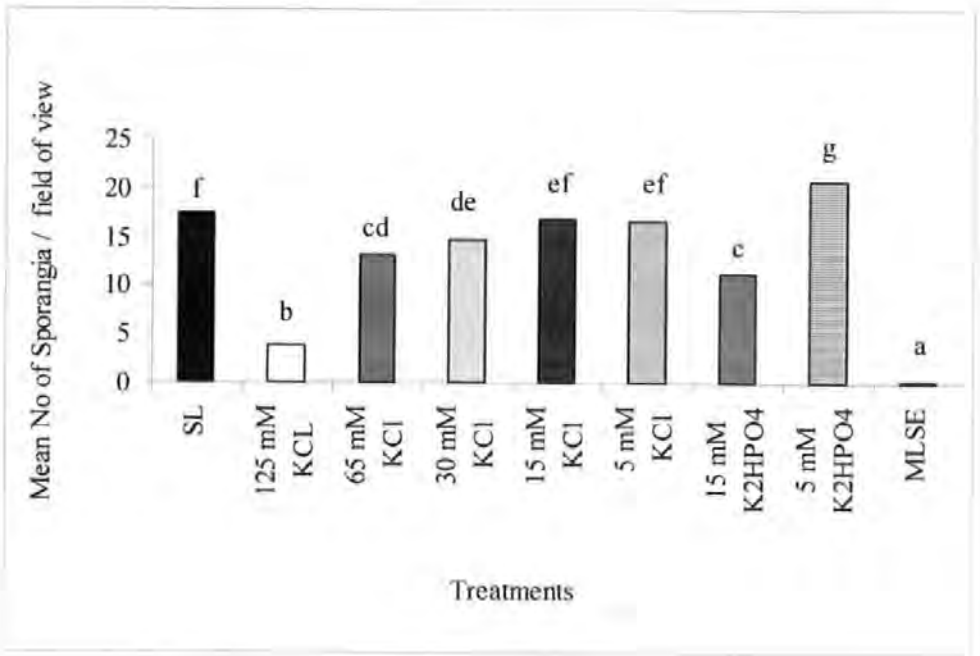
Sporangia formation in the control was almost identical to that previously observed. Sporangia observation showed that the addition of KCl dramatically reduced sporangia formation with a dose response to concentration of KCl and no significant effect at 5 and 15 mM KCl (Fig. 2.6).

Fungal growth and subsequent production of sporangia were similarly reduced by the addition of $K_2HPO_4$ at 15 mM but not at 5 mM sporangia production was higher than in control plates. Interestingly where potassium was supplied as the phosphate salt it had a significantly greater suppressive effect than where supplied as chloride salt (viz 30 mM KCl vs 15 mM $K_2HPO_4$). The results suggest that potassium salts were only detrimental to *P. fragariae* growth when present at high concentrations. These results are in agreement with previous reports that indicated that potassium could measurably interfere with the normal behaviour of the *P. cinnamomi in vitro* (Irving & Grant, 1984 and Byrt *et al*, 1982) where in low concentrations it was not detrimental to the growth of the fungus. These authors also reported that the negative effects of potassium at high concentrations could be prevented by adding other cations to the medium.

The results for treatment with MLSE in this assay confirm the observations of previous experiments as the seaweed extract caused a very significant ($P<0.0001$) reduction on sporangia numbers formed. Chemical analysis revealed that the potassium content of MLSE is high and similar in concentration to to about 135 mM KCl. The pH and EC of MLSE treatments were however higher than 125 mM KCl suggesting that if all the potassium in MLSE was in the ionic form than it would account for approximately 70% of the EC. Since MLSE is a complex mixture of organic compounds and other ions it was not surprising to record a higher EC. The higher pH of MLSE suggests the presence of high levels of Ca or Mg cations. There was a statistical significant difference between the response to 125 mM Potassium and treatment with MLSE.

It could therefore be inferred from these results that potassium was probably not the only component present in the seaweed extract responsible for the observed changes in the behaviour of *P. fragariae*.

Fig. 2.6 - Effect of treatments with MLSE and potassium salts on *Phytophthora fragariae* sporangia production in soilless leachate (SL). Columns with the same letter are not statistically significantly different from each other (P>0.05).



## 2.h. *In vitro* Experiment 8 - Response of *Phytophthora fragariae* to treatments with Maxicrop Concentrate Liquid Seaweed Extract and Beta-glucan in Liquid Medium

Results of Experiments 6 and 7 suggested that potassium was probably not the sole component of MLSE responsible for the morphological responses of *P. fragariae* observed in all *in vitro* assays in liquid medium. It is possible that one or more of the organic components may also be involved.

Seaweed extracts, such as Maxicrop concentrate, based in *Laminaria* species are rich in laminarins (Jensen, 1993) which are a group of the beta-glucan polysaccharides. Meeting *et al* (1990) implicated beta-glucans in the reduction of soil-borne fungal pathogens infectivity and it has been shown that they can directly influence *Phytophthora* by affecting zoospore production. Furthermore, Esquerré-Tugayé (2000) and Grant *et al* (1985) have reported that soluble polysaccharides have a role in the activation of plant defense mechanisms. It was hypothesised that the beta-glucans present in MLSE could form part of its components involved in the suppressive effects on *P. fragariae* observed in the previous experiments. An experiment to study the responses of *P. fragariae* to beta-glucan and MLSE was therefore carried out.

The treatments studied were:

1- Soilless leachate (SL)

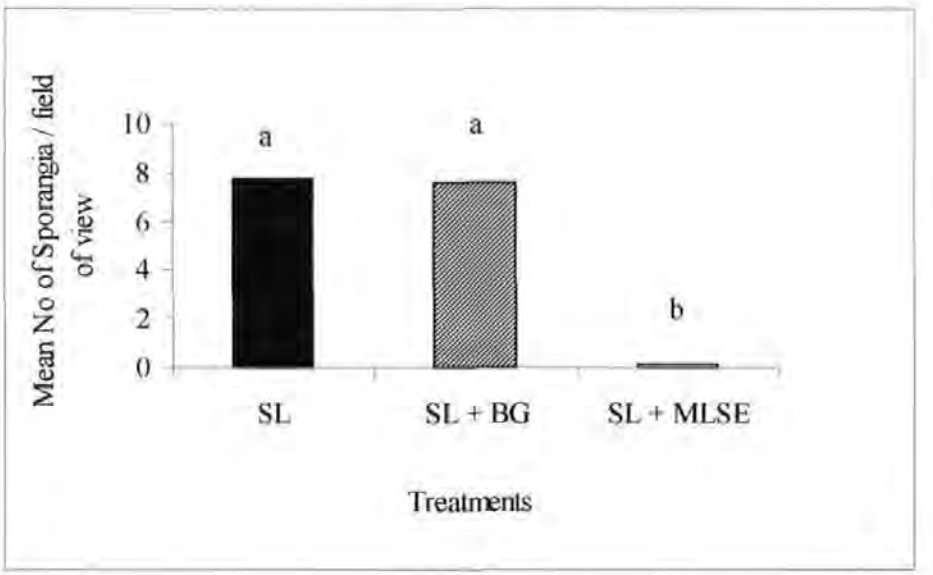
2- SL + Beta-glucan (0.04g $l^{-1}$ SL)

3- 50% MLSE (2.5 ml $l^{-1}$ SL)

**Results**

The addition of beta-glucan to the soilless medium did not have a detrimental effect on the growth of *P. fragariae* mycelium and it did not affect the production of sporangia. Data confirmed previous results obtained with the Maxicrop seaweed extract treatments as a very significant ($P<0.0001$) reduction on numbers of sporangia produced occurred in plates amended with 50% MLSE (Fig. 2.7) although sporangial numbers were only half the level observed in most previous experiments.

Fig. 2.7- Effect of treatments with MLSE and Beta-Glucan (BG) on sporangia production by *Phytophthora fragariae* in Soilless Leachate (SL). Columns with the same letter are not statistically significantly different from each other ($P>0.05$).



## 2.i. *In vitro* Experiment 9 - Response of *Phytophthora fragariae* to treatments with Maxicrop Concentrate Liquid Seaweed Extract, Glucans and Glucanase in Liquid Medium

Previous research work by Walsh (1997) suggested that Maxicrop seaweed extracts can stimulate beta-glucanase activity in the soil. Since beta-glucans are essential components of the cell walls of *Phytophthora* a stimulation of soil beta-glucanase activity could play a part in the suppression of these fungi in the rhizosphere. Walsh (1997) suggested that this could be one of the mechanisms by which the MLSE extract could inhibit observed *P. cinnamomi* activity in the soil. In this experiment the effect of increased concentration of beta-glucan, laminarin and beta-glucanase were compared to the soilless leachate and MLSE.

The following treatments were investigated:

1- Soilless leachate (SL) (Control)

2- SL + beta-glucan (2g $l^{-1}$)

3- SL + Laminarin (0.5g $l^{-1}$)

4- SL + Beta-glucanase (0.05g $l^{-1}$)

5- 100% MLSE (5 ml $l^{-1}$ SL)

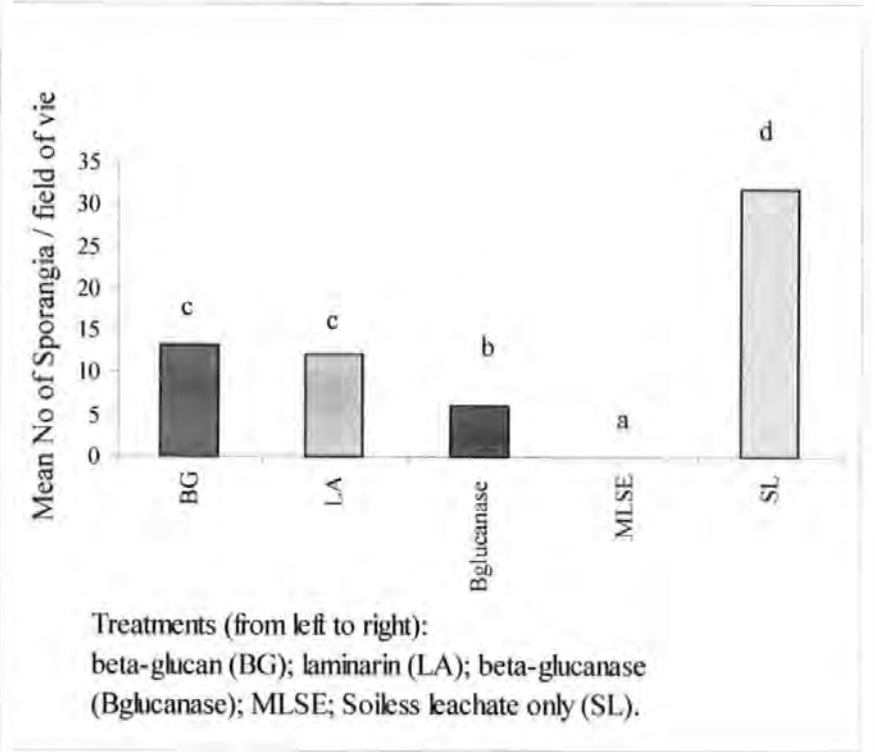
**Results**

Levels of sporangia production in this experiment were high exciding levels registered in all other previous assays. All of the treatments under study depressed the growth of *P. fragariae* in the liquid medium, with a significant reduction on sporangial production (Fig. 2.8). Amendment with MLSE had the most detrimental effect ($p<0.0001$)

and at the termination of the experiment only very few sporangia were observed in plates treated with the seaweed extract.

Additions of beta-glucan and laminarin reduced the numbers of sporangia by half compared to the control. The result for beta-glucan in this experiment contradicts that observed in Experiment 8 but the concentration used here was higher. The beta-glucanase had a stronger suppressive effect on *P. fragariae* than either of the two polysaccharides, causing the fungus to produce approximately 75% less sporangia than in the control. The enzyme seemed to have caused direct damage to the initial mycelium present in the plugs as the hyphal strands observed showed signs of digestion and no mycelial growth could be seen throughout the experiment supporting Walsh's (1997) hypothesis.

Fig.2.8- Effect of treatments with MLSE, Beta-Glucan, Beta-Glucanase and Laminarin on sporangia production by *Phytophthora fragariae* in Soilless Leachate. Columns with the same letter are not statistically significantly different from each other (P>0.05).



**Conclusions of *in vitro* experiments**

The experiments demonstrated a good repeatable *in vitro* assay technique for assessing the effects of various amendments on sporangia and zoospore production. Consistency of response of control treatments was good.

It was demonstrated that *P. fragariae* mycelia growth was severely reduced and as a consequence sporangia formation was significantly and progressively reduced with applications of MLSE to the growth medium. Although a small number of sporangia were at times formed when MLSE was present at low concentrations, the number of zoospores released was smaller and their locomotion pattern was abnormal.

The addition of potassium salts also suppressed *P. fragariae* growth when applied in high concentrations but did not significantly affect the pathogen at the lower concentrations tested. Since the level of potassium found in the MLSE amended liquid growth media was high and comparable to the high salt concentrations studied it could be argued that the fungal responses observed could, at least partially, be due to the potassium ion content of the extract.

The polysaccharides beta-glucan and laminarin were also detrimental to *P. fragariae in vitro* although only a limited range of concentrations were studied. It would be useful to investigate these responses further in the future in order to determine, for example, the concentrations of laminarin in the MLSE and its influences on the pathogen.

MLSE prevented the completion of the pathogen's assexual stage by suppressing mycelium growth. This, in turn, led to significant reductions in the production of one of its most important reproductive structures – the sporangia. Furthermore, zoospores formed and released from mycelium challenged with Maxicrop concentrate seaweed extract did not present a normal locomotion pattern. Since the efficient locomotion of zoospores is

essential for the pathogen to localize and invade a host in the soil environment it was hypothesized that Maxicrop seaweed extract could have the ability to affect the pathogenic activity of *P. fragariae in planta*.

## 3. Effect of Maxicrop Concentrate Liquid Seaweed Extract on *Phytophthora fragariae* infection of Strawberries – Hydroponic Experiments

Responses of *P. fragariae* to Maxicrop Concentrate seaweed extract in the *in vitro* studies indicated that the extract had a fungicide-like effect. It was essential, as a consequence, to investigate whether the responses observed *in vitro* would reflect on the interactions between the pathogen and its host. Before moving to soil-based *in vivo* situations a semi *in vivo* controlled environment system was studied first. This was a hydroponically based system where sterility of materials used was maintained.

### General Materials and Method

### Production of Strawberry Plants and Hydroponic System

The strawberry variety Baron Solemacher (Johnsons Seeds) was chosen because it has high sensitivity to *P. fragariae* and it generally presents clear disease lesions (Dr. Pitt personal communication). Seeds were sown into trays of a mixture of John Innes No 2 and perlite (3:1 by volume). When the seedlings were approximately 24 d old they were transferred to 250 ml plastic beakers filled with perlite medium grade to which 100 ml of Phostrogen (0.325 $gl^{-1}$) nutrient solution had been added. One seedling was placed in the centre of each beaker and covered with a plastic lid in which a hole had been made to allow the strawberry seedling to grow out. Beakers were placed in a controlled environment Phytotron (Fi-totron PG 660, Gallenkamp, Sanyo) with a 16h day length (light intensity – 200 $\mu molM^{-2}S^{-1}$), at approximately 20°C day and 16°C night and a relative humidity of 80%. Plants were allowed to re-establish in the new environment for 2-3 d before the zoospore inoculum was administered (Plate 7).

**Production of inoculum**

*P. fragariae* zoospore cultures were prepared as previously described for the *in vitro* experiments. The soil leachate solution containing the zoospores was decanted from the Petri dishes into a sterile flask and the number of zoospores counted under the microscope. The final solution used as inoculum was adjusted to approximately $10^3$ zoospores / ml. One ml of the inoculum was pipetted over the crown of each plant.

**Watering, Fertigation and Disease Assessment**

Immediately after inoculation 15 ml of MLSE or nutrient solution (depending on treatment) were pipetted over each plants crown. Nutrient solution was then supplied every two days and diluted MLSE (5 ml $l^{-1}$) treatments (15 ml) were applied twice each week. Two experiments were conducted and there were four replicates for each treatment. Plants were harvested after 3 weeks and red core symptoms assessed. Root/shoot base infections were evaluated using an assessment key (Appendix 1). Symptoms were categorized, with 0 representing a healthy plant and 5 the most severely infected plants. After this, the plants were harvested, the shoots severed and weighed (fresh and dry weights) to investigate the correlation between plant growth the level of disease infection.

### 3.a. Hydroponic Experiment 1

The following treatments were studied:

1- Nutrient Solution (NS)

2- NS + MLSE (5ml $l^{-1}$ DW)

3- NS + zoospores

4- NS + MLSE (5ml $l^{-1}$ DW) + zoospores

5- NS + MLSE (10ml $l^{-1}$ DW) + zoospores

### *Results* - Hydroponic Experiment 1

Data obtained in this experiment showed that the inoculum used was active and some of the plants presented severe disease symptoms. Severity was particularly high in plants not treated with the seaweed extract. These showed severe disease symptoms where stuntedness and wilting were so rigorous that plants were effectively approaching death. In comparison, at harvest, the severity of lesions found in the roots of plants amended with MLSE was significantly ($P<0.05$) lighter (Fig. 2.9.1). Results showed that disease was suppressed in plants submitted to both the treatments with MLSE but there was no significant difference between the two concentrations of extract applied. Whilst the reduction caused by MLSE was statistically significant the degree of reduction was only one unit on the score scale and the MLSE plants were still quite heavily infected with disease.

Plant fresh weight was significantly reduced ($P<0.05$) as a consequence of disease infection (Fig. 2.9.2). MLSE in the presence of disease significantly improved plant

weight but did not re-establish plant weight back to the level of the controls. MLSE did not improve weight in the absence of disease (Plate 9). Mean plant fresh weight was negatively correlated with disease severity.

Fig. 2.9.1- Effect of MLSE on *Phytophthora* symptoms on Strawberry plants - Hydroponic Experiment 1. Columns with the same letter are not statistically significantly different from each other (P>0.05).

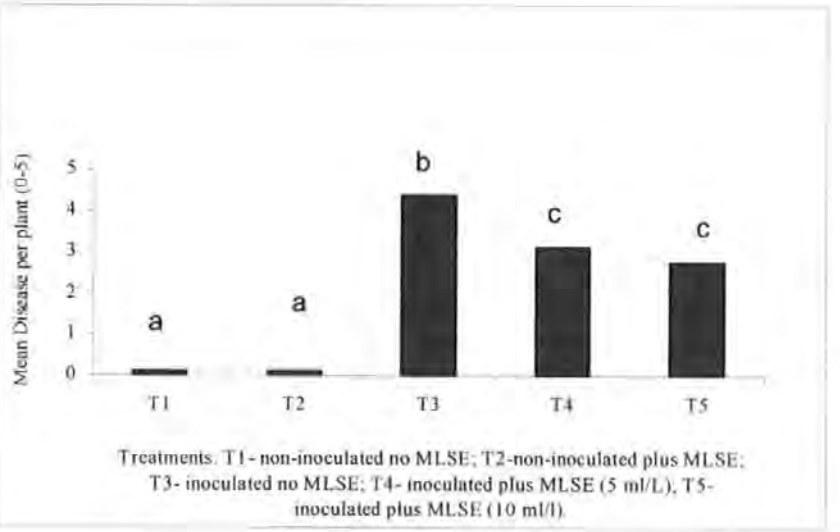


Fig. 2.9.2.- Effect of MLSE on Strawberry plants - Hydroponic Experiment 1. Columns with the same letter are not statistically significantly different from each other (P>0.05).

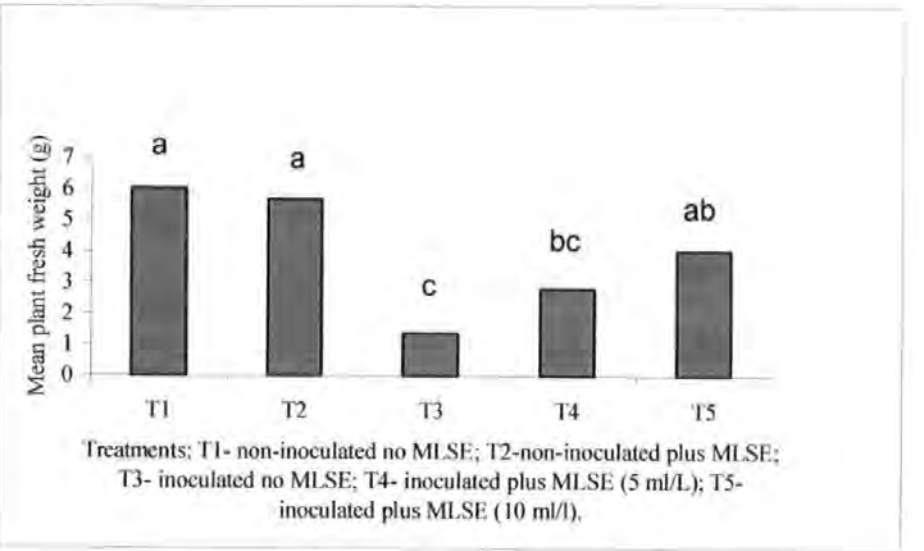


Plate 9- Strawberry plants grown in hydroponics system.

Plate 10- Strawberry plants grown in hydroponics system inoculated with *Phytophthora fragariae*. Left plant not treated with MLSE and right plant treated with MLSE (10ml $l^{-1}$).

### 3.b. Hydroponic Experiment 2

In this experiment there were six replicates of the following treatments:

1- Nutrient Solution (NS)

2- NS + MLSE (5ml $l^{-1}$ DW)

3- NS + zoospores

4- NS + MLSE (5ml $l^{-1}$ DW) + zoospores

**Results**

Results confirmed the responses to applications of MLSE observed in experiment 1. *P.* fragariae symptoms observed in the plants treated with the MLSE were significantly lighter ($P<0.05$) than in non-treated plants (Fig.2.9.3) although complete control was not obtained.

As in Experiment 1, inoculated plants showed a poorer growth than non-inoculated plants and at harvest, on average, their fresh weight was 50% less correlating negatively with disease index. Also as in Experiment 1, MLSE did not seem to have an effect on growth of non-inoculated plants (Fig. 2.9.4).

Fig. 2.9.3- Effect of MLSE on Red Core symptoms on Strawberry plants – Hydroponics Experiment 2. Columns with the same letter are not statistically significantly different from each other (P>0.05).

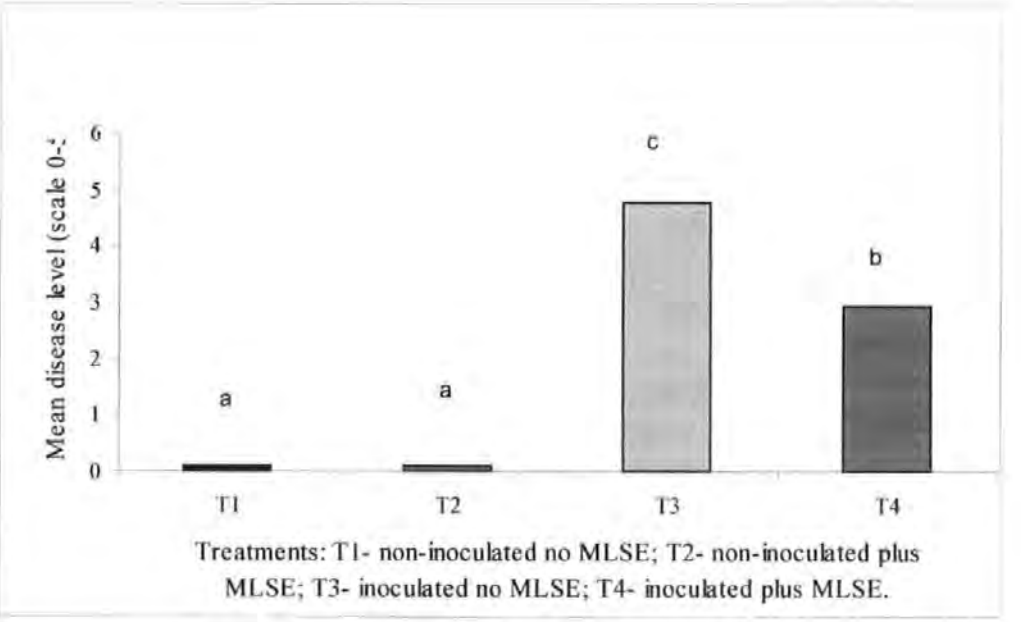
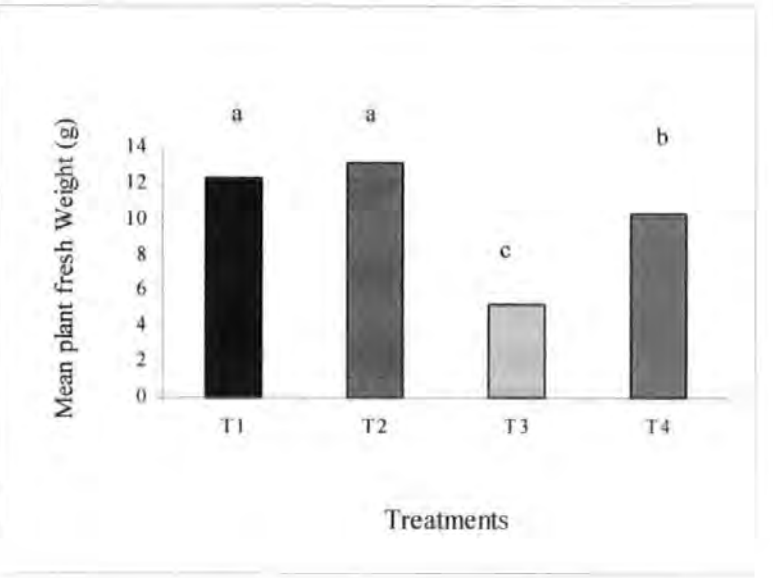



Fig. 2.9.4- Effect of MLSE on Strawberry plants – Hydroponics Experiment 2.



**Discussion and Conclusions**

The hydroponic system was demonstrated as a suitable system for studying disease infection and control of *P. fragariae* on strawberry plants. In the absence of disease plants grew well with a normal morphology. Inoculation of these plants with a zoospore inoculum led to clear and severe disease symptoms.

The experiments showed that infections sustained by plants treated with MLSE were significantly less severe than in untreated plants but disease was not eradicated. It is possible that the reduced disease observed in MLSE treated plants could be that the infectivity of the zoospores applied as inoculum was impaired by the extract. It was demonstrated *in vitro* that the zoospores locomotion was affected by the seaweed extracts, and it is likely that a similar effect would have been reproduced in the hydroponic system. In this way the zoospores would have been slowed down, in their endeavour to localise and parasitise the roots of strawberry plants. This would mean fewer loci of root infection leading to a lower disease score.

Alternatively, the presence of the MLSE might have slowed down mycelial growth in the roots post-infection. Results of the *in vitro* experiments would suggest that re-sporulation and secondary infection would clearly be reduced in the presence of the MLSE.

The dilution of MLSE should not be overlooked in these experiments with 15 ml of MLSE being diluted in 100 ml nutrient solution making it initially 1/7 the strength of solutions used in the *in vitro* experiments where a dose response was observed.

## 4. Effect of Maxicrop Concentrate Seaweed Liquid Extract on *Phytophthora fragariae* infection of Strawberries - Growth Chamber Experiments

### METHOD AND MATERIALS

#### Production of Strawberry Plants

Strawberry seedlings were grown as described in Section 3 and then pricked out into square plastic pots (7×7 cm) containing a mixture of John Innes No 2 compost and perlite (2:1 by volume) and then transferred to the growth chamber. Plants were allowed to re-establish in the new environment for 2-3 d before the zoospore inoculum was administered. Strawberry plants were then inoculated with a solution containing the *P. fragariae* zoospores prepared as described previously for the hydroponics experiments (Section 3). Temperature in the growth chamber was set for 20 ± 2° C by day and 16 ± 2° C by night with a 16 hour photoperiod.

#### Sampling and assessment techniques

Strawberry plants were assessed for disease symptoms, plant shoot fresh and dry weight and number of leaves. Plants were harvested and the roots washed prior to analysis of root/shoot base infections according to the assessment key (Appendix 1). Symptoms were categorised, with 0 representing a healthy plant and 5 the most severely infected plants.

Four plants were planted in each pot and there were four replicates per treatment. The experiment was repeated four times, each with a duration of 6 weeks. MLSE (5 ml $l^{-1}$) was administered in the form of fertigation on weeks 2, 3 and 4. The MLSE was applied (15 ml) to the compost in each pot, water being the control.

The following treatments were applied.

T1 - no Zoospores; no MLSE (Control)

T2 – MLSE (5 ml $l^{-1}$)

T3 – + Zoospore solution

T4 – + Zoospore solution + MLSE (5 ml $l^{-1}$)

**Results**

A full range of disease severity was evident in these experiments resulting in visible stunting effects on plant growth (Plate 11). As in the hydroponic experiments, the MLSE treatments significantly suppressed ($P<0.001$) the level of red core infections (Fig. 2.10.a). Un-inoculated plants showed some slight symptoms indicating either a small amount of cross contamination or misdiagnosis of symptoms. Plant growth characters measured showed variability but no statistically significant effects (Figs. 2.10.b – 2.10.d).

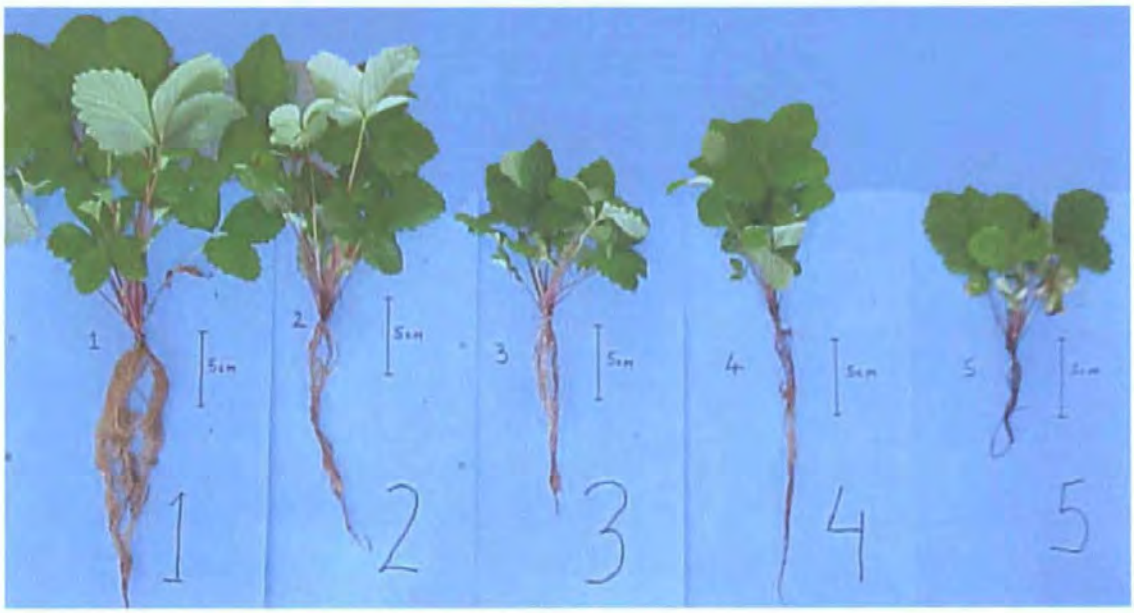



Plate 11- Strawberry plants showing symptoms of Red Core disease. From left to right disease symptoms from light to severe (according to assessment key adopted). Note that a reduction in the size of the plant accompanies the increase in disease severity. Score 5 represents the most severe infection as the root system was almost totally necrotic.

Fig. 2.10.a- Effect of MLSE on Red Core of strawberry plants. Columns with the same letter are not statistically significantly different from each other (P>0.05).

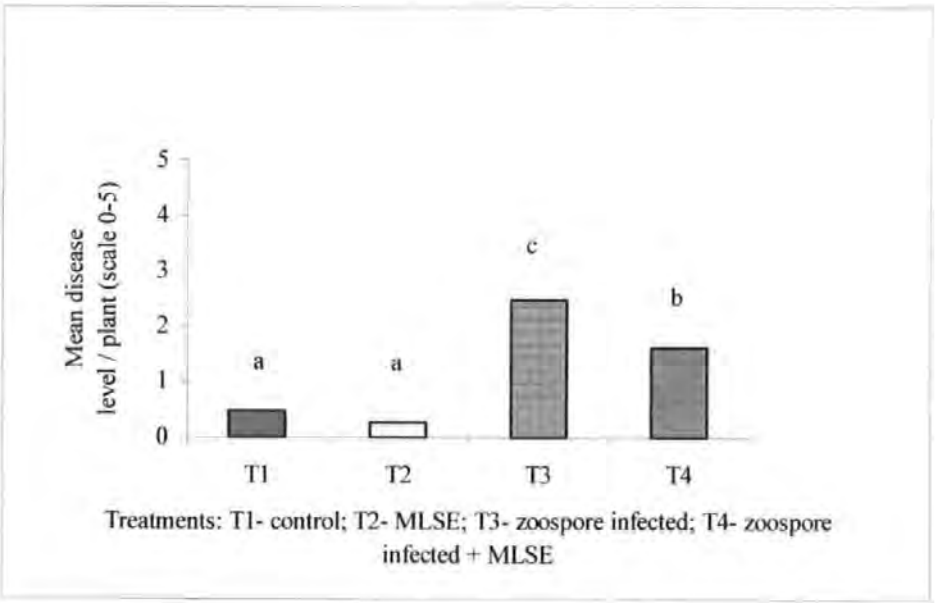



Fig. 2.10.b- Effect of MLSE on strawberry plants. Columns with the same letter are not statistically significantly different from each other (P>0.05).

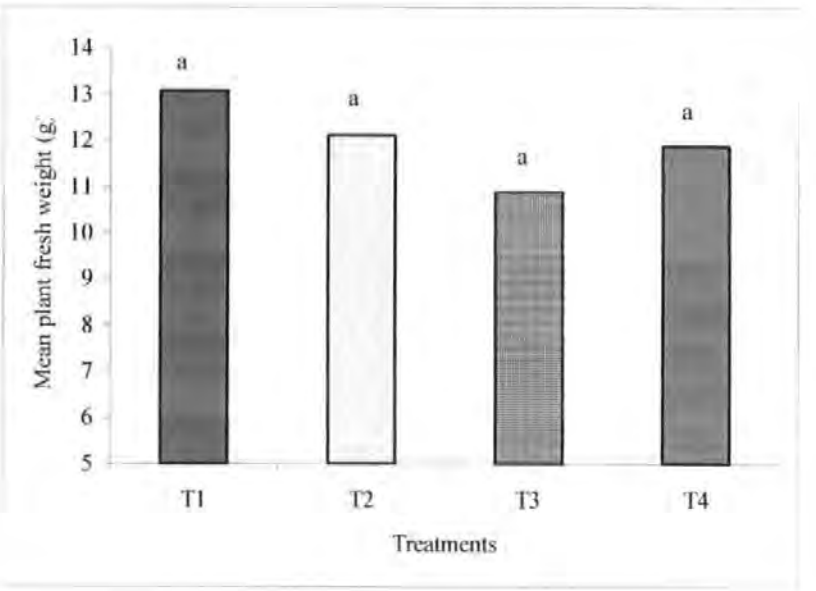


Fig. 2.10.c - Effect of MLSE on strawberry plants. Columns with the same letter are not statistically significantly different from each other (P>0.05).

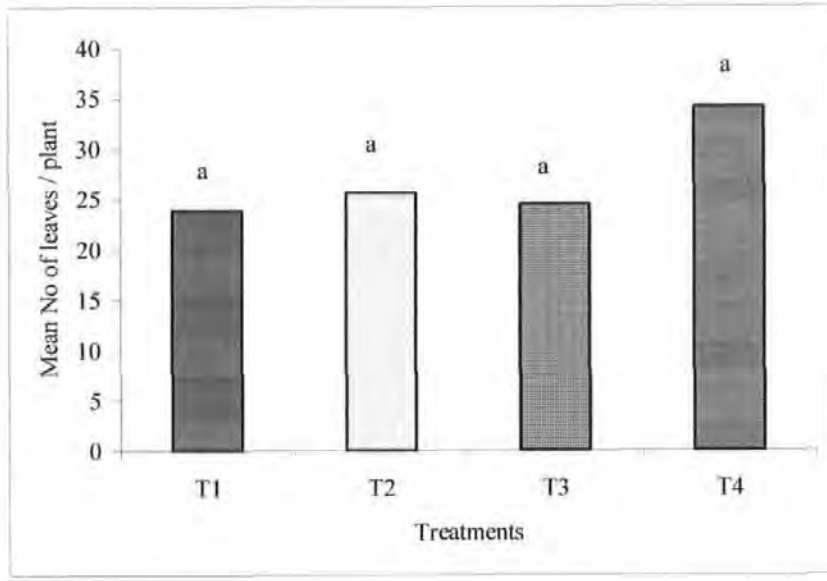
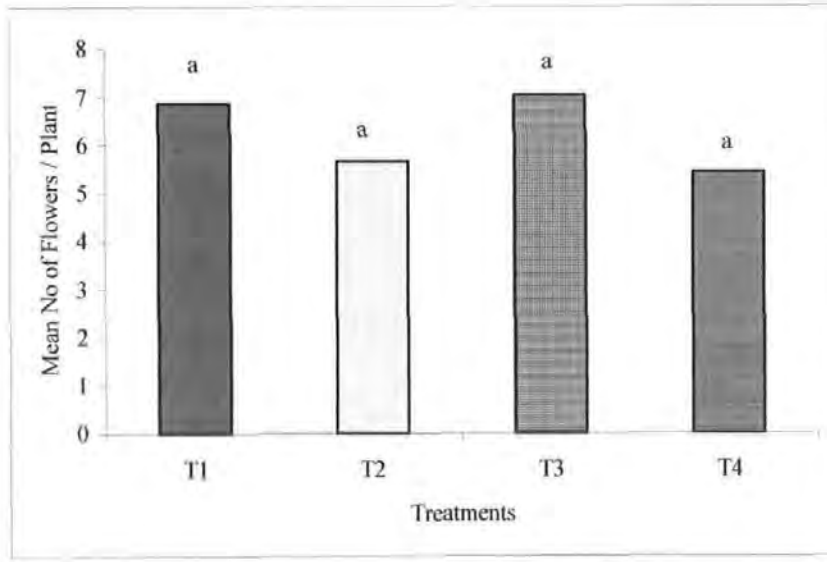



Fig. 2.10.d - Effect of MLSE on strawberry plants. Columns with the same letter are not statistically significantly different from each other (P>0.05).



## Conclusions

Experiments conducted in the growth chamber provided good environmental conditions for the development of red core infections and the growth of strawberry plants. The results of these experiments indicated that Maxicrop Concentrate liquid seaweed extract was capable of suppressing *P. fragariae* symptoms on strawberry plants, but was incapable of complete control of the disease. Replicate variability with respect to both disease and plant growth decreased statistical sensitivity.

# CHAPTER IV - GENERAL DISCUSSION

Seaweeds and various types of seaweed extracts are used as fertilisers in numerous parts of the world on a variety of crops. Through the years a range of beneficial effects such as increased yields, increased resistance to abiotic and biotic stress, control of flowering and fruit maturation, longer shelf life of fruit and vegetables and improved seed germination have been attributed to these fertilizers. Scientific research into such attributes has been developed producing evidence that supports some of the claims made by the fertilizer industries (Patier *et al*, 1995; Blunden, 1991; Jolivet *et al*, 1991). Several active ingredients and mechanisms of action have been proposed and are thought to be responsible for the reported benefits, however, a consensus opinion has not yet been reached amongst agricultural researchers. As a consequence, the products commercialised sometimes lack reliable scientific backing for the claimed effects in the plants as the active ingredients may or may not always be present or, if they are, their concentration may not reach the necessary level for relevant activity to occur (Mercier *et al*, 2001).

Seaweed extracts are generally applied as diluted solutions, therefore, extensive investigations into mechanisms of action of the products has focused on the study of plant promoting compounds that were thought to be capable of mediating the beneficial effects reported at such levels. Cytokinins and betaines have received particular attention as they can survive the industrial process of manufacture and it has been shown that *Ascophyllum nodosum* extracts are capable of mimicking the effects of these phytohormones. Such phytohormones have also been found to be present at different concentration levels in seaweed extracts using a range of biochemical techniques (de Nys *et al*, 1990; Farooqui *et al*, 1990; Abetz, 1980).

Since seaweed extracts are often applied to soil or compost it has been argued that effects on the rhizosphere microbial population could possibly be as important as the ones thought to be stimulated in the plants themselves (Walsh, 1997). Previous research has shown that MLSE can increase the microbial activity in compost (Pattison, 1994), including the bacterial populations and in particular of *Pseudomonads* (Cogram, 1994). Cogram (1994) further suggested that beneficial effects of MLSE on wheat growth were dependent on its synergistic activity with the rhizosphere microbial population. Walsh (1997) demonstrated that *Pseudomonas putida* growth could be stimulated *in vitro* through the applications of MLSE and that the extracts could stimulate the release of siderophores by the bacteria.

Activity of microbes in the soil is difficult to measure directly, however, enzymatic activity has been used as a valuable means of estimation. Walsh (1997) showed that MLSE could induce β-1,3-glucanase and amylase activity in compost. He argued that this could probably induce a stimulation of the β-1,3-glucan degrading microorganisms in the soil. He hypothesised that these microorganisms could in turn antagonise and parasitise phytopathogenic fungi that have β-1,3-glucan rich walls such as *Phytophthora* species. If such mechanism were in action it would support the disease suppressive effects observed with the seaweed extract treatments.

Apart from the possible effects of the seaweed extracts on the rhizosphere microbial population, its β-1,3-glucans compounds have also been investigated as potential plant elicitors. Beta-glucans and other carbohydrates of various origins have been shown to stimulate phytoalexin mechanisms and they are thought to act in a similar way to cell wall fungal carbohydrates (Mercier *et al*, 2001; Okinaka, 1995; Patier *et al*, 1995; Basse & Boller, 1992; Keen *et al*, 1983). Burchett (2000), studying the beneficial effects of MLSE on frost stress in barley, found that another mechanism of action for the MLSE extracts

was possibly through the up-regulation of high molecular-weight proteins which could be signalling molecules. The roles of proteins in pathogenesis and other signalling related processes have been extensively studied and it has been found that peptide synthesis normally accompanies plant responses to pathogens and other stresses (Hahn, 1996; Wubben *et al*, 1996; Okinaka; 1995; Basse & Boller, 1992; Woloshuk *et al*, 1991). It is, therefore, possible that a stimulation of protein synthesis in plants by seaweed extracts could help to explain the increased ability of amended plants to withstand pathogen attacks.

Another component of the seaweed extract that could also contribute to a stimulation of the plant defences is potassium, of which it has a high content. Although it has been shown that applications of potassium salts to plants can prevent posterior pathogen infections, through an induction of systemic resistance, little research has been carried out in order to investigate the possible role of potassium in the reported beneficial effects of seaweed extracts on plant health. It is, nevertheless, known that potassium plays several important roles in the plant physiological activities. Thus, potassium and gibberellic acid (GA) act synergistically in stem elongation processes. Potassium is also involved in sugars translocation and in the membrane polarization structure, which are in turn thought to play essential parts in plant signalling mechanisms and the direct response to cell disruption by wounding or invasion by pathogens. Increased susceptibility to fungal attack has been associated with changes in organic compounds and enzyme activity in K deficient plants (Marschner, 1994; Ward, 1985).

Over the years, several researchers have found that potassium could help to suppress diseases of various types in various plant species. Bushnell & Curran (1983) found that $K^+$ solutions could inhibit infection of barley coleoptiles by *Erysiphe graminis* f. sp. Hordei. Work by Inoue *et al* (1994) showed that local and systemic resistance to penetration and

consequent infection of susceptible barley plants by the powdery mildew fungus could be related to the activity of potassium phosphates in the plants. Mucharromah & Kuc (1991) successfully showed that potassium phosphates induced systemic resistance in cucumber plants against diseases caused by fungi, bacteria and viruses. Reuveni & Reuveni (1992), confirmed that potassium phosphate salts could induce local and systemic protection against powdery mildew and growth increase in cucumber plants. These authors (Reuveni & Reuveni, 1998) have later shown that foliar sprays of mono-potassium phosphate could also control powdery mildew on pepper plants and that this protection was both local and systemic. More recently, Becot *et al* (2000) have demonstrated that potassium phosphonate induced local resistance to downy mildew (*Peronospora parasitica*) in cauliflower seedlings when applied before or after inoculation of the plants. The protection obtained was thought not to be systemic but they observed that it could last for several days and that this response was intensified when the product was applied on roots of older plants.

The work of these authors suggests that potassium based elicitation of plant defence mechanisms is non-specific as the ionic solutions can stimulate suppression of varied diseases in various types of plant species. Although these past investigations dealt essentially with foliar diseases, they provide evidence that a broad spectrum mechanism is likely to be responsible for the results obtained. This interpretation suggests that a similar mechanism could be in place for soil-borne pathogens. If that is the case, since seaweeds normally provide high levels of potassium, it could help to explain the beneficial effects observed for these extracts in soil-borne pathogens reported for a range of crops.

In the context of the search for alternative, sustainable crop production systems, products that might have an ability to stimulate natural plant defence mechanisms will probably have an important role to play. They could be used as strategic protection components in schemes to reduce artificial pesticide outputs. In the light of recent

research, seaweed based extracts have been found to have the potential requisites to be used as plant protectants, however, further investigations are still required to determine how such products can be best exploited (Mercier *et al*, 2001). The present work was developed in that context and its main aims were to evaluate the biological effects of Maxicrop seaweed extracts in plants and to investigate whether they could prompt any responses in soil-borne pathogens infecting the plants in study. Recent investigations into the effects of seaweed extracts have shown that they can suppress the impact of several pathogens, including soil-borne fungi (Walsh, 1997; Cogram, 1994; Pattison, 1994). However, suppressive responses were obtained in experiments conducted under very strict environmental conditions and research carried out by Walsh and Dixon (1997) investigating the responses of various soil-borne oomycete fungi to seaweed extracts in pot trials indicated that applications of Maxicrop seaweed extracts after pathogen inoculation did not have an effect on disease severity and could even in some cases aggravate plant infection. It was therefore, intended in the current investigation to further study the responses to MLSE by various types of pathogens under different environmental settings. Results of a number of experiments described here showed that, albeit some inconsistency, levels of take-all of wheat and Red Core of strawberries were either reduced or did not increase where MLSE amendments were applied. In the present studies the MLSE treated plants never showed increased disease even where the extract was applied after inoculation had occurred.

## 1. Investigation of responses to Maxicrop seaweed extracts by wheat and take-all

### 1.1. Wheat infecting *G. graminis*

Investigation of responses of the *G. graminis* wheat infecting soil-borne pathogen indicated that effects of the seaweed extracts under study were variable and that responses

differed with amendments (MLSE and microbial inoculants). Lack of strong statistical significance in many of these experiments was disappointing despite plants amended with seaweed extracts nearly always presenting lighter average symptoms of the diseases. This could indicate that more sensitive experimental techniques are necessary to detect the changes in response.

Investigation of *G. graminis* growth rates over solid media indicated that the seaweed extract induced some changes in the mycelial growth but it did not prove to be fungicidal. Although colony radial growth of *G. graminis* was slightly slower where the extract was applied, this was not significant. Despite the changes in mycelium characteristics observed with the most concentrated MLSE treatments, the hydroponic experiments did not demonstrate that the pathogenic ability of the fungus had been affected by the extract. The lack of fungicidal activity of MLSE is perhaps commercially positive as it lessens the necessity for the MLSE to be cleared through PSD (Pesticide Safety Directorate).

In hydroponic experiments, a reduction in severity of take-all disease infection was observed in seedlings amended with the MLSE solution, this response being statistically significant for some treatments. Analysis of data suggested that the presence of a soil microflora was not essential to obtain the beneficial effects observed. Plants treated with the soil microflora together with the MLSE showed lighter levels of take-all infection than plants amended with the extracts alone but this was not significant. This would imply that, contrary to findings by previous researchers (Cogram, 1994 and Walsh, 1997), in this system, MLSE alone would be accountable for the significantly reduced level of take-all infection obtained in some experiments.

The disease suppression obtained with MLSE was, however, variable and this was more evident in experiments where optimisation of growing conditions was reinforced in order to prevent drought stress. In those experiments where water and nutrient supply

were refined, average disease level in MLSE amended plants was still lower but statistical significance was lost. It could be hypothesised that stress conditions might have acted synergistically with the seaweed extract in the stimulation of the defence mechanisms of the plants in the first experiments thus increasing their ability to withstand/respond to the pathogenic attack.

Investigation of responses to the seaweed extracts extended in glasshouse experiments showed that similar responses were induced in terms of disease infection in that environment. A statistically significant suppression of disease was obtained in some experiments with sprays at the lowest rate (5 $ml l^{-1}$), however, this was not consistent throughout and, in some cases, no evidence of disease inhibition was found.

Effects of a soil microflora and of a mixture of a pure *P. fluorescens* culture were studied in both hydroponics and glasshouse trials. Results of experiments conducted in hydroponics indicated that these additions did not have any effects on plant growth or take-all disease expression and this was also observed in studies of activity in the compost. Although in hydroponics plants treated with the soil microflora plus the MLSE showed, on average, lighter take-all infections than plants amended with the seaweed extracts alone, this was not significant. This would imply again that, contrary to findings by previous researchers (Cogram, 1994 and Walsh, 1997), in our systems, MLSE alone was responsible for the reduced levels of disease found. It could be argued that the soil solution applied did not provide an adequate beneficial microbial population, despite the fact that an abundant number of fluorescent *Pseudomonads* was found to be present in the solutions applied. It has been reported that *Pseudomonads* can be actively beneficial to plants through various mechanisms such as the production of siderophores and the release of antibiotics and other compounds that might either be directly detrimental to harmful microbes or may stimulate plant defence mechanisms. The production of such substances by the *Pseudomonads* is,

however, dependent upon biological and environmental conditions therefore it could be argued that although the bacteria were present they may have not have biocontrol activity under the conditions of the experiments.

Weather conditions were adverse through out the field trials, particularly during the 2nd season, affecting plant establishment and growth, therefore, results were analysed with some reservations. Positive responses were observed for treatments with early MLSE sprays and of 200g MEG where a trend for lower take-all infections was obtained. Measurements of agronomic characters in both experiments showed a trend indicating that multiple sprays and granule amendments at low rates may improve wheat plant growth. Despite the trend for positive effects to some of the seaweed treatments, the responses obtained were only slight which does not allow for a confident discussion of results and for conclusions to be drawn. It could be speculated that the activity of the extracts could probably be optimized if experiments could be repeated under more regular environmental conditions. Further research would have provided more useful insights to the study, but, the time limits of the project did not allow a pursuit of this hypothesis.

**1.2. Investigation of wheat plant growth in response to Maxicrop seaweed extracts**

Although wheat plants grown in the glasshouse amended with MLSE often produced more tillers and had higher average weights, the MLSE did not have consistent statistically significant effects in plant growth as measured by the number of tillers and shoot fresh and dry weight. Data of glasshouse experiments also showed that MEG can have beneficial effects on wheat plant growth if supplied in small amounts, such as 1g / 450g compost.

The chemical analysis carried out on the EMG treated compost and the granules themselves showed that the product is rich in potassium and magnesium. This suggests

that the positive responses observed could have been promoted by a slight elevation of the availability of these macronutrients. The experiments indicated however, that there is a risk of causing detrimental effects on wheat plants when the extracts are applied at high rates, this being observed for 20g EMG and higher applications. Glasshouse experiments confirmed that the responses of wheat plants treated with EMG plants were dependent on the dose rates of granules applied. Thus, very small amounts (eg. 1g / 450g compost) induced slight growth improvement but a clear reduction in growth was demonstrated for high (eg. 30g / 450g compost) dose rates. These results are in accordance with observations made by Walsh (1997) that suggested that this formulation was not beneficial for plant growth when used at higher rates.

The negative growth responses to MEG observed could be explained by the rise in the compost electrical conductivity (EC) that they produced. It has been demonstrated that potassium plays an important part in the ionic fluxes established in the rhizosphere which can be in turn be measured as EC (Rowell, 1994; Li, 1993). Chemical analysis revealed that EMG released high levels of potassium when dissolved in water. The rise in EC in compost amended with EMG could thus be explained by a similar effect of the granules in the compost. The high potassium content of the granules could also directly contribute to the detrimental effect on plant growth observed for high rate treatments. There is evidence (Marschner, 1995; Gair, 1990; Archer, 1988 & Finck, 1982) that potassium is a nutrient that, when present in high levels in the rhizosphere, is absorbed in excess of the plants needs in detriment of the absorption of other nutrients such as magnesium, calcium and sodium. Such luxury absorption can cause an unbalanced nutritional status that will express itself in poor plant growth even when the other nutrients are present at the required level in the substrate (Marschner, 1995). The poorer wheat plant growth observed for high

rate EMG treatments could thus possibly be explained by a potassium luxury absorption and a consequent obstruction of the absorption of other essential nutrients.

It should, nevertheless, be taken into consideration that EC is unlikely to remain constant through-out a pot experiment as, for example, the granules will not all dissolve simultaneously in that environment and electric ionic dependent flows in the rhizosphere vary with environmental factors (Li, 1993). The effect of applications of MEG in EC of a pot rhizosphere or soil is, therefore, likely to be less dramatic than the one obtained in the laboratory experiment. The study of the effects of MEG over a compost or soil rhizozphere EC throughout the plants' life cycle would probably provide useful information in this respect. The experimental data showed, however, that the highest rates of EMG applied prompted clear negative plant growth responses. If commercialisation of this product is to be pursued a careful study of the rates of application should be carried-out to ensure its optimal use in different plant crop production systems.

Studies into the protein content of wheat plants indicated that in MLSE treated plants there was some up-regulation of high molecular weight proteins. Correspondingly, analysis of total nitrogen levels indicated that where this happened a significantly higher level of nitrogen was present. Phosphorous and potassium were also present at higher rates in MLSE treated plants and the increase of the latter was highly statistically significant. Data obtained in experiment 2, did not confirm these results but K content in MLSE treated plants was always higher than in control plants.

Nitrogen and phosphorous are essential constituents of proteins and nucleic acids and potassium is fundamental for the processes of osmoregulation, maintenance of electrochemical equilibrium in cells and regulation of enzyme activities (Devlin & Witham, 1983). All these physiological processes are fundamental for normal plant growth and they determine their response to external challenges, such as water or salt

stress and exposure to disease agents. Marschner (1995) reported that plants suffering from nitrogen and phosphorous deficiency showed less tolerance towards invasion by take-all. Furthermore, high concentrations of potassium on the plants' substrate increased the resistance to obligate and facultative parasites.

The response to MLSE described above could thus help to explain the suppression of plant diseases by Maxicrop extracts presented here and elsewhere (Cogram, 1994, Pattison, 1994, Walsh, 1997). It could be suggested that applications of MLSE may possibly alter the plants' uptake of nitrogen, phosphorous and potassium. This could in turn form part of a signalling strategy that could be adopted to withstand stress of an abiotic or biological nature, however, further studies would be necessary to verify/refute this hypothesis.

**2. Investigation of strawberry and *Phytophthora fragariae* responses to Maxicrop concentrate seaweed liquid extract**

*In vitro* experiments carried out in solid media showed that no significant effects on the growth of *P. fragariae* occurred where MLSE was applied. Other researchers have investigated the responses of different *Phytophthora* species to seaweed extract treatments in liquid media (Walsh, 1997; Pattison, 1994). Here similar studies were conducted to investigate the reactions of *P. fragariae* to the product. It was found that mycelial growth was severely reduced with MLSE treatments at various concentrations and that this inhibition was absolute in 100% concentration (i.e. 5 ml MLSE concentrate $l^{-1}$). This morphological response resulted in a significant limitation of sporangia formed. The inhibition observed was positively correlated to the increasing concentrations of seaweed extract. Research carried-out by Pattison (1994) indicated that morphological responses of *Phytophthora* to MLSE varied from species to species, however, he did not observe a fungicide-like effect on any of the species studied. He demonstrated that *P. cinnamomi* switched from the production of sporangia to the production of chlamydospores when challenged with MLSE. These resting spores are commonly produced under unfavourable environmental conditions (Ribeiro, 1996). His studies showed that the chlamydospores formed in MLSE treated media were still viable as they were capable of producing germ tubes. Our studies, however, indicated that although sporangia could be formed when MLSE was present in the media at the lower concentrations, zoospore release was less abundant and their locomotion pattern was either permanently impaired or abnormal under those circumstances.

In an attempt to identify the MLSE compound responsible for the morphological changes observed in *P. cinnamomi*, Pattison (1994) and Walsh (1997) studied the effects of several chemicals on the fungal growth in liquid media. Several polysaccharides could

induce morphological changes and laminarin was observed to induce distorted hypae giving rise to low chlamydospore numbers (Walsh, 1997). It was demonstrated in the present study however, that *P. fragariae* responded differently. Treatments with laminarin did not significantly affect the fungus and neither did beta-glucan or beta-glucanase. Potassium salts, however, were markedly unfavourable to *P. fragariae* fungal growth. It was shown that potassium added at concentrations similar to the ones found in MLSE extracts stimulated responses of magnitudes that correlated to the ones observed for the MLSE treatments.

It has been demonstrated by other researchers that ion levels are essential for the normal growth of *Phytophthora* and other fungal species (Becot *et al* 2000; Ribeiro, 1996; Irving & Grant, 1984; Byrt *et al*, 1982). Byrt *et al*, (1982) found that *P. cinnamomi* zoospores were immobilized by applications of $K^+$ but this did not result in the formation of true cysts normally developed before host penetration. Bushnell & Curran (1983) observed that $K^+$ solutions could cause failure of *Erysiphe graminis* f. sp. Hordei to infect barley coleoptiles due to the fungus being inhibited from producing haustoria. Hill *et al* (1998), found that the viability of sporangia of *P. infestans* was severely affected by ion chelators and calcium-modulating treatments. More recently, Becot *et al* (2000) have shown that potassium phosphonate ($K_2HPO_3$) could significantly suppress downy mildew in cauliflowers. In their investigation, the germination of the pathogens' spores was significantly reduced by the potassium phosponate.

In the present study, the completion of *P. fragariae*'s life cycle sexual stage was impaired by the MLSE and potassium salts through the significant reductions in numbers of one of its most important reproductive structures – the sporangia. Furthermore, zoospores formed and released from sporangia challenged with MLSE did not present a normal locomotion pattern that is essential to localise and invade a host in the soil

environment. The effects of the seaweed extracts on the zoospores were not further investigated due to time constraints, therefore, it is not known whether the abnormal behaviour was due to influences on sporangia, on the zoospore or on both. It was, however, demonstrated that the seaweed extracts had the ability to affect *P. fragariae* growth in liquid media *in vitro* and it was therefore hypothesised that they might have the ability to affect the pathogenic activity of the fungus *in planta*.

Data from experiments in hydroponics and growth chamber with strawberry plants showed that red core infections in plants treated with MLSE were significantly less severe. Although the degree of suppression varied between replicates in these systems, on average, they were statistically significant and also sustained improved plant growth of infected plants. Data suggested that MLSE applications did not have any effects on the growth of non-infected strawberry plants.

Considering the suppressive effects of the MLSE on the growth of *P. fragariae* in the *in vitro* assays, the responses obtained *in planta* could imply that the fungus was similarly affected by the seaweed extracts in the hydroponic substrate and in compost. Also possible, would be a reduction in the infective efficacy of zoospores applied as inoculum brought about by the MLSE as found in the *in vitro* studies. If this effect was reproduced in the nutrient solution of the hydroponic system, zoospores would have been unable, or at least slowed down in their endeavour to localise and parasitise the roots of strawberry plants. Another possibility is that, in similarity to the *in vitro* responses, the pathogen was not capable of producing sporangia with the consequent limited occurrence of secondary disease infections. The occurrence of secondary infections is fundamental for the disease proliferation after the initial infection event.

A reduction in release and spread of the causal agents of infection would explain a limitation on infected root mass. It is also possible, though, that the reduced disease

observed in wheat and strawberry plants could be explained by a heightened stimulation of the plant defence mechanisms triggered by the MLSE. It has been shown that certain components of seaweed extracts can have an elicitor effect on plant cells and plants that allows them to respond faster to pathogen attack thus reducing its impact. Algal polysaccharides, such as laminarin and carrageenans, have been found particularly active and these are similar to the polysaccharides found in the cell walls of some fungal pathogens, such as *Phytophthora* (Mercier, 2001; Okinaka, 1995; Patier *et al*, 1995).

Walsh (1997) showed that in order to obtain a suppression of damping-off (*Pythium ultimum*) in *Brassica oleracea* applications of the seaweed extract should be made prior to inoculation of plants. This suggests that the stimulus obtained with the product was not immediate and probably involved switching and build up biochemical/physiological mechanisms in the plant. This would agree with a beneficial action of the seaweed product being implemented through the stimulation of systemically acquired resistance mechanisms.

In our experiments the greater beneficial effects of the seaweed extract in the strawberry/Red Core system, as compared to the wheat/Take-all system for example, seems to have coincided with the simultaneous application of the inoculum and the extract. While in the wheat/soil-borne pathogens the inoculum was present in the rhizosphere for one to two weeks before any MLSE treatment was applied, in the strawberry/red core system the seaweed extract was supplied immediately after the inoculation. It is suggested that because of this simultaneous application both pathogen and extract could have had an elicitation effect that would additively stimulate the plants' defences. A re-enforcement or a build up of plant defence strategies could have consequently resulted and a containment of the pathogen invasion more quickly achieved.

Work by Featonby-Smith and Van Staden (In: Cogram, 1994), showed that seaweed extracts were most active in terms of stimulation of plant growth when applied at early vegetative stages. The objective of the present studies was not to establish the best timing for seaweed applications, therefore, timing for extracts application was chosen bearing in mind results of past investigations. In face of the non significant increase in plant growth in response to treatments with the Maxicrop extracts obtained in almost all the experiments, it could be argued that the stages chosen were not the ones at which a maximized performance could be obtained. This is a line of research that might be worth further exploration following the investigation described here.

It was shown in previous research (Walsh, 1997; Pattison, 1994; Cogram, 1994) that there were considerable variations between batches of Maxicrop liquid seaweed extracts with respect to their effects on plants, microbial organisms and also the biochemical activity of compost. Through out this research project it was decided to use the same batch of Maxicrop liquid seaweed concentrate, thus, it could be argued that this batch was not one of the most active. Variations in product performance may be due to differences in seaweed harvesting time, that has been shown to affect the quality of extracts obtained, (de Nys *et al*, 1990), or to slight differences in extraction procedures. Normalization of products may be essential if a standard output is to be achieved, however, this requires a better knowledge of constituents and their effects on plant and microbial populations alike. Nevertheless, since seaweed extracts are complex mixtures of various compounds, which may have different effects on diverse organisms, an exact standardization may not be possible or even advantageous.

## OVERALL CONCLUSIONS

- MLSE did not significantly improve growth of non-infected wheat and strawberry plants.
- It was found that EMG applied at low rates did not have a significant effect on the wheat, however, it was shown that at high rates it could significantly reduce wheat growth.
- MLSE did not significantly affect *G. graminis* mycelial growth in agar.
- MLSE showed variable capacity, ranging from significant to none, to suppress the infection of wheat by *G. graminis*. Although *G. graminis* infections of wheat were significantly reduced in some experiments in hydroponics and glasshouse, only very marginal responses were observed in the field trials.
- MLSE did not affect growth of *P. fragariae* in agar but it significantly suppressed the growth of the fungus in liquid soilless solution. It was found that the number of sporangia was significantly reduced by seaweed extract treatments and the zoospores released evidenced a defective locomotion. Studies of MLSE components that might be responsible for the responses obtained showed that laminarin, β-glucan and β-glucanase were probably not involved. Responses to applications of potassium salts, however, effectively mimicked the behaviour observed for the MLSE treatments.
- MLSE applications significantly suppressed *P. fragariae* infections of strawberry plants grown in hydroponics and in compost in the growth chamber. The disease suppression obtained sustained improved plant growth of infected plants.

The experimental results presented here suggest that MLSE can have beneficial effects on plant health but that the responses to the extract are variable. MLSE did not have a fungicide-like effect on *G. graminis* and the effects of the product on the establishment of this and the other wheat diseases also studied (results not presented here) suggest that the suppression obtained might be due to a stimulation of the plant defence mechanisms rather than a direct effect on the pathogens. The responses obtained were variable and they seemed to have lost significance as the complexity of the system increased as observed in the transition from hydroponics to glasshouse and field.

MLSE showed a consistent fungicide-like effect on *P. fragariae* growth in the *in vitro* studies and an effective significant reduction of the disease symptoms of plants grown both in hydroponics and growth chamber. It is possible that in this system the mechanism of action of the MLSE was additive, that is to say, it affected both the pathogen and the plant, consequently a more consistent reduction on the disease level occurred. Further investigations of the mechanisms involved in the strawberry/Red Core system might be useful, particularly, if a study can be carried out in the field. The disease can pose great difficulties in the strawberry production and an additional measure to reduce its impact in the field would prove valuable.

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# APPENDICES

## APPENDIX I – ASSESMENT KEYS

The following disease assessment key was used to evaluate the symptoms caused by *Gaeumannomycis graminis* in wheat seedlings grown in hydroponics.

(The rating system was applied to each individual seminal root).

0- no infection visible

1- very light infection: small discrete (<1cm) light brown discoloration.

2- light infection: small discrete (<1cm) dark brown lesions present.

3- moderate infection: dark brown or black lesions >1cm present in more than one root.

4- severe infection: several roots completely black associated to severe plant stuntiness.

5- very severe infection: >50% of roots infected and stem base blackened: associated to severe wilting and yellowing or death from take-all.

The following disease assessment key was used for measuring symptoms caused by *Gaeumannomycis graminis* in wheat plants grown in the glasshouse and the field.

0- no infection visible

1- very light infection: 5-10% of roots infected; light brown discoloration.

2- light infection: >10%<25% of roots infected; small discrete (<1cm) dark brown lesions present.

3- moderate infection: >25%<50% of roots infected; dark brown or black lesions >1cm present in more than one root.

4- severe infection: >50% of roots infected; several roots completely black associated to severe plant stuntiness.

5- very severe infection: >50% of roots infected and stem base blackened: associated to severe wilting and yellowing or death from take-all.

The following disease assessment key was used to evaluate symptoms caused by *Phytophthora fragariae* in strawberry plants.

0- no infection visible

1- very light infection: necrotic tips + red and rotting of steles in < 3% % of length adventitious roots;

2- light infection: > 4 % < 25% rotting and red steles;

3- moderate infection: > 26% < 50% rotting and red steles;

4- severe infection: > 51% < 75% rotting; yellowing and wilting leaves;

5- very severe infection: > 75% rotting; plant stunted or dead;

## APPENDIX II – EXAMPLE OF ANOVA ANALYSIS CARRIED OUT IN EXCEL

Analysis of data obtained for experiment 2.b described in page 133.

Table 6 – Average number of sporangia per field of view.

| Percentage of MLSE (5ml $l^{-1}$) in SL | 0 | 25 | 50 | 75 | 100 |
|---|---|---|---|---|---|
| Rep 1 | 20.5 | 4.33 | 2.33 | 0.167 | 0.167 |
| Rep 2 | 22.5 | 4.67 | 4.5 | 3 | 0.167 |
| Rep 3 | 5.5 | 5.33 | 3.33 | 1.33 | 0.167 |
| Rep 4 | 14.33 | 7.83 | 2.5 | 1.83 | 0 |
| Average | 15.7075 | 5.54 | 3.165 | 1.58175 | 0.12525 |

Table 7- Anova analysis of data presented in Table 6 obtained in Excel.

| *Summary* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Row 1 | 5 | 27.494 | 5.4988 | 73.32489 |
| Row 2 | 5 | 34.837 | 6.9674 | 78.65337 |
| Row 3 | 5 | 15.657 | 3.1314 | 5.629065 |
| Row 4 | 5 | 26.49 | 5.298 | 33.97817 |
| | | | | |
| Column 1 | 4 | 62.83 | 15.7075 | 58.39889 |
| Column 2 | 4 | 22.16 | 5.54 | 2.503067 |
| Column 3 | 4 | 12.66 | 3.165 | 0.982967 |
| Column 4 | 4 | 6.327 | 1.58175 | 1.379319 |
| Column 5 | 4 | 0.501 | 0.12525 | 0.006972 |

**ANOVA**

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Rows | 37.49705 | 3 | 12.49902 | 0.984713 | 0.432577 | 3.4903 |
| Columns | 614.0254 | 4 | 153.5063 | 12.09373 | 0.000358 | 3.25916 |
| Error | 152.3166 | 12 | 12.69305 | | | |
| | | | | | | |
| Total | 803.839 | 19 | | | | |

The Least Significant Difference between samples (LSD) was calculated to determine which treatments were significantly different using the formula below.

LSD = t√(MSError:number replicates per treatment).

# APPENDIX III - SUMMARIZED ADDITIONAL WORK DEVELOPED THROUGH-OUT THE PhD RESEARCH PROGRAM

A preliminary study of growth responses to Maxicrop seaweed extracts (Maxicrop Concentrate and Maxicrop Extruded Granules) by wheat and pea plants grown in pots in a glasshouse environment was developed, of which results are presented here. In this phase of the research project, in addition to take-all, two other wheat pathogens were studied: fusarium (*F. culmorum*), and eyespot (*P. herpotrichoides* (Fon) Deighton). The infectivity of phoma (*P. medicaginis*) in pea plants was also investigated following applications of the seaweed extracts.

## METHOD AND MATERIALS

### Preparation of inocula

Three inoculation procedures were used according to the demands of each particular type of organism.

*P. herpotrichoides* and *F. culmorum* were grown as sand-cornmeal cultures comprising 100g washed sea-sand, 2 g maize-meal and 30ml distilled water. Sterilised medium was inoculated with plugs of actively growing mycelium taken from 2-3 week old cultures grown on potato dextrose agar (PDA). Composts were inoculated with 10 g of sand-cornmeal pathogen culture (grown for 4-5 weeks at room temperature). Un-inoculated sand cornmeal was prepared for use in the control treatments.

Pea seeds (variety Feltham First, round seeded) were inoculated by dipping for 1h in a suspension of $1.7\times10^{6}$ $ml^{-1}$ pycnidiospores scraped from the surface of sporulating *Phoma* cultures maintained on PDA under natural light. Un-inoculated pea seeds used for control treatments were dipped in sterile distilled water for 1h.

**Preparation of soil inoculum**

A fresh soil inoculum was prepared as described for the hydroponics experiments (Chapter II) and 1 ml aliquots were added to the compost in some experiments as appropriate.

**Planting and growth conditions**

For wheat and pea pot experiments six seeds were sown and plants grown in 11 cm diameter pots maintained in the glasshouse with a 12 h day. It was aimed to maintain a maximum temperature of 22-27°C, but this proved to be extremely difficult during some experiments run in Spring time when wider variations of glasshouse temperature occurred. The pea experiment was started in late spring and although the pots were kept in the glasshouse during the first two weeks it was decided to transfer it to a protected open air area to avoid excessively high temperatures.

The growth medium used was John Innes compost, each pot containing 250 g. Where Maxicrop granulated seaweed extracts were applied, these were mixed with the compost at potting.

**Sampling and assessment techniques**

The parameters analysed in wheat plants were: disease level, plant growth stage, plant height, shoot dry weight and leaf area. This last parameter was collated only in the first two experiments since the procedure adopted revealed to be very time consuming and of questionable use. Pea plants were assessed for disease level, plant shoot fresh and dry weight and number of leaves.

After the appropriate times plants were harvested and the roots washed prior to analysis of root/shoot base infections according to assessment keys (Appendix 1). Symptoms were categorised, with 0 representing a healthy plant and 4 or 5 the most severely infected plants. Results were analysed using the One-way analysis of variance based on mean values for the plants in a pot and were carried out using Excel.

**Preparation of bacterial solution**

A solution of *Ps. fluorescens* was applied in some experiments following the procedure described in Chapter II.

## 1. WHEAT - PRELIMINARY GLASSHOUSE EXPERIMENTS

For the study of the responses of eyespot and fusarium of wheat to applications of the seaweed extracts two repeats of each experiment were conducted. The following treatments were applied using a fully randomised-block design with six replicates per treatment.

Table 8- Treatments applied to wheat plants to investigate the effects of Maxicrop seaweed extracts.

| Treatment | Corn meal | Soil inoculum | + pathogen | 10 g MEG | 20 g MEG | MLSE Spray 5 $ml^{-1}$ | MLSE Spray 10 $ml^{-1}$ |
|---|---|---|---|---|---|---|---|
| Control 1 | - | - | - | - | - | - | - |
| Control 2 | √ | √ | - | - | - | - | - |
| Control 3 | √ | √ | √ | - | - | - | - |
| T1 | √ | √ | √ | √ | - | - | - |
| T2 | √ | √ | - | √ | - | - | - |
| T3 | √ | √ | √ | - | √ | - | - |
| T4 | √ | √ | - | - | √ | - | - |
| T5 | √ | √ | √ | - | - | √ | - |
| T6 | √ | √ | - | - | - | √ | - |
| T7 | √ | √ | √ | - | - | - | √ |
| T8 | √ | √ | - | - | - | - | √ |

MEG = Maxicrop extruded granules
MLSE = Maxicrop Concentrate Liquid Seaweed Extract

Sprays of MLSE were applied at two different growth stages: one week after sowing when plants had reached GS 11 ($1^{st}$ leaf unfolded) and at $4^{th}$ week after sowing when plants had reached GS 22 ($2^{nd}$ tiller visible). The experiments were terminated at the $6^{th}$ week after sowing.

**Results - Fusarium Experiment**

The inoculum used in this experiment gave rise to light to moderate levels of fusarium symptoms (Fig. 3.1). Non-inoculated plants (Control 1 and 2, T2, 4, 6 and 8) also presented slight disease symptoms possibly indicating that cross-contamination occurred. The symptoms found in non-inoculated plants were, nevertheless significantly lighter than in Control 3 (inoculated control). Under favourable environmental conditions fusarium is known to produce a high amount of spores and cross-contamination is a common occurrence in glasshouse experiments (Dr. Pitt, personal communication).

Plants amended with 5ml $l^{-1}$ MLSE (T5) showed a level of disease infection equal to the inoculated control (Control 3). Nevertheless, disease symptoms were reduced where other Maxicrop treatments were applied although differences were not statistically significant.

Results suggested that plants amended with MEG and the highest MLSE spray rate (10 ml $l^{-1}$) had poorer growth than control plants. Thus, plants treated with MEG applications and 10 ml $l^{-1}$ MLSE (T1, T2, T3, T4, T7 and 8) were at a lower GS than plants of other treatments at harvest (differences not statistically significant). A significant reduction in mean plant height (Fig. 3.2) was observed in plants treated with 20 g of MEG (T3 and T4).

Fig. 3.1- Effect of Maxicrop Seaweed extracts on Fusarium disease infection on Wheat. Columns with the same letter are not statistically significantly different from each other (P>0.05).

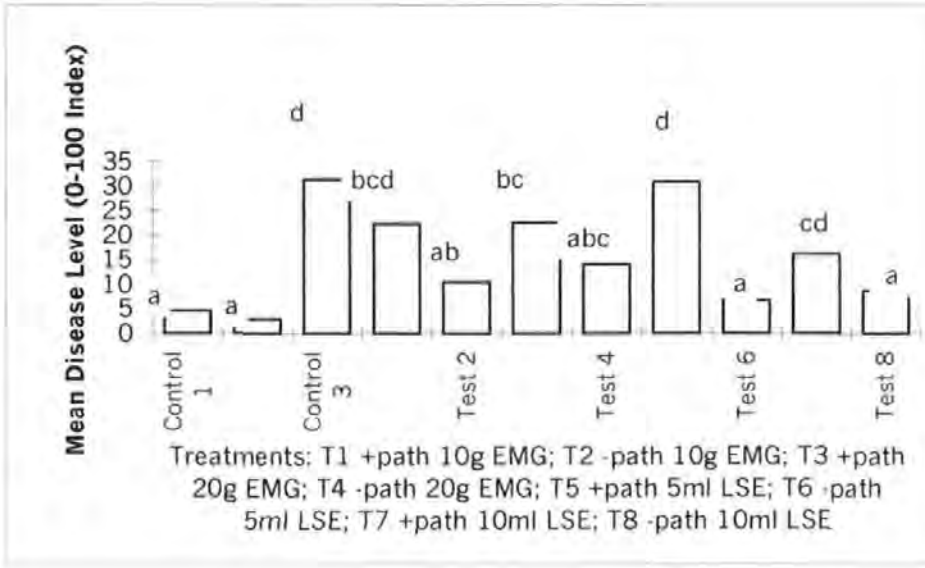



Fig. 3.2- Response of wheat to Maxicrop seaweed extracts. Columns with the same letter are not statistically significantly different from each other (P>0.05).

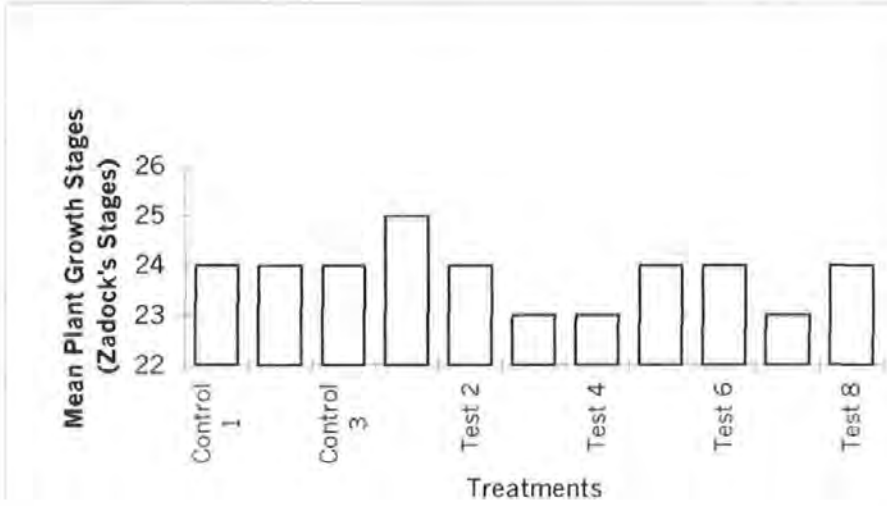
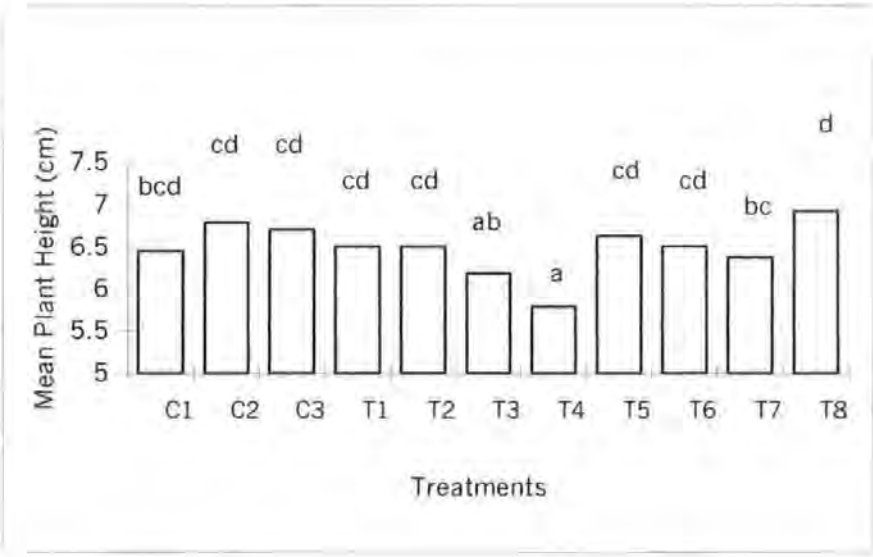


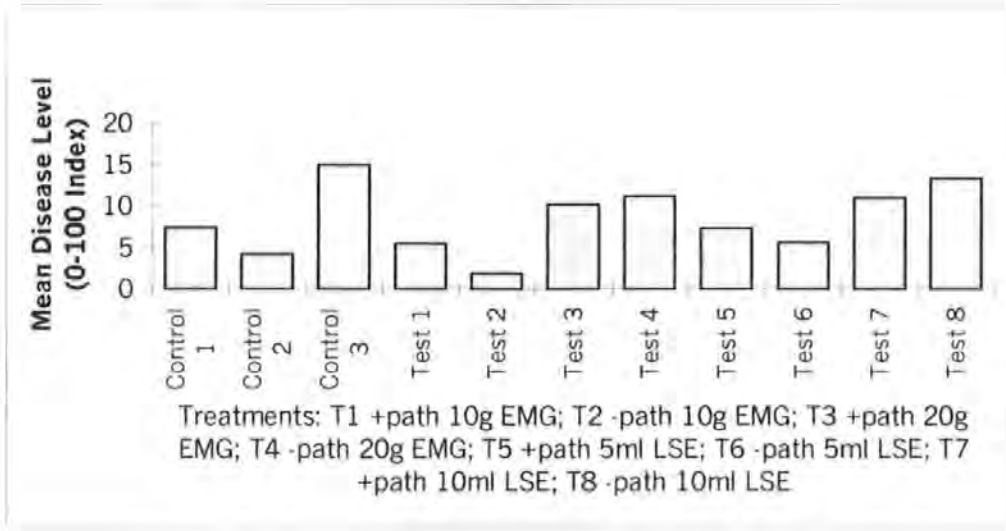
Fig. 3.3- Response of wheat to Maxicrop Seaweed Extracts. Columns with the same letter are not statistically significantly different from each other (P>0.05).




**Results - Eyespot Experiment**

For all the treatments applied only very light levels of eyespot infection were found (Fig. 3.4). Statistical analysis showed that there were no significant differences in infection levels as a response to any of the treatments although all the plants treated with Maxicrop presented lighter symptoms of the disease than Control 3. Plants treated with 10 g of MEG (T5), presented the least severe disease infections.

Fig.3.4- Response of Eyespot to Maxicrop Seaweed Extracts.




There were no significant responses of the wheat plants to MLSE sprays in terms of growth stage, plant height and shoot dry weight. Plant leaf area, however, was significantly reduced where 10 ml $l^{-1}$ MLSE (T8) were applied. Both treatments with MEG (T3 & T4) caused a suppression of plant growth as expressed by growth stage, plant height and leaf area. This reduction was statistically significant for growth stage, leaf area and plant height when compared to Control non-inoculated (Control1 – Figs. 3.5 - 3.7).

Fig. 3.5- Response of Wheat to Maxicrop Seaweed Extracts. Columns with the same letter are not statistically significantly different from each other (P>0.05).

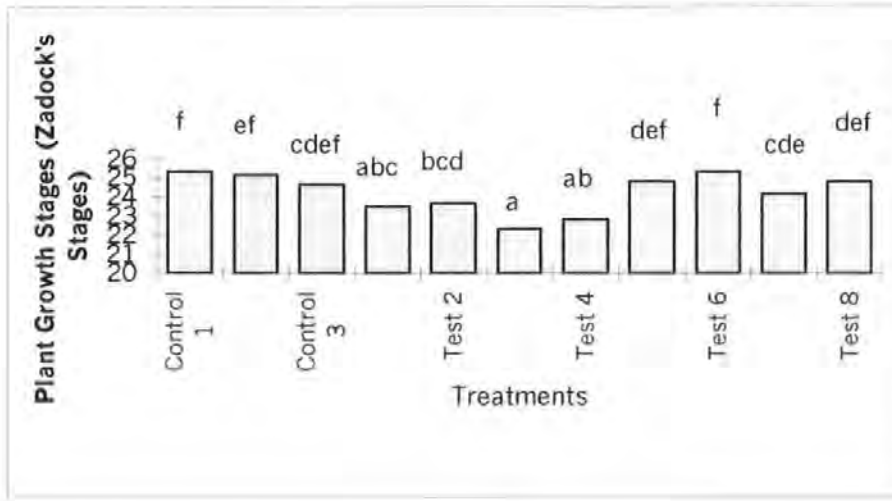



Fig. 3.6- Response of wheat to Maxicrop Seaweed extracts. Columns with the same letter are not statistically significantly different from each other (P>0.05).

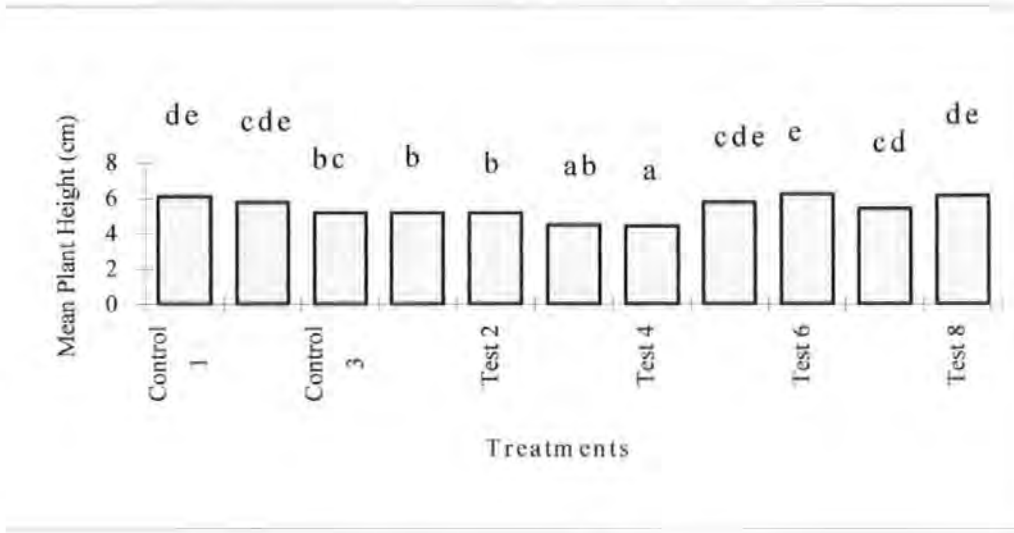
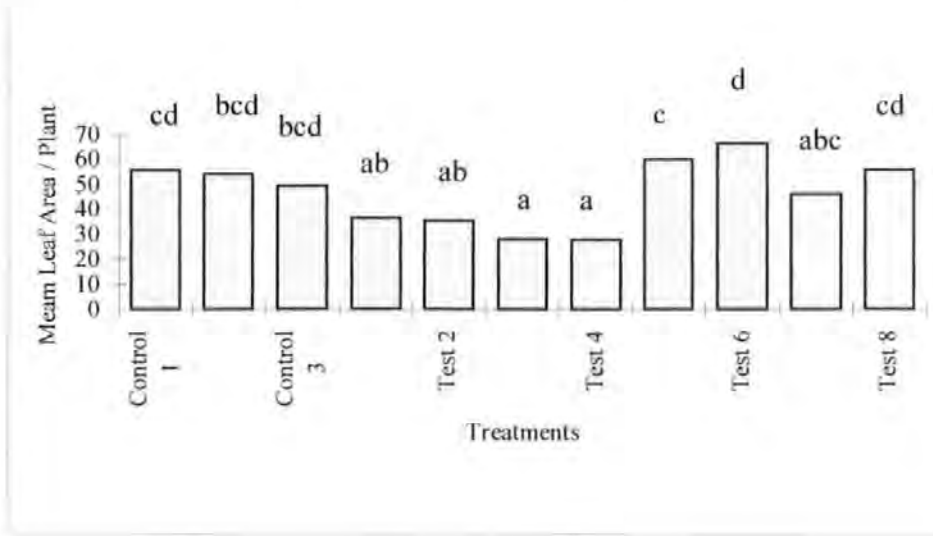


Fig. 3.7- Response of wheat to Maxicrop Seaweed extracts. Columns with the same letter are not statistically significantly different from each other (P>0.05).



## 2. PEAS - PRELIMINARY GLASSHOUSE EXPERIMENTS

To study the responses of pea plants and the infectivity of phoma following the amendment with seaweed extracts one experiment was conducted, the treatments studied are shown in Table 9.

Table 9- Treatments applied to Pea plants to investigate the effects of Maxicrop seaweed extracts.

| Treatment | Soil Solution | + pathogen | 5 g MEG | 10 g MEG | MLSE Spray 5 $ml^{-1}$ | MLSE Spray 10 $ml^{-1}$ |
|---|---|---|---|---|---|---|
| Control 1 | - | - | - | - | - | - |
| Control 2 | √ | - | - | - | - | - |
| Control 3 | √ | √ | - | - | - | - |
| T1 | √ | - | √ | - | - | - |
| T2 | √ | - | - | √ | - | - |
| T3 | √ | - | - | - | √ | - |
| T4 | √ | - | - | - | - | √ |
| T5 | √ | √ | √ | - | - | - |
| T6 | √ | √ | - | √ | - | - |
| T7 | √ | √ | - | - | √ | - |
| T8 | √ | √ | - | - | - | √ |

MEG = Maxicrop extruded granules

MLSE = Maxicrop Concentrate Liquid Seaweed Extract

MLSE sprays were applied at week 1 and 4 when, on average, plants had 2 and 6 leaves respectively.

**Results**

The levels of disease infection achieved were light (Fig. 3.8). Plants treated with the Maxicrop seaweed extracts presented lighter disease symptoms than non-treated inoculated plants (C3). Although plants treated with 10g of MEG (T6) presented significantly lighter lesions, the plants did not show improved growth (Fig. 3.9). On

average, plants treated with either type of Maxicrop seaweed extract showed a reduction in height and dry weight (not statistically significant). Growth stage was very similar for all plants, except for plants sprayed with 10 ml $l^{-1}$ MLSE which were at a lower stage (not statistically significant).

Fig. 3.8- Effect of Maxicrop Seaweed extracts on Phoma of peas.

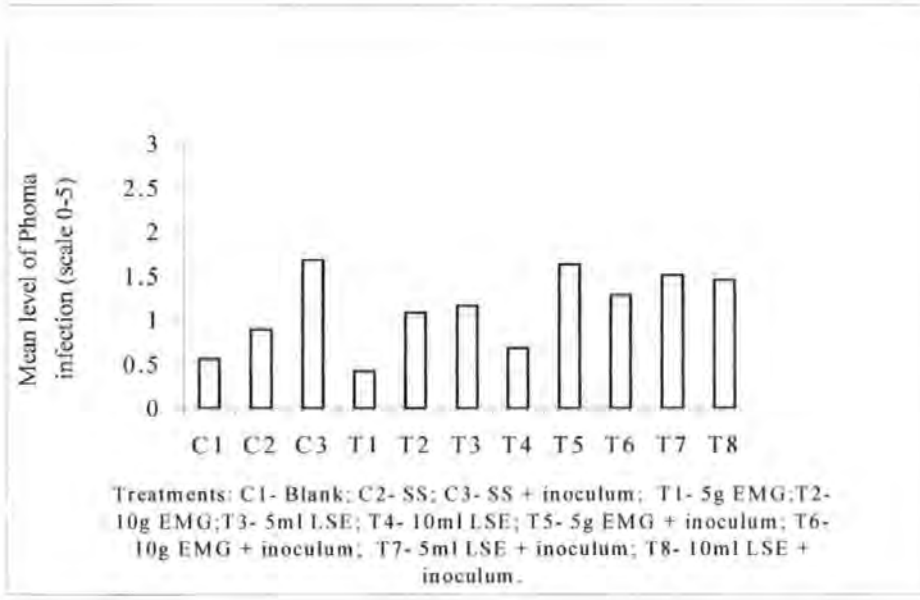


Fig. 3.9- Response of Pea plants to Maxicrop Seaweed extracts.